http://dx.doi.org/10.4314/bajopas.v14i1.15S

Bayero Journal of Pure and Applied Sciences, 14(1): 87 - 93
ISSN 2006 – 6996

SYNTHESIS, CHARACTERIZATION AND *IN VITRO* ANTI-MICROBIAL STUDIES OF SCHIFF BASES DERIVED FROM SOME SELECTED AMINO ACIDS AND INDOLE-3-CARBAZALDEHYDE

[1,3]Sulaiman, Z., [2,3] Umar, A. A. and [3]Ahmad, S. B.

[1]Department of Science Laboratory Technology, School of Science and Technology, Hussaini Adamu Federal Polytechnic Kazaure, P. M. B. 5004, Jigawa, Nigeria.

[2]Department of Science Laboratory Technology, School of Technology, Kano, Nigeria. +234-803-6064-770

[3]Department of Pure and Industrial Chemistry, Faculty of Physical Science, College of Natural and Pharmaceutical Science, Bayero University, P. M. B. 3011, Kano, Nigeria.

[1]Correspondence author: zsjahun@yahoo.com; +234-803-361-3879; +234-803-364-1419

ABSTRACT

Three Schiff bases (L_1 to L_3) were synthesized by refluxing indole-3-carbazaldehyde with three different amino acids (valine, threonine and phenylalanine) in the presence of sodium hydroxide. The synthesized compounds were purified by recrystallization in ethanol and characterized using Infrared spectroscopy, High resolution mass spectrometry, Melting points and CHN microanalysis. The Schiff bases were found to be soluble in polar solvent such as methanol and ethanol but insoluble in non-polar solvent such as hexane. Data from Infrared spectral study indicated that the characteristics band attributed to aldehyde stretching disappeared on the final products and a new absorption band observed at 1629-1635 cm^{-1} was due to the v(C=N) stretching vibration, which is a characteristic band of Schiff base. The CHN analysis of the Schiff bases were consistent with the calculated results from the empirical formula of the proposed structure of each compound. ESI-mass spectra from a methanol solution of the Schiff bases showed singly charged ions at m/z = 289.09, 291.07 and 315.10 which corresponds to $[M+Na]^+$ singly charged adduct ions of L_1 to L_3 respectively. Antibacterial and antifungal assay of the Schiff bases were performed in vitro against Stapylococcus aureus, Streptococcus pneumoniae, Salmonella typhi, Escherichia coli, Trichophyton tonsurans and Tricophyton rubrum.

Keywords: Indole-3-carbazaldehyde, amino acids, Schiff base, Electro spray Ionisation -mass spectrometry.

INTRODUCTION

Schiff bases are one of the important classes of organic compounds which are characterized by the -N=CH- (imine) group. They are sometimes referred to as azomethines or imines and are synthesized by the reaction of aldehyde or ketones with primary amines (Loudon and Parise, 2016). The number of amino and keto precursors available for condensation reactions leading to azomethine compounds is practically unlimited. As such, the judicious selection of the two precursors allows a perfect control over the denticity of the resulting ligands, the nature of the donor atoms and the number of the chelating moieties (Matius, 2015).

Schiff base are important class of compounds due to their large number of applications owing to their characteristic properties such as high synthesis flexibility, varied coordinating ability, thermal stability, novel structures, biological activities and medicinal utility (Pal *et al.,* 2000; Asadi and Sarvestani, 2001; Kumar *et al.,* 2003). A number of Schiff bases have been reported to possess anti-glycation (Taha *et al.,* 2014; Khan *et al.,* 2013), antioxidant (Anouar *et al.,* Sinha *et al.,* (2008) synthesized eight novel heterocyclic Schiff bases derived from the condensation reactions of indole 3-carboxaldehyde with different L-amino acids (histidine, glutamic acid, aspartic acid, leucine) as well as with some 2013; Taha *et al.,* 2013 (a)) anti-leishmanial (Taha *et al.,* 2013 (b)), antifungal (Sundriyal *et al.,* 2006), antitumor (Sinha *et al.,* 2008) and analgesic (Chinnasamy *et al.,* 2010) activities. It has been suggested that the active pharmacophore (-N=CH-) of Schiff bases plays a major role in these significant biological activities (Ahmad *et al.,* 2014). However, the attached neighboring groups may also affect the activity (Satyanarayana *et al.,* 2011).

Additionally, Schiff bases are important intermediates in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate (Liimatainen *et al.,* 2000). Schiff bases can accommodate different metal centers involving various coordination modes allowing successful synthesis of homo and hetero-metallic complexes with varied stereochemistry (Choudhury *et al.,* 2002). This feature is employed for modelling active sites in biological systems (Wang *et al.,* 2011)

Schiff base ligands have been extensively studied in coordination chemistry mainly due to their facile synthesis, tunable steric, electronic properties, and good solubility in common solvents (Shi *et al.,* 2004) aminophenols. The compounds were characterized by various spectroscopic methods (IR, MS, ^{1}H NMR). Sivasankaran and co-workers in 2016 reported the synthesis and characterization of Schiff base ligand Indal-4-AAP, derived from indole-3-carboxaldehyde

and 4-aminoantipyrine and the *in vitro* biological screening effects of the synthesized compounds were tested against various microbial species.

