EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 5
1142-1148
Year: 2018

ZINC (II) COMPLEXES WITH L-ALANINE: SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT AND ANTIDIABETIC EFFECTS

I. P. Tripathi[1] and Aparna Dwivedi*[2]

[1]Dean, Faculty of Science and Environment, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot Satna, [MP].
[2]Research Scholar, Chemistry, Faculty of Science and Environment, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot Satna, [MP].

***Corresponding Author: Aparna Dwivedi**
Research Scholar, Chemistry, Faculty of Science and Environment, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot Satna, [MP].

Article Received on 21/03/2018 **Article Revised on 12/04/2018** **Article Accepted on 02/05/2018**

ABSTRACT
Zn (II) [$ZnSO_4$, $ZnCl_2$ and $ZnCH_3COO$] metal complexes of L-Alanine were synthesized and characterized by standard physico-chemical procedures i.e. elemental analysis, UV-Vis and FTIR techniques. On the basis of these studies a tetrahedral geometry has been proposed for all synthesized complexes. Synthesized metal complexes also tested for their antioxidant and antidiabetic activity to evaluate their inhibiting potential. The results showed that [Zn $(Ala)_2$]2Cl have higher antioxidant and anti-diabetic activity than other synthesized complexes.

KEYWORDS: Metal and metal complexes.

INTRODUCTION

Metal and metal complexes have played key role in development of modern chemotherapy. Metals can play an important role in modifying the pharmacological properties of known drugs after coordinating to a metal. The metal based drugs are also being used or the treatment of variety of ailment viz. diabetes, rheumatoid arthritis, inflammatory and cardiovascular diseases as well as diagnostic agents.[1] Many transition elements have been extensively investigated with regard to their potential as drug for several diseases. Metal containing compounds might be suitable for the development of new chemotherapeutics. Some clinically useful metal-containing compounds and metal complexes known as mettalopharmaceutials that can regulate human health have been developed to treat or cure many types of diseases. Zinc is essential for many metabolic and enzymatic functions in man. Deficiency of zinc in man has now been recognized to occur not only as a result of nutritional factors, but also in various disease states, including malabsorption syndromes, acrodermatitis enteropathica, Crohn's disease, alcoholism and cirrhosis of the liver.[2] It is evident that Zinc has a promising potential as a novel therapeutic agent in diabetes. Studies have also shown that diabetes is commonly accompanied by hypozincemia and hyperzincuria. Furthermore the high prevalence of Zinc deficiency in developing countries could be contributing towards driving the current diabetes epidemic encountered by them. Numerous research studies have been conducted to clarify the molecular mechanisms underlying the action of Zinc in diabetes.[3] Amino acids are suitable ligands for metal complexation due to their synthetic flexibility, their selectivity and sensitivity towards the central metal atom due to presence of amine and carboxylic group.[4] Interaction between transition metals and amino acids are very interesting in the biological applications, metal complexes of transition metal ions can be used as models to study the pharmocodynamic effects of drugs.

In current literature we proposed to synthesize metal complexes of various salt of zinc with L-alanine. Characterization of synthesized metal complexes is done by UV, FTIR and elemental analysis. Antioxidant activity of synthesized complexes has been checked by DPPH and ABTS scavenging activity. Antidiabetic activity of complexes has been checked by α-glucosidase and α-amylase inhibition activity.

EXPERIMENTAL

Materials

Zn acetate, zinc sulphate, zinc chloride, L-alanine, potassium bromide, porcine pancreatic α-amylase, dinitrosalicylic acid, p-nitrophenyl-α-D-glucopyranoside, sodium chloride, sodium diphosphate, disodium phosphate were purchased from SRL, India. While rat intestinal acetone powder was procured from Sigma-Aldrich and DPPH, ABTS was brought from Alfa-Acer. All chemicals viz., DMSO, sodium acetate, sodium

hydroxide, water were synthetic grade and used without further purification.

