International Journal of Research in Pharmacy and Allied Science (IJRPAS)
Published by Ideal Publication
Available at https://idealpublication.in/ijrpas/

Synthesis, Characterization and α-Amylase Inhibition Study of Substituted Schiff Base and Its Metal Complexes

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Article History
Received: 30/11/2023
Accepted: 17/12/2023
Published: 31/12/2023

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Abstract: Mechanochemical synthesis is considered as a green and sustainable approach to chemical reactions since it requires little or no solvent, making the process more environmentally friendly by reducing waste and minimizing solvent related hazards. In this study, substituted Schiff base was synthesized from 2-aminophenol and 2-hydroxy-1-naphthaldeyde via mechanochemical synthesis method. The metal complexes were synthesized by reacting the ligand with metal(II) chlorides in ratio 2:1. The synthesized compounds were characterized by colour, melting point, molar conductivity, FT-IR, and UV-Vis spectroscopy. The ligand and its metal complexes were screened for α-amylase inhibitory activity. FT-IR results showed absorption bands in between the ranges 1628-1632 cm-1 and 1585-1597 cm-1 indicating the formation of azomethine C=N and aromatic C=C respectively. The metal complexes showed M-O absorption bands in between the range 519-579 cm-1 and M-N band in between the range 461-484 cm-1. The synthesized compounds displayed significant inhibitory activities (IC50: 2.00-8.43 mg/mL) against α-amylase comparable to the reference compound, acarbose (IC50: 9.12 mg/mL). These compounds can serve as viable templates in the formulation of new active antidiabetic drugs..

Keywords: α-Amylase. Antidiabetic drugs. Mechanochemical Synthesis. Metal Complexes. Schiff Base

INTRODUCTION

A Schiff base is a nitrogen analogue of an aldehyde or ketone in which the C=O group is replaced by C=N-R group. A Schiff base, named after Hugo Schiff is a class of organic compounds with a functional group namely imine or azomethine (-C=N–) [1]. Schiff base ligands are viewed as special ligands since they are easily synthesized by condensation reaction between aldehyde or ketone derivatives with primary

amines, containing a carbon-nitrogen double bonds (>C=N) with the nitrogen atom connected to an aryl or alkyl group but not hydrogen [2]. The Schiff bases which are synthesized from aromatic aldehydes are relatively more stable than those from aliphatic aldehydes that are unstable and readily polymerizable [3]. Schiff bases are considered as a very important class of ligands that are capable of bonding to almost all metal ions, coordinating to metal ions via azomethine nitrogen [4].

Several methods have been utilized for the synthesis of Schiff Base including conventional synthesis [5], microwave irradiation [6], ultrasound irradiation [7], and mechanochemistry [8]. Although the formation of a Schiff base is reversible, due to the hydrolysis of the imine under certain conditions, it is still straight-forward for the reaction to succeed. It is still unknown why some types of Schiff bases are stable in the presence of water even in acidic solution, while others are very sensitive to water and easily hydrolyze back to aldehyde (or ketone) and amine [3].

To overcome this potential hydrolysis, the reaction of Schiff bases should be done under dry solvent conditions or using some additional procedure to remove the side product (in most cases, water). The lone pair on the nitrogen atom of the imine can supply electrons, which enable the formation of a proper donor bond to a metal ion for complexation to occur. Many Schiff bases have a second functional group, normally OH and SH groups or N atom, which are near the imine group. These functional groups can allow the formation of five or six membered chelate rings when coordinated with different metal ions [9].

The aim of this research is to synthesize, characterize and evaluate the α-amylase inhibitory potentials of a Schiff base and its metal complexes.

MATERIALS AND METHODS

MATERIALS

All reagents and chemicals are of analytical grade and were used as received without further purification. 2-aminophenol, 2-hydroxy-1-naphthaldehyde, metal salts; copper (II) chloride, manganese (II) chloride, and iron (II) chloride were obtained from Sigma Aldrich, Germany. All solvent; ethanol, methanol, acetone, n-hexane, dimethylsulfoxide (DMSO), and ethyl acetate were also obtained from Sigma Aldrich, Germany. The samples were analyzed using FT-IR Spectrophotometer (FTIR-8400S), Shimadzu, Japan. Stuart SMP10 Digital Melt Point was used for the melting point determination. The maximum absorption was measured using UV-Vis spectrophotometer (UV-1650PC), Shimadzu, Japan. The electrical conductivity was determined using Jenway 4010 conductivity meter. AR2130 Analytical balance purchased from Ohaus, USA was used for mass measurement. Thin Layer Chromatography (TLC) plate and UV lamp of 254 nm was used for chromatographic techniques.

METHODS

Synthesis of Schiff base Ligand

The method reported by Sani and Siraj [10] was adopted and modified for the synthesis of the Schiff base. 2-hydroxy-1-naphthaldehyde, (0.3444 g; 1 mmol) and 2-aminophenol (0.1091 g; 1 mmol) were weighed into a mortar and ground with pestle for 45 min. A small amount of n-hexane (1 mL) was added

to allow the formation of a yellow powder crystal. The reaction was monitored by thin Layer Chromatography (TLC). The product obtained was air dried and kept in a desiccator for further analysis.