To contribute to these studies, three new Schiff bases were prepared by separately condensing indole-3-carboxaldehyde with each of the selected amino acids (valine, threonine and phenylalanine) via reflux in ethanolic solution. In addition to the characterization of the Schiff bases by physicochemical and spectrophotometric techniques, biological activities of the synthesized Schiff bases were examined against some microbial strains for evaluation of antibacterial and antifungal activities.

MATERIALS AND METHOD

Reagents

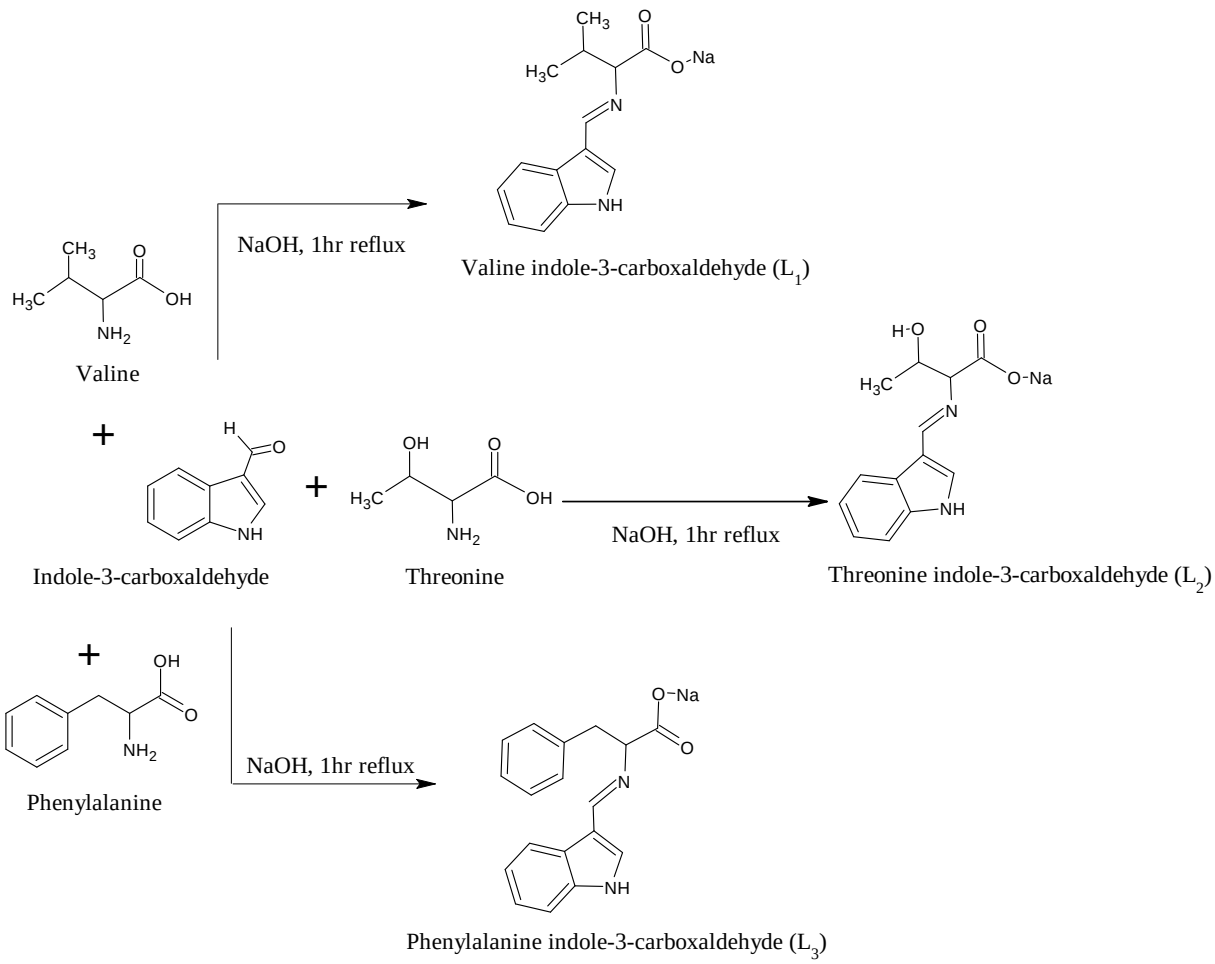
Chemicals (reagent grade) were purchased from sigma Aldrich and Alfa Aesar and were used without further purification. L-valine, L-threonine, L-phenylalanine, indole-3-carboxaldehyde and sodium hydroxide were used as starting materials for the preparation of the Schiff base. All glass wares used were thoroughly washed with detergent, soaked in 5% nitric acid, rinsed with distilled water and dried in an oven.

