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ENZYMATIC INHIBITION AND FREE RADICAL SCAVENGING ACTIVITY OF SOME COMPLEXES OF COPPER(II) WITH L-AMINO ACIDS

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ABSTRACT The colored complexes were prepared by the salts of copper(II) to a solution of ligands as L-amino acids. In conclusion, the structures of the obtained complexes were characterized by elemental analysis, FT-IR, UV- and cyclicvoltameteric spectral studies. The synthesized metal complexes were investigated for biological activities. Enzymatic inhibition activity has been done by using Rat intestinal acetone powder as enzyme and also antioxidant activity by DPPH and ABTS assay. All tested metal complexes reveal effective biological activities against enzyme inhibition andfree radical scavenging activity significantly.

KEYWORDS : amino acids, copper(II), UV, IR, CV. enzymatic activity, antioxidant activity.

Introduction

The metal complexes have become the subject of intense research interestwith coordination chemists as they are simple to prepare, have excellent complexation ability with both transition and non-transition elements and the complexes have interesting structural characteristics and possible analytical applications. This interest and wide range of applications resulted in a large number of papers and several reviews on complexes with L-amino acids.In addition, these complexes have received considerable attention due to their broad profile of pharmacological activity, as they afford a diverse variety of compounds with different activities (Beraldo et al 2004 and Quirago et al 2004). These complexes are used as model molecules for biologicaloxygen carrier systems (Gaballa et al 2007). Numerous metal ions are recognized to play specific and important roles in biological processes in the human body. In particular copper(II) ion, the third most abundant transition metals in human, is essential for many organisms. This metal is required for aerobic metabolism, and it can be found as active site or as structural component of a large number of enzymes (Taperio et al 2003 and Wilkinson 1987). Also, Lamino acids are important class of ligands and played an important role in the development of coordination chemistry as they readily form stable complexes with most transition metals (Sherif et al 2009 and Gup et al 2005). There have been several reports on metal complexes of the L-amino acid ligands having a variety of applications including biological, clinical, analytical and industrial in addition to their important roles in catalysis and organic synthesis(Auyanget al 2002 and Balakrishanan et al 2002).As continuation of the work in this field, we report here the synthesis, characterization and biological

activity of copper(II) with L-leucine, L-isoleucine and L-serine as ligands. Evaluation of enzyme inhibition activity by using acetylcholine enzyme and also antioxidant activity by using in vitroassays such as 2, 2'-diphenyl-1-picryl-hydrazyl (DPPH) and 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity for the newly synthesized metal complexes was undertaken.

Experimental

Materials and Methods

Chemicals:Acarbose, α-glucosidase Rat intestinal powder was procured from Sigma Aldrich, USA. All solvents were HPLC grade and used further purification. $CuSO_4.5H_2O$, $Cu(NO_3)_2.H_2O$, $CuCl_2$, $Cu(CH_3COO)_2$, CH_3COONa were purchased from alfaacear, Great Britain.

Synthesis of complexes: These complexes were prepared from the different salts of copper and amino acids (L-leucine, L-isoleucine and L-serine) as ligand. 2 mM of amino acid was added in 20 ml of aqueous solution with containing 2 mM of sodium acetate and allow it continuous string. Then 1 mM of metal salt in 2 ml of triple distilled water was added into this solution with continuous string for 3 hours. A clear deep blue colored solution obtained and kept for crystallization. After few days shining deep blue colored and needle shaped crystals obtained.

Infrared Spectroscopy: 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 Glober IR source, KBr beam spillter and detector. For each spectrum, 16 scans were obtained with the resolution of 4 cm^{-1}. The obtained IR spectra were proceeding by mean of the program OPUS 7.0.

UV-VIS spectroscopy: The UV-visible transmittance spectraof the complexes were recorded at 25°C on aShimadzu UV-Vis 160 spectrophotometer, inquartz cells at the desired wave length region.3 mM solution of complexes in DMSO was used in all UV -visible measurements.