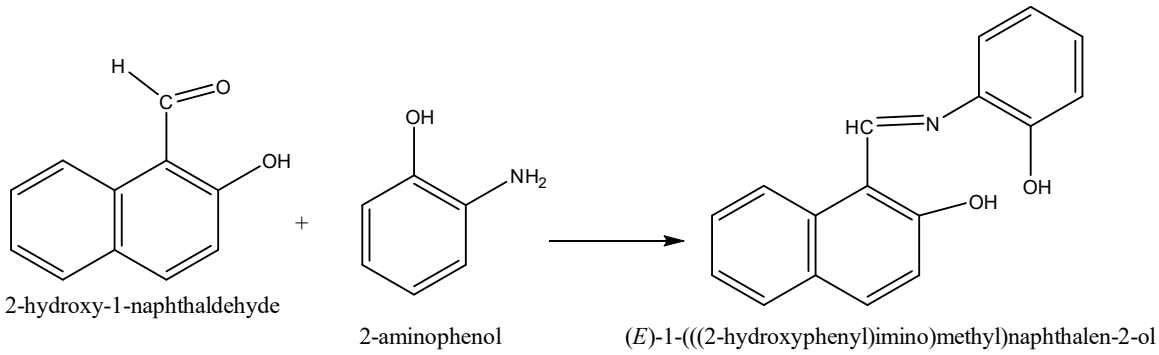


Figure 1. Synthesis of the Schiff base

Synthesis of the metal complexes of the Schiff base

The method reported by Sani and Siraj [10] was adopted and modified for the synthesis of the Schiff base metal complexes. The Schiff base [(*E*)-1-(((2-hydroxyphenyl) imino) methyl) naphthalen-2-ol] (0.5263 g; 2 mmol) was reacted with anhydrous $NiCl_2$ (0.1296 g; 1 mmol), $FeCl_2$ (0.1622 g, 1 mmol) and $MnCl_2$ (0.1258 g; 1 mmol). The respective reactants (Schiff base and metal salt) were weighed into a mortar and ground with pestle for 45 min. A small amount of methanol (1 mL) was added to allow the formation of a powder crystal. The reaction was monitored by thin layer chromatography (TLC). The products obtained were air-dried and then kept in a desiccator for further analysis.

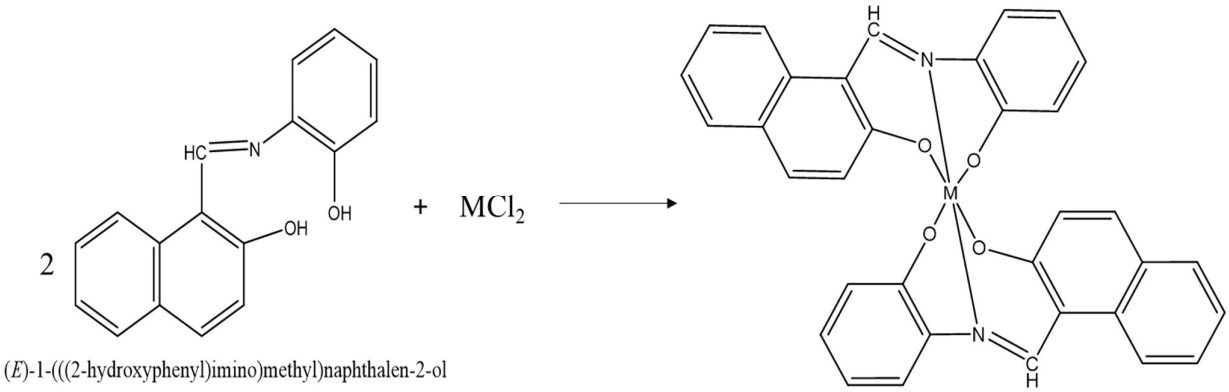


Figure 2. Synthesis of the Schiff base metal complexes (M=Fe, Mn and Ni)

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was carried out using FTIR-8400S Shimadzu equipment. KBr (Potassium bromide, spectroscopy grade) was ground into powdery form, pelletized with hydraulic press and scanned with instrument as background. Then small amount of Schiff base and metal(II) complexes were mixed with

KBr and were pelletized using hydraulic press, inserted into the instrument and scanned in transmittance mode at a frequency range of 4000–400 cm^{-1}.

Solubility Test

Water and some common organic solvents; acetone, ethanol, methanol, dimethylsufoxide (DMSO) and n-hexane were used to determine the solubility of the Schiff base and its metal complexes.

UV-Visible Spectroscopy

The UV–Vis spectroscopic measurements were recorded using a UV–Vis spectrophotometer to obtain the absorbance profile. The concentration ($1x10^{-4}$ M) of each synthesized compound was prepared. The UV–Vis spectrum of each synthesized compound was recorded in the range of 200-800 nm using a 1 cm quartz cuvette with ethanol as solvent [11].

Electrolytic conductivity

The method reported by Uba et al. [12] was adopted and modified for the electrolytic conductivity measurements. Electrolytic conductivity of the metal complexes were done in DMSO ($1x10^{-3}$ M). The electrolytic conductivity meter was calibrated by distilled water, then the molar conductance of Fe(II), Mn(II) and Ni(II) complexes were measured at room temperature.

Melting Point

Melting point was determined using a Stuart model SMP10 digital melting point apparatus. Small amount of Schiff base and the metal complexes were inserted into a capillary tube in which one end was sealed. The capillary tube containing the sample was inserted into the melting point apparatus till it melts.