Instruments

All weighing was carried out on electric mettle balance model AB54, Melting points were determined using a digital WRRS-IB Microprocessor melting points apparatus. Infrared spectral analyses were recorded using Perkin Elmer Infra-red Model 337. High-resolution mass spectra were recorded by electro spray ionization (ESI) technique on Apex-III mass spectrometer.

Synthesis of the Schiff Bases

Ethanolic solution (20 cm^3) of the respective amino acid (valine, threonine or phenylalanine) (0.01M) were added with continuous stirring to indole-3-carboxaldehyde (0.01M) dissolved in 10 cm^3 ethanol. 0.4g of NaOH was added to the mixture and was refluxed for l hour. The reaction mixture was cooled on an ice bath. The product obtained was filtered, washed with ethanol (5cm^3) then with diethyl ether (10cm^3) and dried. The crude product was recrystallized from aqueous ethanol to give the corresponding Schiff base (Zahid *et al.,* 2007).



Scheme 1: Synthetic Reactions of the Schiff bases (L_1-L_3)

Anti-microbial screening

The synthesized Schiff base and its corresponding metal complexes were screened for antibacterial activity against bacterial species: *Stapylococcus aureus, Escherichia coli, Streptococcus pneumoniae* and *Salmonella typhi* and antifungal activity against *T. tonsurans* and *T. rubrum.*

Anti-bacterial test

Using an inoculation loop, enough material from an overnight culture of the test organism was transferred

into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 Mcfarland standard (as a reference to adjust the turbidity of bacterial suspensions) as described by clinical laboratory standard institute (CLSI), (2020). The standard Inocula of the isolate were swabbed on to the surface area of the prepared agar plates. Nutrient agar was used as the required medium. The Schiff bases were dissolved in dimethyl sulfur oxide (DMSO) to produce four different concentrations 40, 20, 10 and 5µg per disc. A sterilized forcep was used to place the prepared disc of the Schiff bases on the already inoculated agar plates at various intervals and then incubated at 37 °C for 24hrs (Yusha'u and Salisu, 2011).

Anti-Fungal test

For anti-fungal activity, potato dextrose agar (PDA) was used as the required medium. A sterilized forcep was used to place the prepared disc of the Schiff bases on the already inoculated agar plates at various intervals and was left for 3days at room temperature (Hassan *et al.,* 2006). The inhibition zone of the Schiff base and the corresponding metal complexes were measured (in millimeter) around the disc and were compared ketoconazole as the standard drug.

RESULTS AND DISCUSSIONS

Reaction between indole-3-carboxaldehyde and the selected amino acids (valine, threonine or phenylalanine) resulted in the formation of yellow to pale brown Schiff bases (L_1-$L_{3,}$) which are soluble in water and some polar solvents such as methanol, ethanol, DMSO etc but insoluble in hexane. Percentage yield of the synthesized compounds is high within the range 91.05-94.51% as presented in table 1. The observed sharp melting point of the Schiff bases is within the range 161-168 ^oC which indicates purity of the ligands. The results of elemental (CHN) analysis (Table 2) are in good agreement with those calculated for the suggested formula of the compounds.

Table 1: Physical Properties of the Schiff bases

Compound	Molecular Formula	Colour	Melting Point (^oC)	Percentage Yield (%)
L_1	$C_{14}H_{15}N_2NaO_2$	Yellow	161	94.51
L_2	$C_{13}H_{13}N_2NaO_3$	Pale yellow	168	91.05
L_3	$C_{18}H_{14}N_2NaO_2$	Pale brown	164	92.07

Where L_1 = Valine-indole-3-carboxaldehyde Schiff base
L_2 = Threonine-indole-3-carboxaldehyde Schiff base
L_3 = Phenylalanine-indole-3-carboxaldehyde Schiff base

Table 2 : Elemental analysis of the Schiff bases

Compound	% Elemental analysis Observed (Calc)		
	C	H	N
L_1	63.16(63.18)	5.64(5.68)	10.53(10.53
L_2	58.61(57.65)	4.88(4.72)	10.45(10.81)
L_3	68.57(68.80)	4.45(4.81)	8.89(8.92)

Where L_1 = Valine-indole-3-carboxaldehyde Schiff base
L_2 = Threonine-indole-3-carboxaldehyde Schiff base
L_3 = Phenylalanine-indole-3-carboxaldehyde Schiff base

