

Synthesis and Bioactive Appraisal of Mixed Complexes Mn (II) and Salicylhydroxamic Acid using Oxalic Acid

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Abstract

Several synthesized metal ions of transition metals from mixed ligand form complexes due to coordination bond which can be soluble in polar or non- polar solvents depending on conditions. This study was aimed to synthesis ligands of salicylhydroxamic acid (SHA) and oxalic acid that formed Mn (II) ion complexes. The solubility test on yielded complexes showed positive effects on methanol, ethanol while Di-methyl sulfoxide (DMSO) became inert under distilled water and chloroform. Melting point test revealed that mixed complexes and SHA are within the temperature range of 180°C to 300°C respectively. The functional group present in complexes investigated using FTIR showed C=O, C=C and C-O stretching's with O-H and M-O coordination's. The complexes was characterized using UV-visible where peaks of the ligands and the mixed complexes in DMSO showed the maximum absorbance at 300 nm, whose concentrations have uniform horizontal values that indicated the $n \rightarrow \pi^*$ transition of the ligand SHA. The antimicrobial activity tested on the ligand SHA ($C_7H_7NO_3$), complexes and reference drug investigated against four bacterial species; staphylococcus aureus, Escherichia coli, Klebsilla pneumonia and Salmonella typhi and two fungi species candida albicans and Aspergillus niger that involved agar disc diffusion methods showed diameter of inhibitions zone of individual sample analyte are dignified. A momentous activity of Mn (II) complexes observed on investigated micro-organisms of the mixed complexes syntheses showed effective anti-microbial agent in this research.

Keywords: Synthesis, Mixed complexes, Ligands, Salicylhydroxamic, oxalic

INTRODUCTION

Complexes of transition metal ions syntheses transpired due to coordination bonding of ligands have stability effects on some reagents. They transpired by their functional group present, and play importance role antimicrobial activity on both Bactria and fungal medium. (Adegoke *et al.*, 2019). Modern Researchers confirmed that synthesis mixed ligand complexes

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were either stable or unstable on solubility test due variations in temperature (Wankhede *et al.*, 2014).

Coupled complexes emanated from ligands have been transformed into very vital agents for antimicrobial activity compared to the derived ligands. They are binary complexes in association with transition metal mixed ligands. They also played important role in tackling enormous microbial infections especially in human body systems and organs by healing to normal health status (Sharma *et al.*, 2012).

Several coupled ligands complexes derived from transition elements showed different phases of natural activities exhibited due to biochemical reactions performed in body systems such as antimicrobial, gastro vascular formations, cancer deficient cells, anti-mycobacterial, that lead to many infectious diseases. Anti-inflammatory and vital pesticides activity were extensively concern (Moustafa and Magda, 2017).

Manganese (II) plays vital role as a biocompatible element that sustain wide application in various fields. Salicylhydroxamic acid and oxalic acid are ligands whose metal complexes of these ligands have various economic importance with related application in speeding faster chemical reactions in many organic synthesis.

This research focus on synthesis, practical emerging compounds with bioactive evaluation of mixed complexes of Mn (II) and salicylhydroxamic acid using oxalic acid. Concept of synthesis with regards to mixed ligand complexes in modern research have been modified to a great extent towards applied technology of tremendous importance to the humans. The roles of coupled ligand complexes of transitional metals in biological process have well defined yield. The interest may be due to the ease of preparation of these complexes and their wide applications in various field of human activities.

METHODS ADOPTED

Sample Collection

All the reagents and materials used were commercially available and were purchased at the recognized and licensed dealers and are used with purity, with appropriate grade utilized by methods adopted by researchers.