Synthesis of metal complexes

The Zn (II) metal complexes were prepared from various salts of zinc ($ZnSO_4$, $ZnCl_2$ and $ZnCH_3COO$) and L-alanine as ligand. For synthesis 2 mM of L-alanine was added in 20 ml of aqueous solution which containing 2 mM of sodium acetate and allow it to clear solution with continuous stirring. Then 2 ml aqueous solution of 1mM metal was added drop by drop with continuous string for three hours. After stirring the solution were transferred in a petridish to remove solvent in a incubator at 35 ^{0}C after 4-5 days solid white color complex obtained.[5]

Physical measurement of metal complexes

The electronic spectra of the complexes were recorded at 25 ^{o}C on a shimadzu UV-VIS 160 spectrophotometer, in quartz cells at the desired wave length region. 3 mM solution of complexes in DMSO was used in all UV–visible measurements at Faculty of Science and Environment, MGCGV Chitrakoot, Satna [MP].[6]

Infrared (IR) spectra were obtained by the KBr method using a Bruker Alfa-T model Fourier transform (FTIR) spectrometer (Bruker Instrument, Germany).The spectrometer was equipped with a Global IR source, KBr beam splitter, and detector. For each spectrum, 16 scans were obtained with the resolution of 4 cm^{-1}. The obtained IR spectra were processed by means of the program OPUS 7.0 at Faculty of Science and Environment, MGCGV Chitrakoot, Satna [MP].[7]

Elemental analysis were carried out on an Euro vector element analyzer at CDRI Lucknow [UP]
To know the percent of carbon, hydrogen and Nitrogen in synthesized complexes.

DPPH scavenging activity

This assay is based on the measurement of the scavenging ability of antioxidant substances towards the stable radical. The free radical scavenging activity of the test samples of Zn(II) complexes and L-alanine examined in vitro using DPPH radical as described by Sasikumar et al. with slight modification. 125µl of various concentrations of test samples were mixed with 125µl of DPPH (4mg/100ml methanol) solution and 50 µl of tris HCl solution (pH 7.4). The mixture was incubated for 30 min and the absorbance was measured at 517 nm against a reagent blank in a micro titer plate reader (Bio-TEK, USA). Ascorbic acid was used as standards. All determinations were carried out in triplicate.[8]

IC_{50} value was quantified using formula, Y= 0.341(x) +20.597, R^2 = 0.964. The percentage of DPPH inhibition by the sample was calculated by the following formula
% Inhibition = {[(AC - AS)/AC] ×100}

Where, AC is the absorbance of the control and AS is the absorbance of the tested sample. The concentration of inhibitor required to inhibit fifty percent of free radical activity under the mentioned assay conditions is defined as the IC_{50} value.

α- glucosidase inhibition activity

The α-glucosidase inhibitory activity assay was performed by following the method of Tripathi et al. In brief, Rat-intestinal acetone powder was dissolved in 100 ml of saline water and sonicated properly at 4°C. After sonication, the suspension was centrifuged (3,000 rpm, 4°C, 30 minutes) and the resulting supernatant was used for the assay. A reaction mixture containing 50 µl of phosphate buffer (50 mM; pH 6.8), 50 µl of rat α-glucosidase and 50 µl sample of varying concentrations (100-800 µg/ml) was pre-incubated for 5 min at 37°C, and then 50 µl of 3 mM PNPG was added to the mixture as a substrate. After incubation at 37°C for 30 min, enzymatic activity was quantified by measuring the absorbance at 405 nm in a micro titer plate reader (Bio-TEK, USA). Acarbose was used as standard and experiments were done in triplicates.[10]

IC_{50} value was quantified using formula, Y= 0.3576(x) + 28.825, R^2 = 0.9519. The percentage of enzyme inhibition by the sample was calculated by the following formula
% Inhibition = {[(AC - AS)/AC] ×100}

Where, AC is the absorbance of the control and AS is the absorbance of the tested sample. The concentration of inhibitor required to inhibit fifty percent of enzyme activity under the mentioned assay conditions is defined as the IC_{50} value.

RESULTS AND DISCUSSION

Characterization of synthesized metal complexes

The Complexes were prepared with L-alanine using three salt of zinc i.e ($ZnSO_4.7H_2O$ (A), Zn $(CH_3COO)_2$. $2H_2O$ (B) and $ZnCl_2$ (C)) in aqueous solution. It is assumed that synthesized complex of an L-alanine was same for all three salt so complexes were differentiate to identify by (A), (B) and (C) for $ZnSO_4.7H_2O$, $Zn(CH_3COO)_2.2H_2O$ and $ZnCl_2$ respectively. All synthesized complexes were in white colored, non-hygroscopic and thermally stable solids. The complexes were semi soluble in common organic solvents such as methyl/ethyl alcohol but were fairly soluble in H_2O, DMSO and DMF. Complexes were analyzed by means of elemental analysis, IR and UV-VIS spectroscopy

Elemental analysis

We have done elemental analysis to confirm the percentage presence of carbon, hydrogen and nitrogen in our synthesized complexes. We have also calculated percentage of C, H & N and compare with the found elemental data. Elemental data of the complexes are given in Table-1

Table 1: Elemental analysis data of Zn(II) complexes of L-Alanine.