Infra-red Spectroscopy

The important IR spectral data are given in Table 3. The significant feature in the infrared spectra of the synthesized Schiff bases was presence of bands at 1629, 1632 and 1635 cm^{-1} (Fig. 1a-1c) which falls within the range 1520-1690 cm^{-1} assignment for ν(C=N) stretching vibrations (Nakamoto, 2009). This suggests the formation of the ligands by reaction of indole-3-carbozaldehyde with the respective amino acids (valine, threonine or phenylalanine). The formation of imine linkage (–C=N-) as well as lack of carbonyl (–C=O-) and amino (-NH_2-) groups from original amino acid and aldehydic compounds, also confirms the formation of the Schiff bases. The bands observed at 1496, 1438 and 1432 cm^{-1} in the IR spectrum of L_1, L_2 and L_3 Schiff bases (Fig 1a-1c) respectively are characteristics of COO^- symmetric stretching of carboxylic group. Bands at 1570, 1515 and 1577 cm^{-1} are due to asymmetric stretching of carboxylic group for the Schiff bases L_1, L_2 and L_3 as seen in figures 1a-1c respectively. These values a close to those reported by Joseyphus *et al* (2006) and Pretsch *et al* (2000).

Table 3: Infrared Spectra of the Schiff bases

Compound	*ν*(C=N)	*vas*(COO^-)	*vs*(COO^-)
L_1	1629	1570	1496
L_2	1632	1515	1438
L_3	1635	1577	1432

Where L_1 = Valine-indole-3-carboxaldehyde Schiff base
L_2 = Threonine-indole-3-carboxaldehyde Schiff base
L_3 = Phenylalanine-indole-3-carboxaldehyde Schiff base