Enzyme inhibition study of the Schiff base and its metal complexes

The method reported by Balan et al. [13] was adopted and modified for the α-amylase inhibitory activity study of the Schiff base and its metal complexes. A total of 50 µL of the sample (20–500 µg/mL) was placed in wells of a microplate and 50 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing -amylase solution (0.5 mg/mL) was added. This solution was preincubated at 25 °C for 10 min, after which 50 µL of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added and then further incubated at 25 °C for 10 min. The reaction was terminated by adding 100 µL of dinitrosalicylic acid (DNS) reagent. The microplate was then incubated in boiling water for 5 min and cooled to room temperature. The absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the sample with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition as follows;

$$\text{Inhibition (\%)} = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \times 100$$

Each experiment was carried out in triplicate with adequate blanks in between. The IC_{50} values were hence calculated.

RESULTS AND DISCUSSION

The physical properties of the synthesized Schiff Base and its metal complexes were presented in Table 1. The percentage yield of the Schiff base was 50 % while that of the metal complexes were 91 %, 76 % and 82 %. The interaction between 2-hydroxy-1-naphthaldehyde and 2-amino phenol gives the yellow-coloured Schiff base [12]. The Fe(II), Mn(II) and Ni(II) complexes were dark brown, green and yellow in colour respectively. The purity and stability of the Schiff base and metal complexes were established by the observance of sharp melting point. The melting point of the Schiff base was 192 ^{0}C while the Fe(II), Mn(II) and Ni(II) complexes exhibited 234 ^{o}C, 236 ^{o}C and 239 ^{o}C melting points respectively, indicating thermal stability. Electrical conductivity of the metal complexes were presented in Table 1. The molar conductance of the Fe(II) complex was 0.2 Ω^{-1} cm^2mol^{-1} while Mn(II) and Ni(II) had zero value of conductance.

Table 1. Physical Properties of the Schiff Base and its Metal Complexes

S/N	Ligand and metal complexes	Molecular formula	Colour	Yield (%)	Melting point (^{o}C)	Molar conductance ($Ohm^{-1}cm^2$ mol^{-1})
1	2A2HS	$C_{17}H_{13}NO_2$	Yellow	50	192	-
2	2A2HSFe	$C_{17}H_{13}NO_2Fe$	Dark brown	91	234	0.2
3	2A2HSMn	$C_{17}H_{13}NO_2Mn$	Green	76	236	0.0
4	2A2HSNi	$C_{17}H_{13}NO_2Ni$	Yellow	82	239	0.0

Solubility Test

The solubility of a compound in various solvents depends on the nature of the compound, type of bonding and solvent [12]. The solubility of the synthesized compounds are presented in Table 2. The solubility test was carried out in methanol, ethanol, dimethylsulphoxide (DMSO), water, n-hexane and acetone. The Schiff base and metal complexes were found to be soluble in DMSO, methanol and ethanol. This is because polar solvents dissolve polar compounds due to similar attractive force between them. The Schiff base is insoluble in water and n-hexane which is similar to the report of Uba et al. [12].

Table 2. Solubility Test of the Schiff Base and its Metal Complexes

S/N	Ligand and metal complexes	Methanol	Ethanol	Acetone	DMSO	Distilled H_2O	n-Hexane
1	2A2HS	Soluble	Soluble	Sparingly soluble	Soluble	Insoluble	Insoluble
2	2A2HSFe	Sparingly soluble	Sparingly soluble	Soluble	Soluble	Insoluble	Insoluble
3	2A2HSMn	Soluble	Sparingly soluble	Soluble	Soluble	Insoluble	Insoluble
4	2A2HSNi	Sparingly Soluble	Sparingly soluble	Soluble	Soluble	Insoluble	Insoluble

The results obtained is similar to that of Sani and Siraj [10]. However, the compounds are slightly soluble in acetone because many non-polar solvents can dissolve compounds containing oxygen atoms which is used in forming a bond with polar hydrogen of the carbon-hydrogen in the solvent as reported by Uba et al. [12].

FT-IR

The FT-IR spectra of the Schiff base and the metal complexes are presented in Figures 3-6 respectively. The interpretation and major bands of the FT-IR spectra of the Schiff base ligand and its metal complexes were presented in Table 3. The IR showed an absorption band at 3375 cm^{-1} which could be attributed to *ν*(O-H) vibration frequency of the Schiff base. This is in line with the results obtained by Sani and Siraj (3385 cm^{-1}) [10] and Uba et al. (3365 cm^{-1}) [12]. In the spectra of the metal complexes, these bands were obtained at a lower wavenumber except Fe(II) complex that had 3395 cm^{-1}.