S. N.	Complex	Empirical formula	Mole-cular weight	Color	Elemental analysis Calculated (Found)				
					C%	H%	N%	O%	M%
1.	$[Zn(ala)_2][A]$	$C_6H_{14}N_2O_4Zn$	243.58	White	5.79 (6.70)	29.59 (26.47)	11.5 (10.5)	26.27	26.85
2.	$[Zn(ala)_2][B]$	$C_6H_{14}N_2O_4Zn$	243.58	White	5.79 (6.29)	29.59 (25.48)	11.5 (10.2)	26.27	26.85
3.	$[Zn(ala)_2][C]$	$C_6H_{14}N_2O_4Zn$	243.58	White	5.79 (6.35)	29.59 (25.27)	11.5 (9.93)	26.27	26.85

Electronic Spectrum

The electronic spectra data of the complexes were recorded in 100% DMSO and their assignments are given in Table-2. One representative spectra of complex $[Zn(ala)_2]2Cl$ is shown in Fig.-1. In UV spectra of Zn(II)complex with L-alanine λ_{max} showed around 200-205 nm. Normally, the geometry of the metal complexes around metal ion can be found out using the d-d transitions in the electronic spectroscopy above 400 nm. However, the Zn(II) complexes due to the completely filled d^{10} configuration, do not exhibit d-d transition and show no absorption band above 400 nm. Nevertheless, the four coordinated Zn(II) complex may be assigned a tetrahedral geometry.[15]

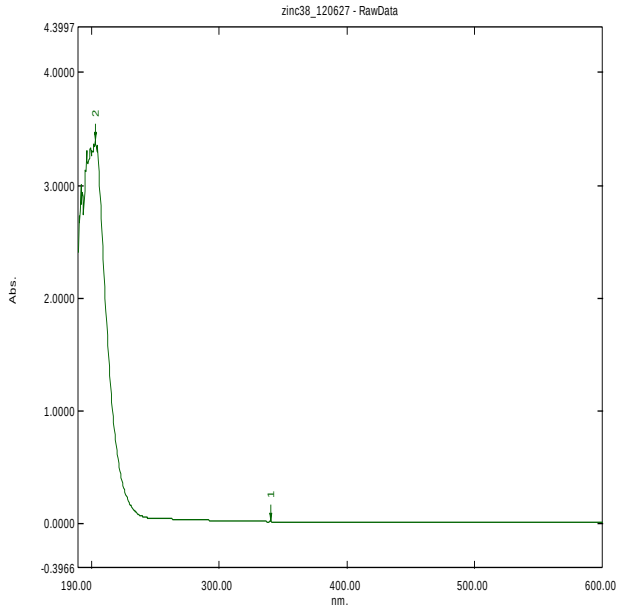

Fig. 1: UV spectrum of $[Zn(ala)_2]2Cl$.

Table 2: λ_{max} (nm) values (in 100% DMSO solution) for Zinc(II) complexes.

S. N.	Complex	λmax(nm)
1.	$[Zn(ala)_2][A]$	205
2.	$[Zn(ala)_2][B]$	200
3.	$[Zn(ala)_2][C]$	203

Infra Red spectrum

Complexes of Zinc with essential amino acids were synthesized in aqueous solution and analyzed by means of their IR and electrochemical properties. The IR spectra show that the amino acids act as bi-dentate ligands with co-ordination involving the carboxyl oxygen and the nitrogen atom of amino groups. Infrared studies on coordination of amino acids have shown that the coordination of metal with ligand making it a useful tool in structural studies.[12]

In the IR spectrum of Zn(II) metal complexes of amino acids the spectra exhibited a marked difference between bonds belonging to the stretching vibration of (N-H) of the amine group in the range between 3469-3382 cm^{-1} shifted to higher frequencies by 92-27 cm^{-1} suggesting the possibility of the coordination of ligands through the nitrogen atom at the amine group.[13] In order to get further information about the coordination behavior of the ligand with metal ion, the N-H starching vibration at 3334 cm^{-1} in the complex was shifted to higher frequencies with the complexes, suggesting that the coordination of the metal ion with the ligand was via the nitrogen atom.[14,15].