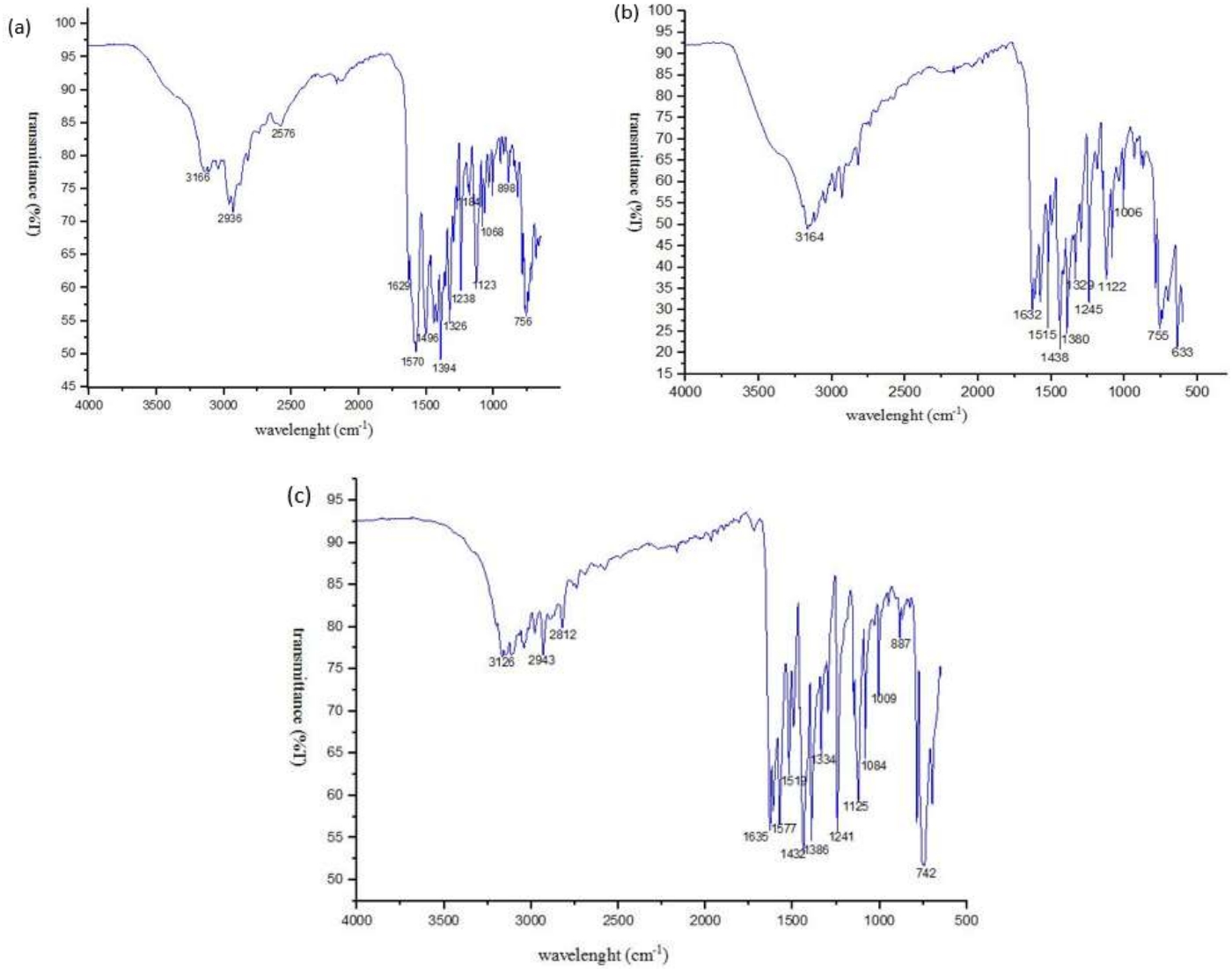


Figure 1: Infrared spectra of (a)Valine-indole-3-carboxaldehyde (L_1) (b)Threonine-indole-3-carboxaldehyde (L_2) (c) Phenylalanine-indole-3-carboxaldehyde (L_3)

Mass Spectrometry-ESI

The high resolution ESI mass spectrum of valine-indole-3-carboxaldehyde (L_1) (Fig. 2a) shows a well-defined peak at m/z = 289.09 which corresponds to singly charged sodium adduct $[M+Na]^+$ (Table 4). Additionally, base peak at m/z = 440.17 corresponds to singly charged potassium adduct in the form $[M+4K-3H]^+$. Similar adduct ions were reported by Anneli and Karl (2017), Sricker, *et al.* (2021) and Sulaiman *et al.,* (2022). The esi-mass spectrum of Thereonine-indole-3-carboxaldehyde (L_2) is shown in figure 2b. The base peak at m/z = 291.07 corresponds to the sodium adduct ion $[M+Na]^+$. The high intensity of this peak indicates great stability of this ligand in the gas phase (Fig. 2b). In the esi mass spectrum of Phenylalanine-indole-3-carboxaldehyde (L_3) schiff base (Fig. 2c), the molecular ion peak was observed at m/z = 315.10 corresponding to the molecular weight of the shiff base (L_3). It can be noted that sodium adduct ion $[M+Na]^+$ at m/z = 337.09 is also present (Juribašić *et al.,* 2011; Natalia *et al.,* 2015).

Table 4: ESI-Mass Spectral data for theSchiff bases

Compound	Found m/z	Expected m/z	Assignment
L_1	289.09	289.27	$[M+Na]^+$
L_2	291.07	291.24	$[M+Na]^+$
L_3	315.10	314.22	$[M+Na]^+$

Where L_1 = Valine-indole-3-carboxaldehyde Schiff base
L_2 = Threonine-indole-3-carboxaldehyde Schiff base
L_3 = Phenylalanine-indole-3-carboxaldehyde Schiff base
M= Molecular weight of the Schiff base