Blending of Salicylhydroxamic Acid (SHA)

SHA was coupled in phases whereby methyl salicylate initially started with esterification process in salicylic acid using methanol. Then salicylic acid with concentration of 0.2 mol was carefully measured and 28 cm³ portion of the acid was mixed with 81cm³ portion of methanol, then exactly 8 cm³ of H₂SO₄ was poured into coupling mixtures of hydroxylamine and methyl salicylate in two portions. Later hydroxyl amine with concentration of 0, 2 mol where 13.9 g portion of the reagent was weighed using electric balance. It was mixed up with 200 cm³ portion of sodium hydroxide solution with concentration of 10 % and the mixture was cooled and allowed for I hour that formed complexes at normal temperature. Then 152.3g portion of methyl salicylate with a concentration 0.1 mol was poured drop by drop into the mixture followed by constant shaken that achieved vibrant dissociation. The mixture was kept that settled for 36 hours. Then it was acidified with 2 M H₂SO₄ acid and cooled with evolution of precipitate. The precipitate formed was filtered, recrystallized from hot water containing a drop of acetic acid, re-cooled and filtered; white precipitate was collected and dried in a vacuum desiccator over anhydrous CaCl₂ as adopted by Leena *et al.*, (2021)

Synthesis of Mixed Ligands Complexes

Exactly 0.197g aqueous solution of Manganese (II) chloride tetra-hydrate with concentration of 1 mmol are mixed with 5 cm³ distilled water, then 1 cm³ of HCl acid with a concentration of 0.1m was added to the mixture. A ligand salicylhydroxamic acid (SHA) with concentration 1 mmol was correctly weighed using electric balance with only 0.153g was utilized and dissolved in ethanol in 10 cm³. Then oxalic acid crystals with concentration of 1mmol was carefully measured at exactly 0.090 g which was added dissolved with 5 cm³ distilled water. The solution pH modifications at 5-6 was done by pouring few drops of 0.1M NaOH. The ligands SHA solution was added into Manganese (II) chloride tetra-hydrate solution drop by drop with constant stirring followed by pouring oxalic acid solution. The mixture was continuously stirred for 30 minutes, which was then kept in a beaker for 2-3 hours at normal temperature that formed complexes. The obtained precipitate complex was filtered, washed with ethanol first then cold water and finally dried in a vacuum desiccator over anhydrous CaCl₂ as adopted (Saifun *et al.*, (2020).

Solubility Test

The solubility test of the ligands and the mixed complexes was carried out in water, ethanol, methanol, acetone, chloroform, dimethylsulfoxide and dimethylformamide in which 0.2g of each sample were tested in 10 cm³ of each solvent (Kawkab and Al-Ali, 2010).

Melting Point Test

The melting point of the ligand and the metal complexes were determined using melting point apparatus with capillary tube as adopted (Ibrahim *et al.*, 2020)

Molar Conductance Measurements

Exactly 0.001mol of each ligand was withdrawn from the bottle and the mixed complex were dissolved in 10 cm³ of dimethylsulfoxide (DMSO) and the corresponding specific conductance values were recorded, From the specific conductance value recorded, the molar conductance of each ligand and the mixed complex was calculated (Moamen *et al.*, 2011).

$$\text{Molar conductance} = \frac{\text{specific conductance}}{\text{ionic concentration}} \times 100$$

UV -Visible Analysis

UV-Visible spectra of the complexes formed was recorded at various wavelengths range between 200nm to 800nm that achieved stable peaks. The corresponding concentrations was plotted against the wavelength after determining the maximum absorbance as adopted by (Nasiru *et al.*, 2023)

Fourier Transform Infrared (FTIR) Analysis

FTIR analysis from the determined functional group presents on both the synthesized ligand and the mixed complexes, the methods employed was adopted (Nasiru *et al.*, 2023).

X-Ray Diffraction (XRD) Analysis

XRD analysis was done in order to find out the average crystalline size. Debye Scherer equation was calculated where the average crystalline size and plane of Symmetry angle of the synthesized mixed complexes was investigated. The equation of illustrated values was adopted (Nasiru *et al.*, 2023).

$$D = \frac{K\lambda}{\beta \cos \theta}$$

Whereby D represents Particles size = 0.94 while K equals to Constant volume, Λ = X-ray wavelength (0.154nm), then P equals to Line broadening at half the maximum intensity finally Θ equals to Bragg's angle (in degree).