Table 3. FT-IR Analysis of the Schiff Base and its Metal Complexes

S/N	Ligand and metal complexes	v(OH)	v(C=N)	v(C-H) Aromatic	v(C=C) Aromatic	v(C-O)	v(M-O)	v(M-N)
1	2A2HS	3375	1632	3050	1585	1271	-	-
2	2A2HSFe	3395	1628	2955	1597	1298	579	461
3	2A2HSMn	3121	1631	3028	1585	1272	519	483
4	2A2HSNi	3120	1632	3027	1585	1272	549	484

The Schiff base ligand shows absorption band at 1632 cm^{-1} which is attributed to v(C=N). In the spectra of the metal complexes, the band was observed at lower wavenumber except the nickel complex which had 1632 cm^{-1} as the Schiff base. These observations correspond to those reported by Neelofar et al. (1626 cm^{-1}) [15] and Sani and Siraj (1634 cm^{-1}) [10]. The presence of the hydroxy group in the ligand was further substantiated with the appearance of the phenolic C–O stretch band at 1271 cm^{-1} which is similar to that of Bhaskar et al. (1284 cm^{-1}) [9]. The absorption band at 3050 cm^{-1} in the IR spectrum of the ligand was assigned to C-H aromatic. The absorption band which appeared at 1585 cm^{-1} in the IR spectrum of the ligand was attributed to C=C stretching vibration. This is in line with the observation of Al-Adilee and Hassan (1564 cm^{-1}) [18]. The FT-IR spectra of the metal complexes showed M-O band in between the range 519-579 cm^{-1} and M-N band in between the range 461-484 cm^{-1}. There is absence of M-N and M-O band in the spectrum of the Schiff base which further confirmed the synthesis of the metal complexes. The results obtained for the complexes were related to the result of Alhakimi et al. [19], who reported the presence of M-O absorption band between 506-567 cm^{-1} and M-N band between 489-507 cm^{-1} for Ni(II), Fe(II) and Mn(II) complexes.

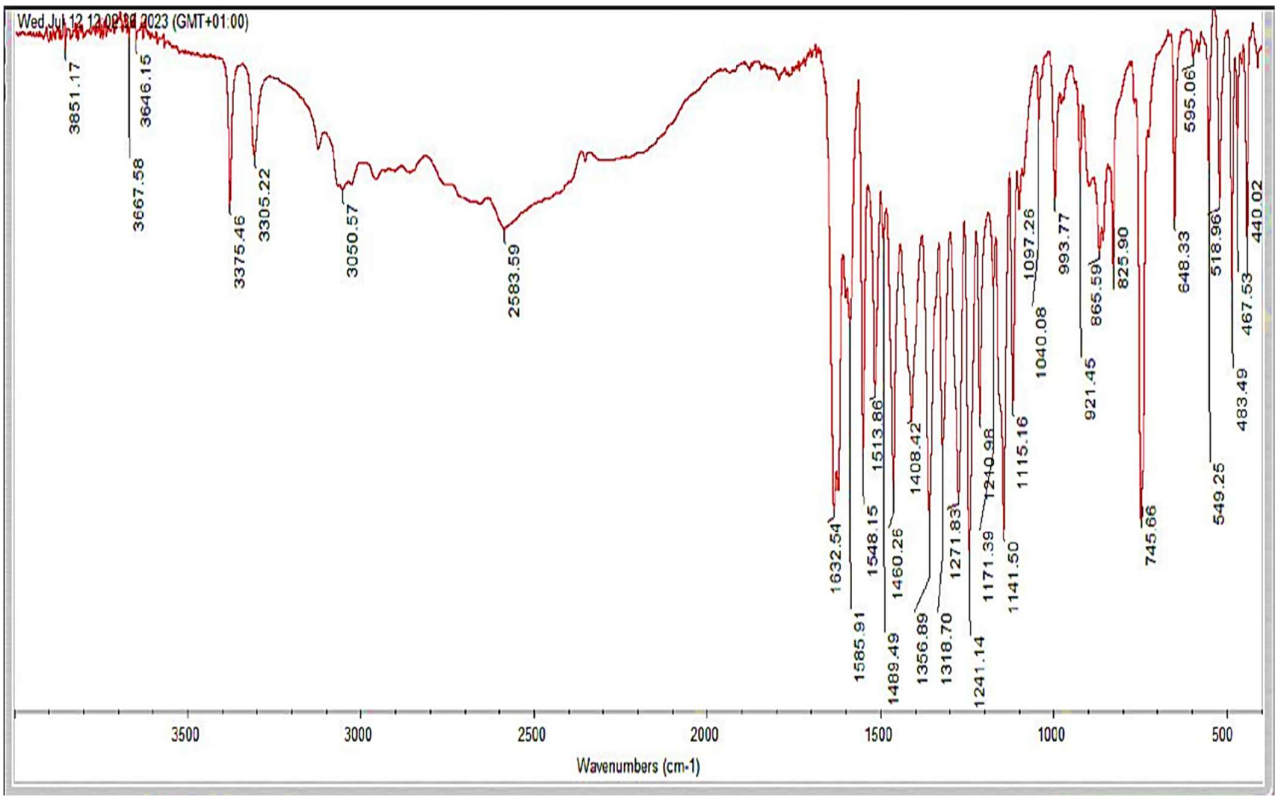


Figure 3. FT-IR spectrum of the synthesized Schiff base (**2A2HS**)