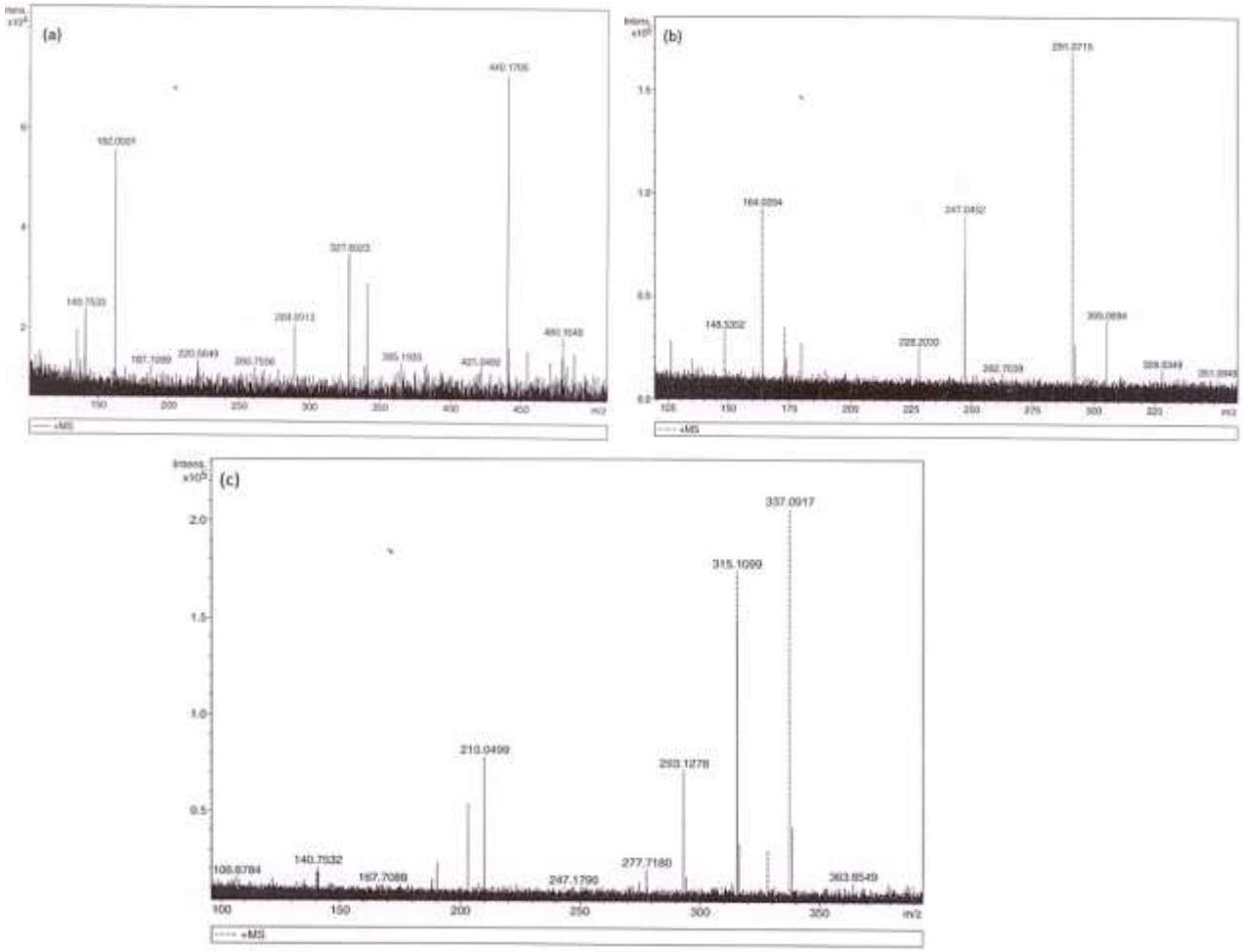


Figure 2: ESI-Mass spectra of (a)Valine-indole-3-carboxaldehyde (L_1) (b)Thereonine-indole-3-carboxaldehyde (L_2) (c) Phenylalanine-indole-3-carboxaldehyde (L_3)

Biological Activity

The synthesized indole-3-carboxaldehyde amino acid Schiff bases were found active against all the tested bacterial strains. Valine-indole-3-carboxaldehyde (L_1) is the most active of the three ligands with inhibition zones ranging from 16mm for *Staphylococcus aureus* and *Streptococcus pneumoniae* 15mm and 14mm for *Escherichia coli* and *Salmonella typhi* respectively, at 40µg/disc (Table 5). Thereonine-indole-3-carboxaldehyde (L_2) is found most active against *Streptococcus pneumoniae* and *Staphylococcus aureus* at 40µg/disc (Table 5) having inhibition zone of 16 and 15mm respectively. Phenylalanine-indole-3-carboxaldehyde (L_3) showed significant activity against *Salmonella typhi* and *Escherichia coli* (Table 5). In general, the antibacterial activity results revealed that all the three Schiff bases shows weak to good activity when compared to control (ciprofloxacin). It is also seen that the Schiff bases are most active against *Staphylococcus aureus* and *Streptococcus pneumoniae* bacterial strains which are the Gram positive type. This is because gram positive bacteria are more susceptible to antibiotics due to absence of outer membrane (Lackna, 2017).

In the case of anti-fungal activity, Ketoconazole was used as positive control and DMSO as negative control. All the Schiff bases were found to be active against both fungal species at all concentrations. And activities increases with increase in concentration of the Schiff bases. The results for anti-fungal activities are presented in Table 5.

Table 5: Growth Inhibition Zone of Microbes

Compounds	Concentration (µg/disc)	Zone of inhibition (mm)					
		Anti-Bacteria				Anti-Fungi	
		S. aureus	*S. pneumoniae*	*S. typhi*	*E. coli*	*T. tonsurans*	*T .rubrum*
L_1	40	16	16	14	15	13	13
	20	14	15	12	14	11	11
	10	13	13	11	12	9	10
	5	11	10	10	11	8	8
L_2	40	15	16	14	12	14	16
	20	12	11	11	10	10	14
	10	10	10	9	9	9	11
	5	9	9	8	8	8	9
L_3	40	14	10	15	16	13	14
	20	12	9	11	13	11	12
	10	10	8	9	11	9	8
	5	9	-	8	10	-	-
Ciprofloxacin	10	14	20	32	37	-	-
Ketoconazole		-	-	-	-	34	44
DMSO	-	-	-	-	-	-	-

Where L_1 = Valine-indole-3-carboxaldehyde Schiff base
L_2 = Threonine-indole-3-carboxaldehyde Schiff base
L_3 = Phenylalanine-indole-3-carboxaldehyde Schiff base

CONCLUSION

Three Schiff bases were synthesized by refluxing indole-3-carbazaldehyde with amino acid in the presence of sodium hydroxide. The compounds were characterized by solubility tests, melting points, elemental analysis, infrared spectroscopy and ESI-mass spectrometry. IR spectral analysis shows bands in the range 1629-1635 cm^{-1} which suggest the formation of imine linkage (-C=N-) between indole-3-carbazaldehyde and the selected amino acids. Data from elemental analysis and high resolution electrospray mass spectrometry confirms the molecular weight and structures of the Schiff bases. The Schiff bases were found to be active against both bacterial and fungal species at all concentrations, and their activities increases with increase in concentration of the Schiff bases.