Cyclic voltametry: The cyclicvoltametric measurements were carried out with a Metrohm Instrument (Germany) having an electrochemical cell with a three-electrode system. The reference electrode was an Ag/AgCl2. Platinum wire used an as a working electrode, Platinum wire electrode used as an auxiliary electrode. The 3 mg of complex were dissolved in supporting electrolyte 25 ml of 0.01 M solution of KCL solution. The voltamograme, peak position and area were calculated using NOVA 1.9 software.

Biological Activities of Metal Complexes

α-Glucosidase Inhibition : Method for determination ofα-Glucosidase was adopted from Tripathi et al (2013). Rat intestinal acetone powder (Sigma chemicals,USA) was sonicated properly in normal saline (100:1 w/v) and after centrifugation at 3000 rpm×30 minutes the supernatant was treated as crude intestinal α-Glucosidase. 50 µl various dilutions in DMSO (0.1mg /ml solution) were mixed and incubated with 50 µl of enzyme in a 96-well micro plate for 5 minutes. Reaction mixture was further incubated for another 10 minutes with 50 µl substrate (5 mM, pnitrophenyl-α-D-glucopyranoside) prepared in 100 mM phosphate buffer (pH~6.8) and release of nitrophenol was read at, 405 nm spectrophotometerically (MultimodeSynergyH4 micro plate reader, BioTek instrument, inc.Winoosci, VT, USA). All the samples were run in triplicate and acarbose was taken as standard reference compound. Several dilutions of primary solution (5mg/ml DMSO) were made and assayed accordingly to obtain concentration of the test sample required to inhibit 50% activity (IC50) of the enzyme. Quantification was performed with respect to the standard curve of acarabose ($Y = 26.63X + 46.26$, $R^2 = 0.958$) results were expressed as milligram of acarbose equivalent per ml of extract.

DPPH scavenging activity : free radical scavenging assay

The assay for free radical DPPH was done by using 2, 2-di¬phenyl-1-picrylhydrazyl (DPPH) method. In brief, a 96-well microplate, 25 µl of various dilutions (10-100 µg/ml) of meth-anolic extract 125 µl of tris–HCl buffer (0.1M, pH 7.4) and 125 µl of DPPH solution (0.004% w/v in methanol) were added. The reaction mixture was shaken well. The DPPH decolourization was recorded at 518 nm on a BioTekSynergy H4hybrid multimode micro plate reader (BioTek instruments, IncWin¬oosci, VT, USA.), after 30 min incubation in dark. The percent¬age of DPPH scavenging by complex dilutions

obtained in terms of ascorbic acid equivalent concentration. Quantification was performed with respect to the standard curve of Ascorbic acid (y = 0.731x+14.60; R2 = 0.947). Results were expressed as milligram of Ascorbic acid equivalent per ml of extract.

ABTS free radical Scavenging activity : For ABTS assay, the procedure followed the method of BibhabasuHazra et al. with modification suitable for micro well plates. The ability to test samples to scavenge ABTS+ radical cation was compared to ascorbic acid standard the ABTS+ radical cation was pre generated by mixing 7mM ABTS stock solution with 2.45mM potassium persulphate and incubating for 18 hrs in dark at room temperature until reaction was complete and absorbance of ABTS+ cation solution was 0.637 (+ 0.02) by diluting with water at room temperature then 20µl of test samples with different concentration were mixed with 180µl of ABTS solution and absorbance was measured at 734nm after 5min. Quantification was performed with respect to the standard curve of ascorbic acid (Y = 0.517X + 40.06, R2 = 0.985). Experiments were done in triplicates. The concentra¬tion was calculated using the following equation: scavenging effect (%) = Ao – A1 / Ao X 100. Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample.

Results and Discussion:

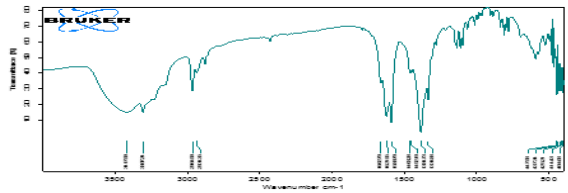
Characterization of metal complexes

Elemental analysis : The elemental analysis confirms the stoichiometric of the compounds, the composition corresponded to a metal-ligand ratio of 1:2. The results of these investigations are presented as following.