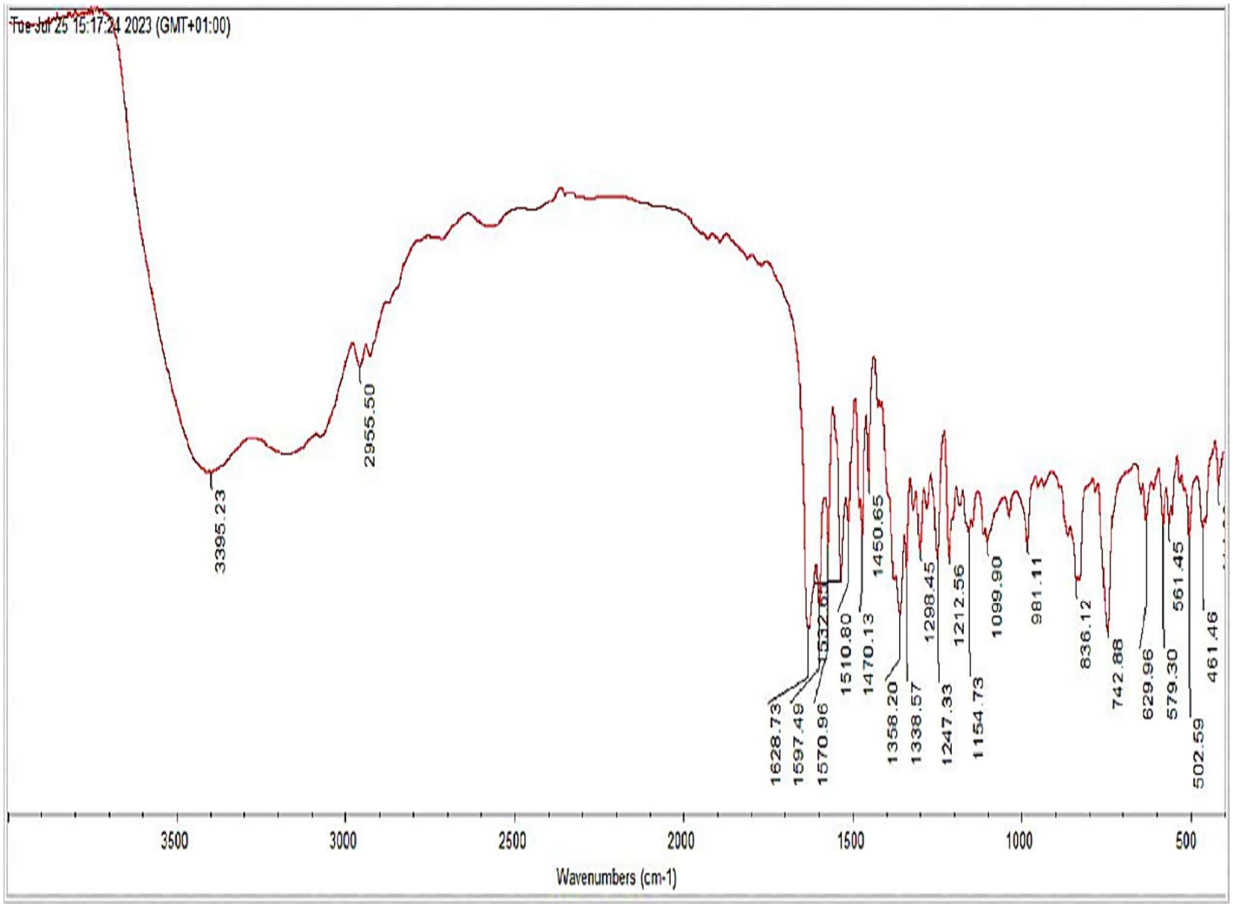


Figure 4. FT-IR spectrum of the Fe(II) complex (**2A2HSFe**)