Acknowledgement

This work was Funded by Tertiary Education Trust Fund (TETFund) Nigeria. The authors are very grateful to University of Sussex Uk, Department of Life Science for the FTIR, Mass spectrometry and elemental (CHN) analysis.

REFERENCES

Ahmad, N. A., Muhammad, T., Nor Hadiani I., El Hassane, A., Sammer Y., Waqas, J. Khalijah, A., Norizan A., Khalid M. K. and Syed M. K., (2014). Synthesis, Crystal Structure, DFT Studies and Evaluation of the Antioxidant Activity of 3,4-Dimethoxybenzenamine Schiff Bases. *Molecules* 19, 8414-8433; doi:10.3390/molecules19068414

Anneli, K. and Karl, K. (2017). Adduct Formation in ESI/MS by Mobile Phase Additives*. Journal of American Society of Mass Spectrometry*. 28:887-894 DOI: 10.1007/s13361-017-1626-y

Anouar, E. H., Raweh, S., Bayach, I., Taha, M., Baharudin, M. S., Di Meo, F., and Trouillas, P. (2013). Antioxidant properties of phenolic Schiff bases: structure–activity relationship and mechanism of action. *Journal of computer-aided molecular design*, 27(11), 951-964.

Asadi, M. and Sarvestani, A. H. (2001). Synthesis, characterization, and thermodynamics of tertiary phosphine cobalt (III) Schiff-base complexes. *Canadian Journal of Chemistry*, 79(9), 1360-1365.

Aziz, A. N., Taha, M., Ismail, N. H., Anouar, E. H., Yousuf, S., Jamil, W.,.& Kashif, S. M. (2014). Synthesis, crystal structure, DFT studies and evaluation of the antioxidant activity of 3, 4-dimethoxybenzenamine Schiff bases. *Molecules*, 19(6), 8414-8433.

Chinnasamy, R.P., Sundararagan, R., Govindaraj, S. (2010). Synthesis, characterization and analgesic activity of novel Schiff base isatin derivatives. *Research in Social and Administrative Pharmacy*, 1(3):342–347.

Choudhury C. R., Dey S. K., Mondal N., Mitra S., Mahalli S. O. and Abdul Malik K. M. (2001) Synthesis and structural characterization of $[Ni(Me_2NCH_2CH_2CH_2N{=}CHC_6H_4O)_2]$ *Journal of Chemical Crystallography*, 31(1):57-62.

Clinical Laboratory Standards Institute(CLSI) (2020), *Performance Standards for Antimicrobial Susceptibility Testing.* 30th ed., Supplement M100. Wayne, PA.

Hassan, S., Umar, R. A., Lawal, N., Bilbis, L. S., Mohammed, B. Y. (2006). Evaluation of

Antifungal activity of *Ficussycomes L.* (*moraceace). Best Journal*. 3(2):18-25.

Juribašić, M., Bellotto, L., Traldi, P., and Tušek-Božić, L. (2011). Electro spray ionization mass spectrometry of palladium (II) quinolinyl amino phosphonate complexes. *Journal of The American Society for Mass Spectrometry,* 22(10):1815-1825

Kumar D., Syamal A. and Gupta P.K., (2003).Syntheses, magnetic and spectral studies of the coordination compounds of polystyrene-anchored dibasic tridentate Schiff base. *Journal of Indian Chemical Society.,*80 (1), 3

Lakna, P. (2017), Differences between gram positive and gram negative bacteria. http;/pediaa.com/difference-between-garm-positive-and-negative-bacteria/4/3/2017

Liimatainen, J., Lehtonen, A. and Sillanpaa R. **(**2000): *cis*-Dioxomolybdenum(VI) Complexes with Tridentate and Tetradentate Schiff Base Ligands. Preparation, Structures and Inhibition of Aerial Oxidation of Aldehydes. *Polyhedron.*19 (9): 1133–1138.

Loudon, M and Parise J. (2016), Organic Chemistry, 6thEdn., New York, H. Freeman, Macmillan Matius A. (2015): The Exceptionally Rich Coordination Chemistry Generated By Schiff-Base Ligands Derived From O-Vanillin. *Dalton Transactions*, 44, 16633.