The infrared spectra of the complex $[Zn(ala)_2]SO_4$ is given in Fig.-2 in which band of N-H bending at 1616 cm^{-1} while band of N-H stretching occurred at 3324 cm^{-1}. Bands assigned from1235 cm^{-1} -1048 cm^{-1} are representing the C-N stretching bands. In the spectrum of the L-alanine (fig 3) bands are assigned to the symmetric and asymmetric bending vibration of N-H band. Band at 1616 cm^{-1} also indicates the metal-ligand bond formation. The important absorption bands of Zn(II) complexes are listed in Table-3.

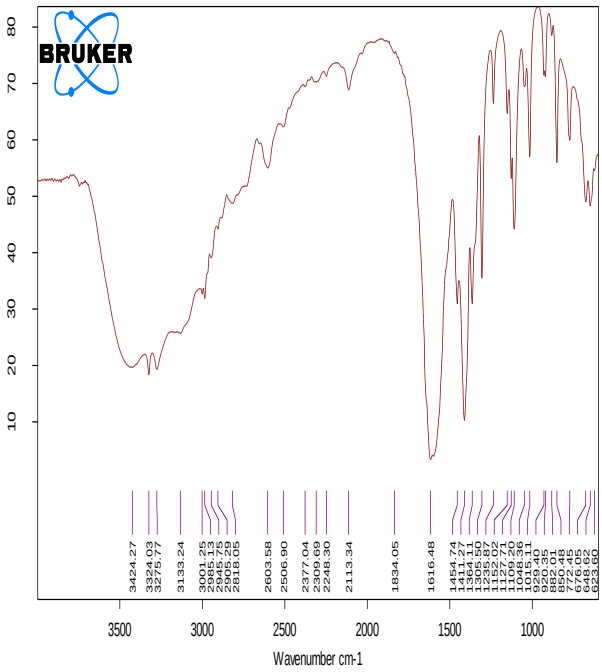

Fig. 2: Infrared spectrum of $[Zn(ala)_2]SO_4$.

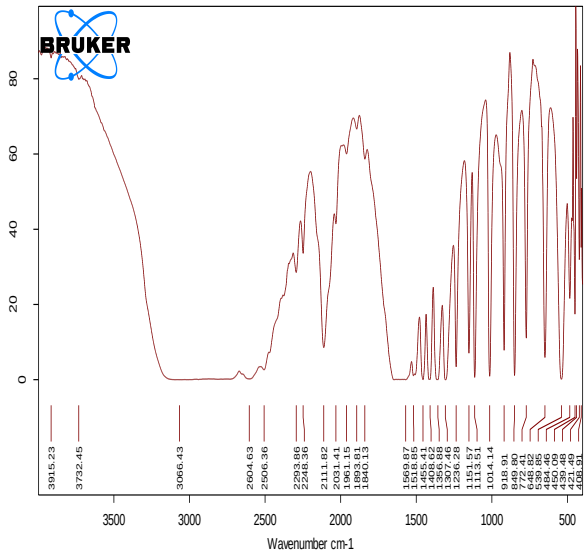

Fig. 3: Infrared spectrum of L-Alanine.

Table 3: IR Frequencies (in cm^{-1}) of Zn (II) complexes of L - Alanine.

S. N.	IR frequency in (cm^{-1})					Group Assignment
	L-Alanine		$[Zn(Ala)_2]$ (A)	$[Zn(Ala)_2]$ (B)	$[Zn(Ala)_2]$ (C)	
	Reported (16,17)	Found				
1	3570-3416	3066	3086	3085	3086	υ_{as} (OH) H_2O
2	2730, 2605, 2546	2406,2506	2346,2377	2377	2346,2377	Sum tone
3	1934,1884	1961,1893	-	-	-	Sum tone
4	1837-1703	1840	1843	1834	1843	Sum tone + υ (C=O) COOH
5	1566, 1539	1569,1518	1616	1614	1616	$\upsilon_{as}(NO_2)$; $\delta_{as}(NH_3^+)$
6	1451	1455	1456	1454	1466	CH_3, C-O str, OH bending
7	1411	1408	1412	1411	1412	CO^- sym. Str.
8	1356	1356	1364	1363	1364	CH_3^+ sym. Def.
9	1308	1307	1306	1305	1306	CH bend
10	1238	1236	1236	1236	1236	NH_3^+ rock
11	1151	1151	1152	1152	1152	CH_3 rock
12	1114	1113	1109	1109	1109	NH_3^+ rock
13	1018	1014	1014	1015	1014	CCN out of phase
14	923	918	930	929	930	CCN in phase
15	854	849	860	860	860	CCN in phase str. + CC str.
16	773	772	772	772	772	CO_2^- bend