Antimicrobial Activity Test

The complexes coupled with derived ligands were subjected for antimicrobial activity by the disc diffusion process. Portions of the mixed complexes coupled with ligands are mixed together plus pure erythromycin in association with Fungusol were poured into DMSO. The culture media employed for the anti-microbial investigation were nutrient agar, for bacteria, and Saboraud's dextrose agar for fungi were tested for antibacterial activity as adopted (Nnamani *et al.*, 2020).

Antibacterial Activity Test

Antibacterial activity of the ligand and its metal complexes were tested on these bacterial species of *Staphylococcus aureus*, *Escherichia coli*, *Klebsilla pneumonia* and *Salmonella typhi*. Exactly 2mg/ml of the ligands and metal complexes extract were poured over in DMSO were subjected to culture for bacterial activity. The disc was impregnated with the complexes and ligands which was incorporated close to the inoculum prior to incubation period at 37°C at 24 Hours awaiting period. The susceptibility test was determined by measuring the zone of inhibition (ZOI) and compared with a standard drug as adopted (Nnamani *et al.*, 2020).

Antifungal Activity Test

The antifungal activity of the ligands and the mixed complexes were tested against *Aspergillus niger* and *Candida albicans* species at 2mg/ml. The suspension of each microorganism was poured on the surface of solidified dextrose agar already poured into petri dishes. The impregnated disc was placed on the surface of the agar plate at 37°C for 48hrs. The activities were determined comparing from the diameter of zone of inhibition and compared with the standard drug as adopted (Nnamani *et al.*, 2020).

RESULTS AND DISCUSSION

Solubility Test of Ligands and Complexes

The Solubility test of manganese (II) complexes was illustrated on Table 1. The complexes of SHA dissolved completely in these solvents; methanol, DMSO with ethanol due to their polarity properties. However the complexes are insoluble in water, and chloroform. Prepared complexes tested upon solubility indicated soluble in DMSO and methanol while insoluble in ethanol, acetone and water which is attributed to polymeric properties of the complexes. The results obtained on solubility was similar to the findings of Hauwa *et al.*, (2024).

Table 1 Solubility property of derived Ligand and the complexes

Samples	methanol	Water	Ethanol	Acetone	DMSO	Chloroform
SHA	S	IS	S	IS	S	IS
Oxalic acid	S	S	SS	IS	S	IS
Mixed complex	S	IS	S	IS	S	IS

KEY: S - Soluble, IS - Insoluble, SS - Slightly soluble.

Melting Point Test of Complexes and Ligands

Melting point ability of the derived SHA and Mixed Complexes are presented on Table 2. The complexes of SHA and Mixed ligands obtained are crystalline in nature, they showed stability to air and light changes at normal temperature, whereby melting points of the ligand SHA = 180°C while that of Mixed complexes showed 300°C. The sharp melting point observed indicated the absence of impurity (or minimal) in the prepared ligand and the high melting in

the mixed complexes may signify the polymeric nature of the complexes and confirmed the formation of the complexes as reported by Adegoke *et al.*, (2019) and Leena *et al.*, (2021).

Table 2 Melting Point of complexes and ligand

Samples	Colour	Melting point (°C)
SHA	White	180
Mixed complexes	White	300

Molar Conductivity Test

The molar conductivity of the mixed complexes and the ligand are shown on Table 3. Mixed complexes revealed molar conductivity of $23.6 \text{ Ohm}^{-1} \text{ cm}^2$ while SHA ligand showed only $14.3 \text{ Ohm}^{-1} \text{ cm}^2$. Other ligands of oxalic acid do not show any influence on molar conductivity. These values were considered low and indicated virtually non-electrolytes nature of the complexes (Hauwa *et al.*, 2024).