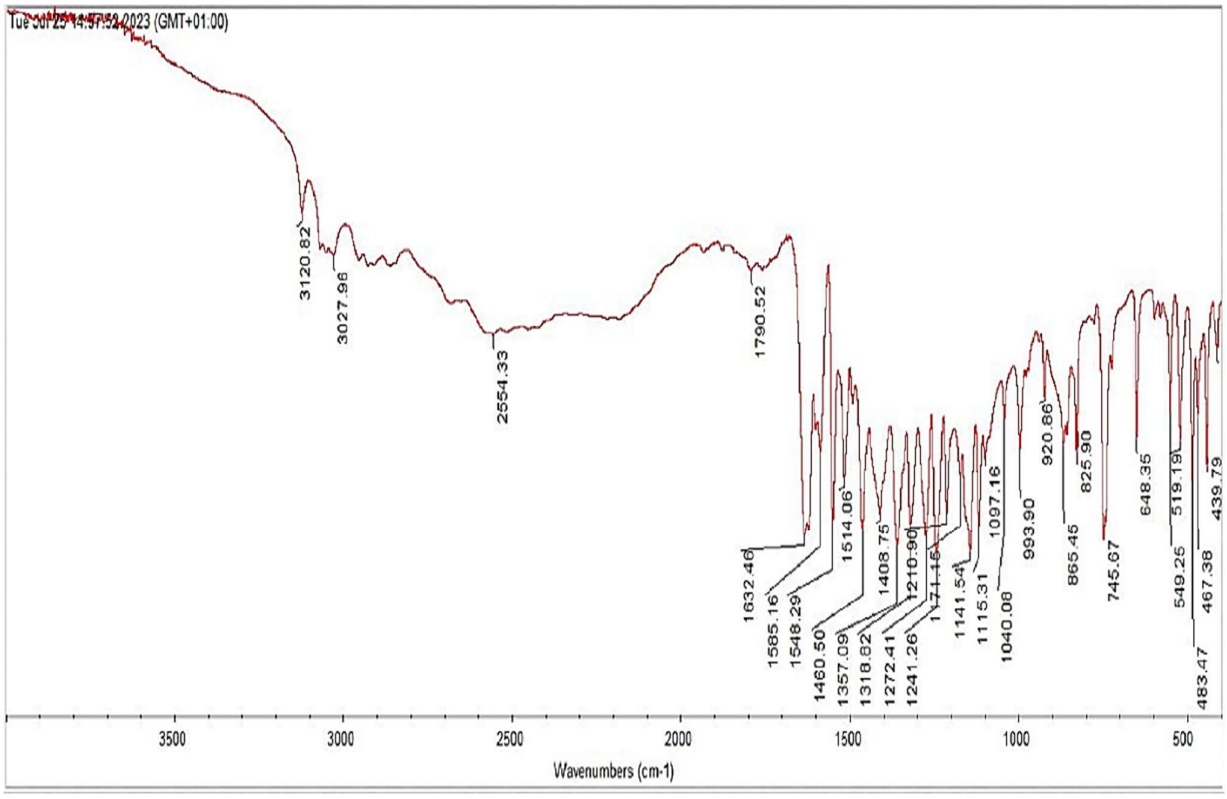


Figure 5. FT-IR spectrum of the Mn(II) complex (**2A2HSMn**)

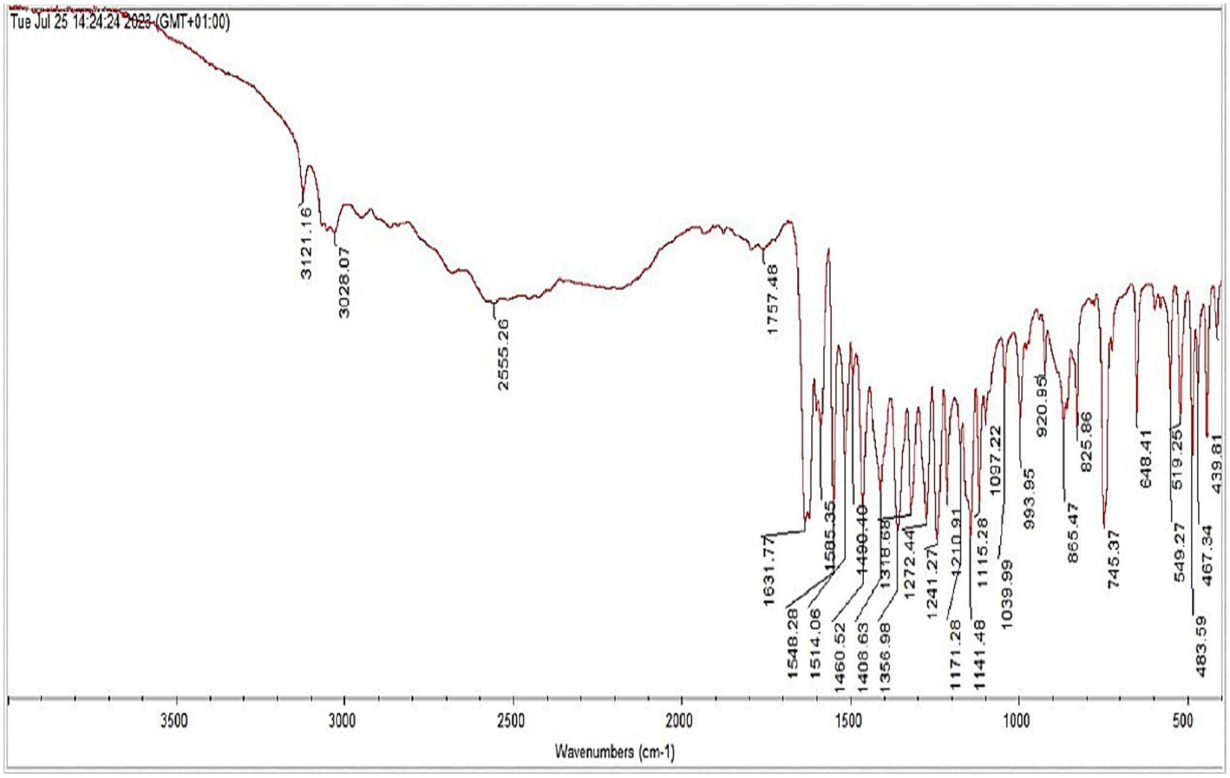


Figure 6. FT-IR spectrum of the Ni(II) complex (**2A2HSNi**)

Ultraviolet-Visible Spectroscopy Analysis

Table 4 showed the maximum wavelength of the Schiff base and its complexes in the UV-Vis spectra in Figures 7-10. The UV-Vis spectrum of the Schiff base ligand (Figure 8) exhibited strong absorption at 320 nm and 380 nm. The first band (320 nm) was assigned to π→π* transition relating to the conjugated C=C. The second absorption band (380 nm) was assigned to n→π* transition relating to the azomethine group (C=N). In the UV-Vis spectra of the metal complexes, three intense broad bands in the region 439-447 nm was assigned d–d transitions.