DPPH scavenging activity

The DPPH radical is a stable free radical with a deep purple color. When a solution of DPPH radical is mixed with an antioxidant, its color turns from purple to pale yellow of the corresponding hydrazine. The reducing ability of antioxidants towards DPPH can be evaluated by monitoring the decrease of its absorbance at 515–528 nm.[18-19] In current work we have screened L-Alanine Zn(II) complexes for their free radical scavenging activity for DPPH. In this activity Zn(II) complexes of L-alanine are used as antioxidants which are scavenging DPPH free radical. Table-4 represents the IC_{50} value for all three complexes and Fig.-4, Fig.-5 shows the inhibition curve of ascorbic acid and Zn(II) complexes respectively.

Table 4: IC_{50} value of Ascorbic acid & Zinc(II) complexes for DPPH scavenging activity.

S. N.	Name of compound	IC_{50} value in µg/ml
1	$[Zn(ala)_2][A]$	1007.487
2	$[Zn(ala)_2][B]$	1024.206
3	$[Zn(ala)_2][C]$	929.48
	Ascorbic Acid	86.225

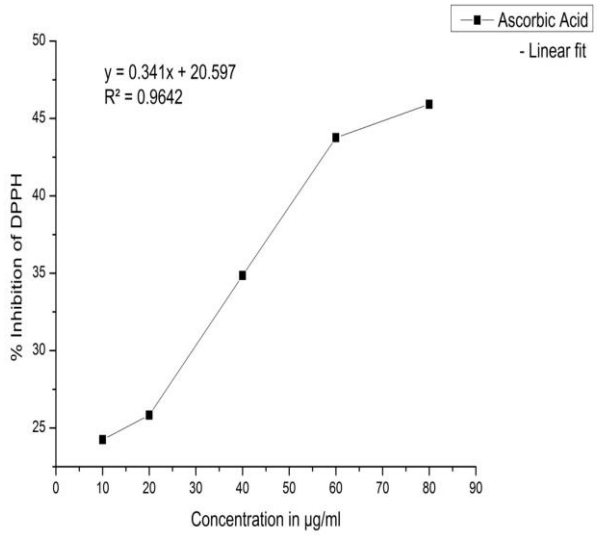

Fig 4: Percentage of inhibition of DPPH by Ascorbic acid.

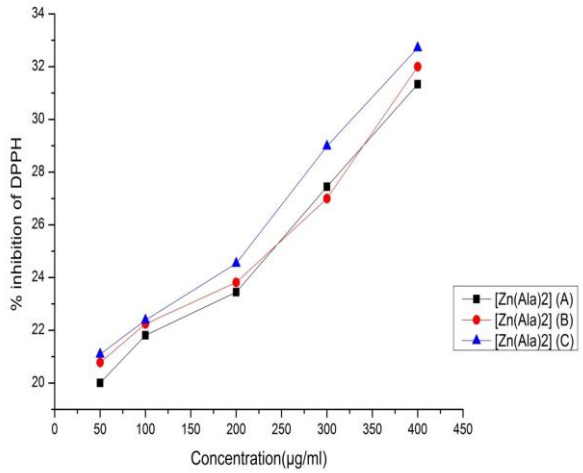

Fig. 5: Percentage of inhibition of DPPH by Zn (II) complexes.