Table 3 Molar Conductance Values of Complexes in DMSO Complex

Complex	Concentration Mol dm^{-3}	Specific Conductance $\text{Ohm}^{-1} \text{ cm}^{-1}$	Molar Conductance $\text{Ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$
Oxalic acid	1.0×10^{-3}	$\times 10^{-6}$	-
SHA	1.0×10^{-3}	14.3×10^{-6}	14.3
Mixed complexes	1.0×10^{-3}	23.6×10^{-6}	23.6

UV-visible Analysis of Ligand (SHA)

The UV-visible analysis shown by synthesized ligand SHA indicated a highest peak intensity band at 300 nm, while the subsequent concentrations have uniform horizontal values on the plotted graph. This indicates that the $n \rightarrow \pi^*$ transition of the ligand SHA was projected. The results obtained was similar to (Hauwa *et al.*, 2024) but the result maximum resul of peak intensity band of 300 nm was contrary to 400 nm maximum band of 400nm but showed the same horizontal values from findings of Usmsn *et al.*, (2023).

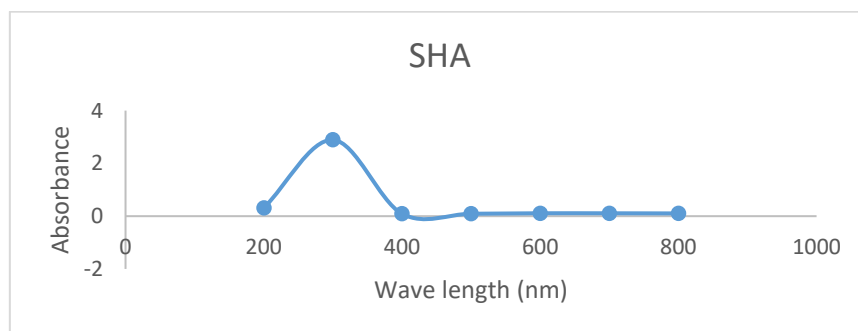


Figure 1 UV-Visible Analysis Results for SHA

UV-visible Analysis Mixed Complexes

The UV-visible analysis of the prepared mixed complexes was shown on Figure 2. The maximum absorbance value appears at 300nm while the subsequent concentrations have uniform horizontal values. This indicates stable complex formation and a ligand to metal charge transfer (LMCT). The Manganese II metal exhibited hybridization of d^5 and a lower level electronic layer of $6s$ at this level no specific crystal shape occurred. The result obtained showed the same graphical horizontal values but different maximum intensities values of 400 nm as reported by Usman *et al.*, (2023). The field intensities are much weaker than for spin-allowed $d-d$ spectra with no variation of crystal shape (Hauwa *et al.*, 2024).

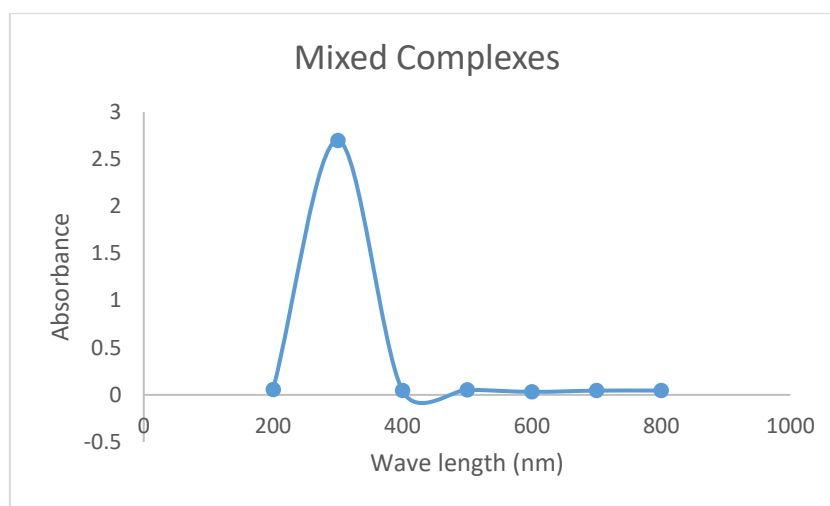


Figure 2 UV-visible Analysis Results for Mixed Complexes

FTIR Analysis of Ligand (SHA)

The FTIR absorption band for the synthesized SHA and is presented on Figure 3. The band observed for the synthesized SHA showed significant band of O-H = 3377 cm^{-1} and N-H = 3227 cm^{-1} , while a band stretching at around 1654 cm^{-1} which designated as (C=C) stretching frequencies. Another peak protruded by 1606 cm^{-1} indicated (C=O) stretching of aromatic ring. The results obtained was in line with a research reported by Sujata, (2020).