Nakamoto K., (2009): Infrared and Raman Spectra of Inorganic and Coordination Compounds, 6th ed. John Wiley and Sons: New York, pp47 – 68. Learning., ISBN 978-1-936221-34-9, pp. 984-985

Natalia, G., Beata, P., Piotr S. and Grzegorz, A. (2015). Studies of the complexation behavior of tetramorpholinylo-PNP-lariat ether with Ag(I), Ca(II), Cd(II), Cu(II) and Pb(II) using Electrospray Ionization Mass Spectrometry *The Royal Society of Chemistry and the Centre National de la Recherche Scientifique* 39, 1761—1772.

Pal, S. K., Krishnan, A., Das, P. K., and Samuelson, A. G. (2000). Schiff base linked ferrocenyl complexes for second-order nonlinear optics. *Journal of Organometallic Chemistry,* 604(2), 248-259.

Pretsch, E., Buhlmann, P. and Affolter, C. (2000): Structure determination of organic compounds: tables of spectral data. 3rd Edn. New York. Springer.Pp 264, 273, 309.

Satyanarayana, V. S.V.; Sivakumar, A.; Ghosh, A. R. (2011). Synthesis, characterization of some new five membered heterocycles based on imidazole moiety and their applications on therapeutics*. Letters in Drug Design and Discovery*, 8(3), 276-283.

Shi, Q., Xu, L., Ji, J., Li, Y., Wang, R., Zhou, Z., Cao R, Hong, M and Chan, A. S. (2004).Syntheses and structures of two anion-templated dinuclear cadmium complexes with diamino-binaphthyl Schiff bases as ligands. *Inorganic Chemistry Communications*, 7(12), 1254-1257.

Sinha, D., Tiwari, A. K., Singh, S., Shukla, G., Mishra, P., Chandra, H., & Mishra, A. K. (2008). Synthesis, characterization and biological activity of Schiff base analogues of indole-3-carboxaldehyde. *European journal of medicinal chemistry*, 43(1), 160-165.

Sivasankaran, N. M. Arish D. and Johnson J. (2016), Synthesis, characterization and biological studies on some metal complexes with Schiff base ligand containing pyrazolone moiety *Journal of Saudi Chemical Society.* 20: S591–S598. http://dx.doi.org/10.1016/j.jscs.2013.04.007

Stricker, T., Bonner, R., Lisacek, F. and Hpfgartner, G. (2021). Adduct annotation in liquid chromatography/high-resolution mass spectrometry to enhance compound identification. *Analytical and Bioanalytical Chemistry*. 413:503-517

Sulaiman, Z., Na'aliya, J. and Usman, B. (2022). Mechanochemical Synthesis, Characterization and *In-vitro* Anti-Microbial Studies of Binuclear Cu(II) and Zn(II) Complexes with Schiff Base Derived from Phenylalanine and Vanillin. *ChemSearch Journal*. 13(1): 9 – 22,

Sundriyal, S., Sharma, R. K., and Jain, R. (2006). Current advances in antifungal targets and drug development. *Current medicinal chemistry*, 13(11), 1321-1335.

Taha, M., Naz, H., Rasheed, S., Ismail, N. H., Rahman, A. A., Yousuf, S., and Choudhary, M. I. (2014). Synthesis of 4-methoxybenzoylhydrazones and evaluation of their anti-glycation activity. *Molecules,* 19(1), 1286-1301.

Taha, M., Ismail, N. H., Jamil, W., Yousuf, S., Jaafar, F. M., Ali, M. I., Kashif, S.M and Hussain, E. (2013)(a). Synthesis, evaluation of antioxidant activity and crystal structure of 2, 4-dimethylbenzoylhydrazones.*Molecules,* 18(9), 10912-10929.

Taha, M., Baharudin, M.S., Ismail, N.H., Khan, K.M., Jaafar, F.M., Samreen,Siddiqui, S. and Choudhary, M.I. (2013)(b) **.**Synthesis of 2-methoxybenzoylhydrazone and evaluation of their anti-leishmanial activity. *Bioorg. Med. Chem. Lett*., *23*, 3463–3466.

Wang, Q., Bi C. F., Fan, Y. H., Zhang, X., Zuo, J., and Liu, S. B. (2011). A Novel Copper(II) Complex with Schiff Base Derived from *O*_Vanillin and L_Methionine: Syntheses and Crystal Structures *Russian Journal of Coordination Chemistry,.*37(3):228–234.

Yuhsa'u, M. and Salisu, F.U. (2011): Inhibitory Activity of Detariummicrocarpum Extract on Some Clinical. Bacterial Isolates. *Biological and Environmental Science Journal for the tropics*, 8(4):113-117.

Zahid, H., Chohan, M., Praveen, A. and Ghaffar, A. (1997). Structural and Biological Behaviorof Co(II), Cu(II) and Ni(II) Metal Complexes of Some Amino Acid Derived Schiff-Base Ligands, *Metal Based Drugs.* 4(5):267-272.