Table 4. UV-Vis absorption of the Schiff base and its metal complexes

S/N	Ligand and metal complexes	UV-Vis (λ max, nm) Solvent: Ethanol
1	2A2HS	320, 380
2	2A2HSFe	325, 355, 439
3	2A2HSMn	325, 355, 442
4	2A2HSNi	325, 352, 447

Enzyme Inhibition Studies

The inhibitory activities of the synthesized Schiff Base and its metal complexes against α-amylase enzyme was studied and presented in Table 5. IC_{50} values of the α-amylase inhibitory was determined.

Table 5. Inhibitory activities of the Schiff Base and its Metal Complexes against α-Amylase

S/N	Ligand and metal complexes	IC_{50} (mg/mL)
1	2A2HS	2.00 ± 1.69
2	2A2HSFe	2.45 ± 0.55
3	2A2HSNi	8.43 ± 0.98
4	2A2HSMn	5.17 ± 1.14
5	Acarbose	9.12 ±1.14

*Values are expressed in mean ± standard deviation value.

The IC_{50} value is defined as inhibitor concentration that inhibit 50 % of enzyme activity under assay conditions. The result provided in this study (Table 5) showed that the IC_{50} inhibitory activities is highest for the reference compound (acarbose) with the value 9.12 ±1.14 mg/mL. The order of the α-amylase inhibitory is Acarbose > 2A2HSNi > 2A2HSMn > 2A2HSFe > 2A2HS. This indicates that 2A2HSNi (8.43±0.98 mg/mL) exhibited significant α-amylase inhibitory activity comparable to the reference compound, acarbose with IC_{50} of 9.12±1.14 mg/mL. The value obtained in this study is related to the result of Deepika and Santhy [20].