α- glucosidase inhibition

α-glucosidase inhibition is an activity which inhibit the α-glucosidase enzyme and phenomena which inhibit the α-glucosidase enzyme called α-glucosidase inhibitor. α-glucosidase inhibitors play an important role to maintain the level of glucose in a diabetic patient. In current work we used Zn (II) metal complexes of L-alanine as α-glucosidase inhibitor. Substituent groups present on ligand may influence the hydrogen bond donor capability of the phenyl hydroxyl group. It may also act as hydrogen bond acceptors to appropriate hydrogen bonds donors of protein side chains and causes variations in the inhibitory potential.[20] In α-glucosidase, side chain of Threonine 215 acts as the hydrogen bond acceptor and the side chain hydroxyl group of Serine 244 serves as a hydrogen bond donor.[21] Bond formation between the metal ion and protein side chain is important for inhibition or activation of the enzyme. Metal complexes as α-glucosidase inhibitor can be further stabilized in the active site through hydrogen bonds with catalytic residues and the establishment of hydrophobic contacts in a cooperative fashion.[22]

IC_{50} values of α- glucosidase inhibition for Zn(II) complexes and acrabose are presented in Table-5 and percentage of inhibition of α- glucosidase by acarbose and Zn(II) complexes represented graphically in Fig 6 and Fig 7 respectively.

Table 5: IC_{50} value of Acarbose & Zinc(II) complexes for α-glucosidase inhibition activity.

S. N.	Name of compound	IC_{50} value in µg/ml
1	$[Zn(ala)_2][A]$	427.7197
2	$[Zn(ala)_2][B]$	448.8191
3	$[Zn(ala)_2][C]$	417.033
	Acarbose	**59.21**

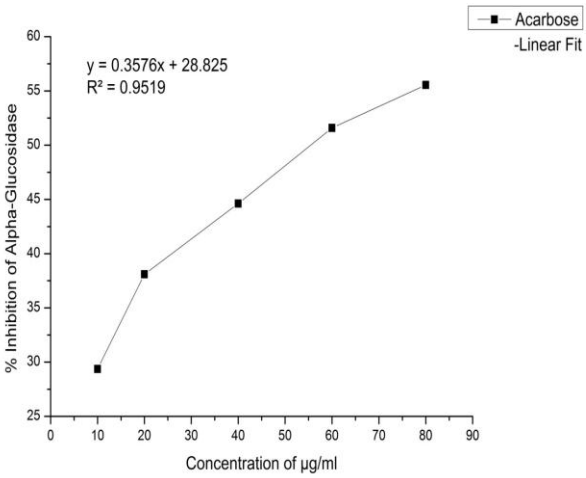

Fig. 6: Percentage of inhibition of α-glucosidase by Acarbose.

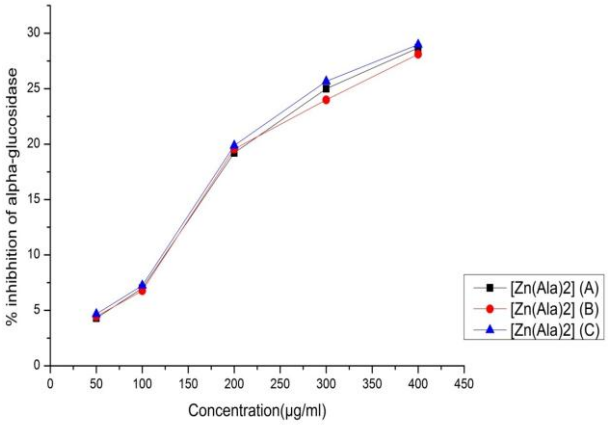

Fig. 7: Percentage of inhibition of α-glucosidase by Zn(II) complexes.

CONCLUSION

Transition metals play an important role in biological activity. We have synthesized Zn (II) metal complexes of L-Alanine and characterized via infrared, ultraviolet spectral properties. Sharp and intense peak at 1616 cm^{-1} in IR spectrum represents metal-ligand bonding which represents that complexes have synthesized. UV spectral bands in region of 200-205 nm are showing $\pi \rightarrow \pi^*$ transition which is indicating involvement of imines nitrogen atom in synthesized metal complexes and assuring metal ligand coordination. We have studied α-glucosidase and DPPH inhibition properties of Zn(II) complexes of L-alanine in which $[Zn(ala)_2]2Cl$ showing most potent result in both studies. It is reported that α-glucosidase inhibitors are also good antioxidants. In our study $[Zn(ala)_2][C]$ shows minimum IC_{50} 417.033 µg/ml for α-glucosidase inhibition activity and 929.48 µg/ml for DPPH inhibition activity. Synthetic α-glucosidase inhibitor may be effective for antidiabetic treatment and other disorders because it is easy to synthesize and also having a great possibility to inhibit α-glucosidase and free radicals.

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