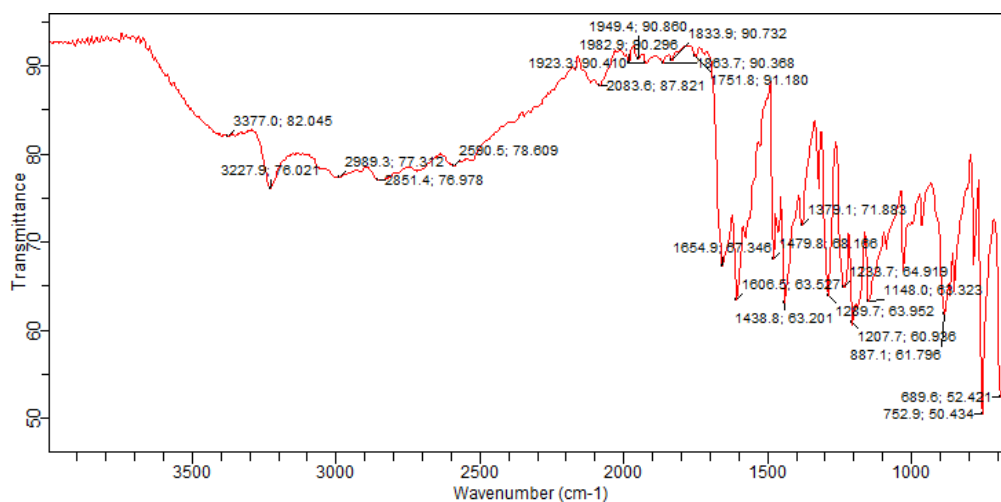


Figure 3 FTIR spectra for the Salicylhydroxamic acid (SHA)

FTIR Analysis of Mixed Complexes

The FTIR absorption band observed for the mixed complexes is presented on Figure 4. Mixed complexes showed wavelength peaks of O-H = 3369 cm^{-1} and N-H = 3239 cm^{-1} stretching frequencies. The bands are broadened. Broadening may be caused by the participation of oxygen from OH group in complex ion, and appearance of O-H of coordinated water molecules. The bands at 1654 cm^{-1} which is unchanged with the band in ligand SHA and is designated for aromatic (C=C) stretching, and at 1610 cm^{-1} stretching band appeared which shifted slightly to a lower significant value revealed a complex ion. This showed relative bonding of attached ligands designated as (C=O) stretching frequencies for the mixed Complexes. A new band at 1244 cm^{-1} designated as (C-O) Stretching. A stretching frequency band appears at 812 cm^{-1} where assigned to (M- O) showing a metal- oxygen coordination and

bands at 784 cm^{-1} assigned M- N coordination, the results of this study was nearly the same as research conducted by Leena *et al.*, (2021).