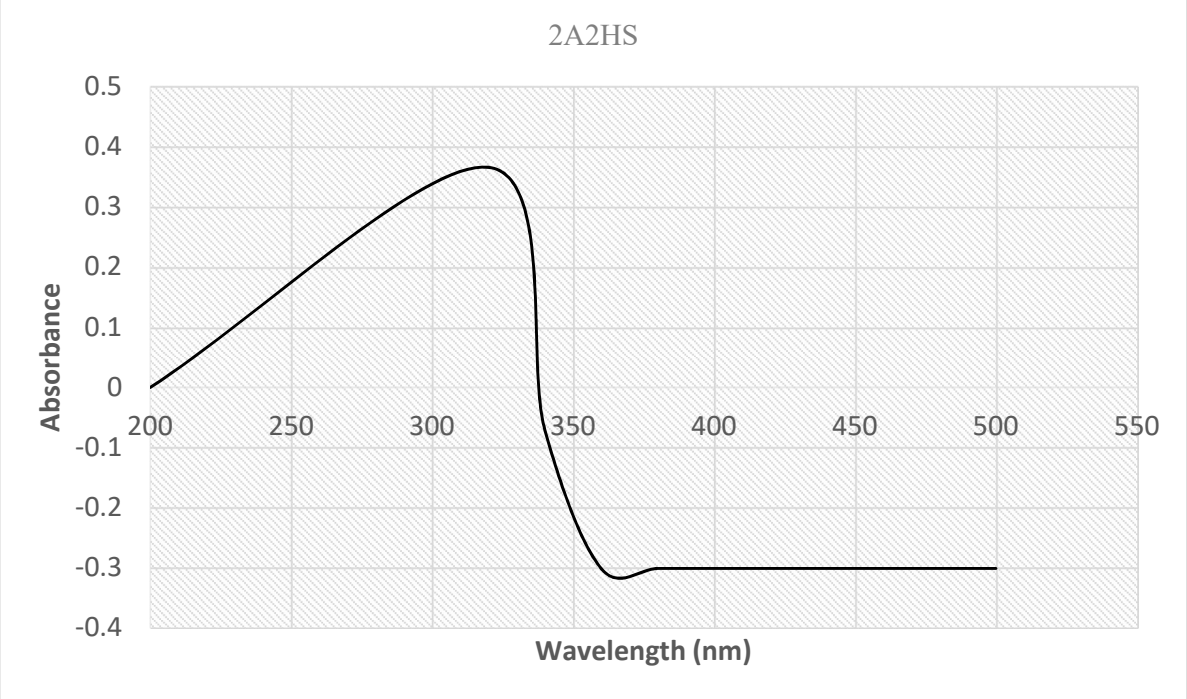


Figure 7. UV-Vis absorption spectrum of the Schiff Base Ligand

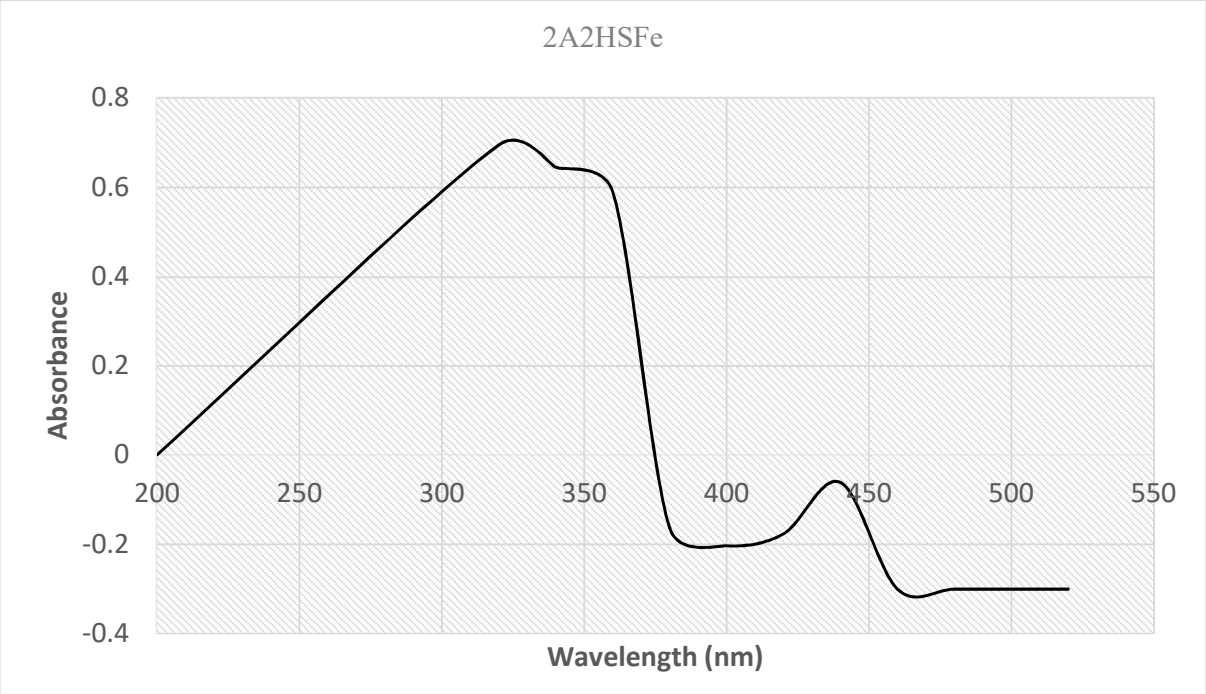


Figure 8. UV-Vis absorption spectrum of the Fe(II) complex

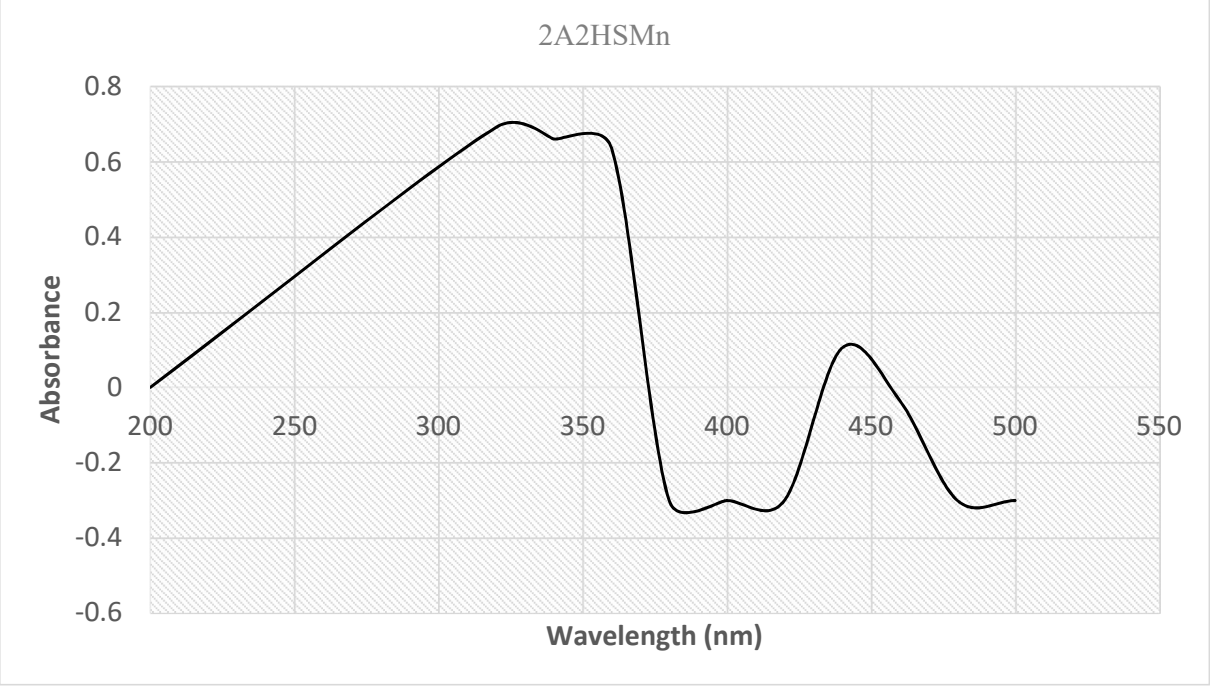


Figure 9. UV-Vis absorption spectrum of the Mn(II) complex



Figure 10. UV-Vis absorption spectrum of the Ni(II) complex

CONCLUSION

In this study, substituted Schiff base and its metal complexes were synthesized and characterized by physical and spectroscopic techniques. The compounds were subsequently tested for their α-amylase inhibitory potentials. The compounds displayed significant inhibitory activities against α-amylase comparable to the reference compound (acarbose). These compounds can serve as viable templates in the formulation of new active antidiabetic drugs.

ACKNOWLEDGEMENTS

The authors appreciated the support of all members of staff in the laboratory at the Department of Chemistry and Industrial Chemistry, Kwara State University (KWASU), Malete, Kwara State, Nigeria.

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