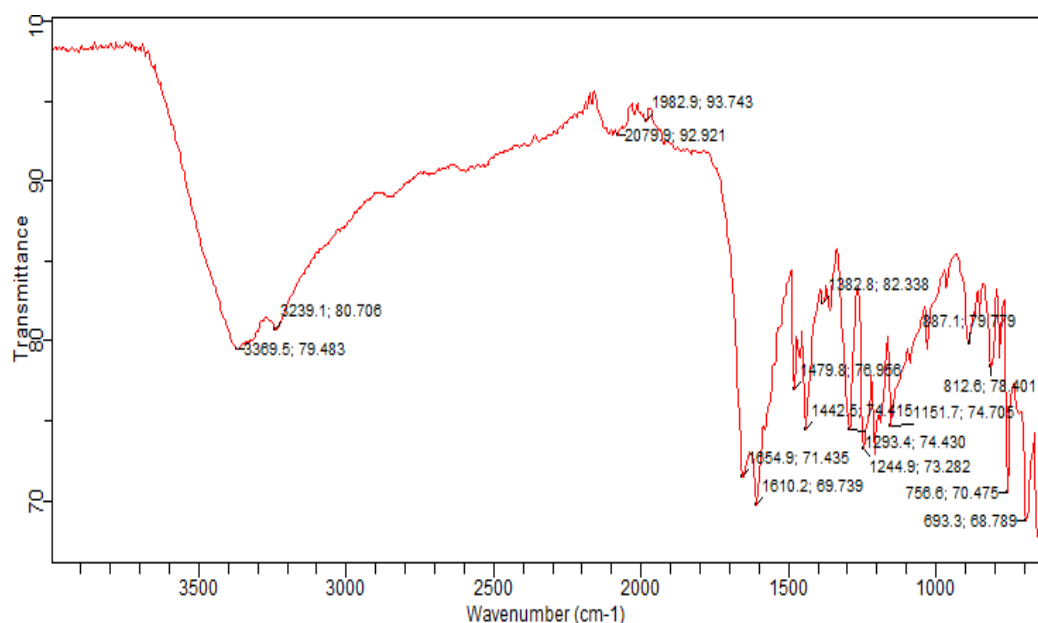


Figure 4 FTIR Spectra for the Mixed Complexes

Table 4 FTIR Wave Length of Mixed Ligand complexes and SHA

Samples	(O-H)	(C=C)	(M-N)	(C=O)	(M-O)
SHA	3577 3227	1654	-	1606	-
Mixed complexes	3369, 3239	1654	784	1610	812

X-Ray Diffraction (XRD) of Mixed Complexes

As illustrated XRD analysis on Figure 5, showed result obtained for the synthesized mixed complexes according to the Bragg's angle range between 11.00° - 36.94° showed lower peaks. The result confirmed six protruded peaks angles were observed at $2\theta = 11.00^\circ$, 13.41° , 17.28° , 33.51° and 36.94° with respect to the plane of symmetry (101), (111), (201), (211), (311) and (320). These values indicated hexagonal crystalline structure with crystalline units of 87.98 nm as calculated by to the Scherer equation. The diffraction peaks are indexed based on JCPDS file number 36-1451, showed closely related values with agreement to the literature reported by Nasiru *et al.*, (2023) and that of (Athifeona *et al.*, (2020).

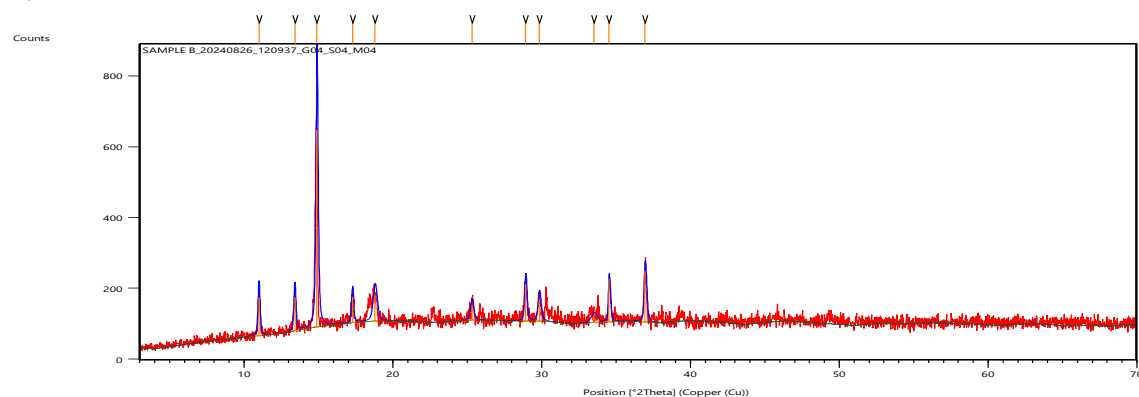


Figure 5 X-Ray Diffraction of Synthesized Mixed Complexes

Antibacterial Activity of SHA, Oxalic Acid and Mixed Complexes

Table 5 showed antimicrobial activities of Mn (II) complexes synthesis. As reported in the table, SHA showed moderate activity towards *Staphylococcus aureus*, *Escherichia coli*, and *Klebsilla pneumonia*. Oxalic acid showed weak inhibition towards all the tested bacteria's. Mixed complexes showed a good activity towards all the tested bacteria's. The results of this study are in line with previous studies carried out by Fazary (2014). The activity of bacteria proceeded to H-bonding by the surface Nitrogen and Oxygen atoms at SHA and the mixed complexes due to the presence of active centers of the cell constituents. The result confirmed higher activity of metal ion in the solution Kamini *et al.*, (2012). It has been observed that mixed complexes exhibited higher activity compared to mobile ligands responses of microorganisms with similar surface conditions Sheela, (2015). Result of activity of the syntheses ligand and complexes are negligible compared to erythromycin.

Table 5 Antibacterial Activity Test

Samples	<i>Stappyllococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsilla pneumonia</i>	<i>Salmonella typhi</i>
	Zone of inhibition			
SHAM	14	14	13	09
Oxalic acid	9	9	5	5
Mixed complexes	25	22	18	19
Standard	26	19	26	24

Weak activity (below 10 mm), Moderate activity (10– 15mm), Good activity (16 – 20mm); significant activity (21 – 26 mm)

Antifungal Activity of Ligands and Mixed Complexes

The result obtained on antifungal studies was represented on Table 6 whereby two fungal species used showed that both the ligands has a moderate activity against the tested fungi species. Mixed complexes showed a good activity against the tested fungi. The results was synonymous with a research reported by Nnamani *et al.*, (2020). However, mixed complexes shows higher zone of inhibition values than the ligands. Compared to the standard, Fungosol has slightly higher inhibition value than the free ligand and the mixed ligands. The results of the analysis was presented in a table below

Table 6 Antifungal Activity Test

Complexes	<i>Candida albican</i>	<i>Aspergillus niger</i>
	Zone of inhibition	
SHA	14	18
Oxalic acid	10	15
Mixed complexes	18	25
Standard	20	28

Weak activity (below 9), Moderate activity (10-19), Good activity (20-29)

CONCLUSION

The primary ligand in this study (salicylhydroxamic acid) was prepared by reacting salicylic acid with methanol forming methyl salicylate and reacting with hydroxylamine hydrochloride. The product became a source of mixed complexes formations due to reaction with manganese (II) chloride tetrahydrate with Ethanoic acid. A complexes occurred due to reaction of oxalic acid that served as secondary ligand. The mixed complexes that emerged undergo solubility, melting point and molar conductance test together with FTIR, UV-Vis,

Antimicrobial, Antifungal activities and XRD analysis. The result obtained showed complexes and ligands are non-electrolytes with lower molar conductance in DMSO. The complexes confirmed soluble in methanol, ethanol, acetone and DMSO but insoluble in water and chloroform, melting point observed on mixed complexes = 300 °C while ligand SHA= 145 °C respectively.

UV/Visible result shows maximum absorbance at 300 nm for both the synthesized ligand and the mixed complexes, FTIR result showed the presence of O-H, C=H, C=C, C-O, M-O and M-N functional groups in the synthesized ligand and mixed complexes, the ligand bonded to the metal ions also carbon, oxygen and hydrogen atoms. The crystal structure showed tetrahedral and octahedral geometry on synthesized complexes which have bioactive ability in treating some ailment bacterial and fungal diseases were tested on some selected Bacteria's and Fungi with positive activities capable of alleviating menace of pandemic diseases.

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