Table (1) : Elemental analysis data of copper complexes with amino acids.

S.no.	Complex	Empirical formula	Molecular weight	Color	Elemental analysis Calculated (Found)			
					M %	C %	H %	N%
1	[Cu(leu)2] 2SO4	C11H36N2O4Cu	511.82	Shining blue	12.41	28.13 (28.45)	07.03(6.57)	5.47(5.72)
	[Cu(leu)2] 2Cl	C11H26N2O4Cu	396.79	Royal blue	16.01	36.29 (36.45)	6.55(6.15)	07.05(7.23)
	[Cu(leu)2] 2NO3	C11H31N3O4Cu	494.86	Shining blue	12.84	29.10 (30.42)	6.26(6.73)	05.65(5.68)
	[Cu(leu)2]2CH3COO	C15H32N2O4Cu	361.99	Deep blue	13.75	36.36 (35.84)	6.92(6.71)	06.06(6.67)
2	[Cu(iso)2] 2SO4	C11H36N2O4Cu	511.82	Shining blue	12.41	28.13 (28.45)	07.03(6.57)	5.47(5.72)
	[Cu(iso)2] 2Cl	C11H26N2O4Cu	396.79	Royal blue	16.01	36.29 (36.45)	6.55(6.15)	07.05(7.23)
	[Cu(iso)2] 2NO3	C11H31N3O4Cu	494.86	Shining blue	12.84	29.10 (30.42)	6.26(6.73)	05.65(5.68)
	[Cu(iso)2]2CH3COO	C15H32N2O4Cu	361.99	Deep blue	13.75	36.36 (35.84)	6.92(6.71)	06.06(6.67)
3	[Cu(ser)2] 2SO4	C6H17N2O6Cu	459.66	Shining blue	13.82	15.66 (15.45)	3.70(3.48)	6.09(6.67)
	[Cu(ser)2] 2Cl	C6H7N2O6Cu	344.68	Royal blue	18.43	20.89 (14.45)	2.03(2.17)	08.12(8.04)
	[Cu(ser)2] 2NO3	C4H15N2O6Cu	442.68	Shining blue	14.35	16.26 (16.74)	2.71(2.87)	09.49(9.66)
	[Cu(ser)2]2CH3COO	C6H13N2O6Cu	409.83	Deep blue	15.50	29.28 (29.87)	3.17(3.02)	06.83(7.16)

Infra red spectroscopy Infrared studies on coordination compounds of amino acids have shown that the coordination of metal with ligand, making it a useful tool in structural studies (Nakamoto et al 2009). The binding mode of amino acid to the Cu(II) centre provide heuristic approach to the study of their coordination chemistry. In this complex the amino acid is presumed to occur in zwitterionic form binding the metal centre in bidentate mode through the carboxylate and amine end. The band observed for all the complexes approximate at 3300 to 3380 cm^{-1} in the spectrum assigned to the υ(N-H), suggesting the possibility of the coordination of ligand through the nitrogen atom at the amine group. While another absorption band appeared at 1632 cm^{-1} range could be explained as υ(COO) where the υ(OCO)sym was noticed at 1420 cm^{-1} range for all the synthesised complexes (Lever et al 1968 and Ning et al 2000). In order to get further information about the metal ligand bonding approximate at 1580 cm^{-1}, this also indicates the involvement of this group in the metal-ligand bond formation. The absorption band at 3119 cm^{-1} mostly in the complexes were shifted to higher frequencies, suggesting that the coordination of the metal ions with the ligand was via the nitrogen atom. The IR spectra show that amino acids act as bidentate ligands with the coordination involving the carbonyl oxygen and the nitrogen atom of amino group.The important absorption at assignments are listed in Table-2 and its spectral graphs are represented as Fig-1(a), 1(b) and 1©



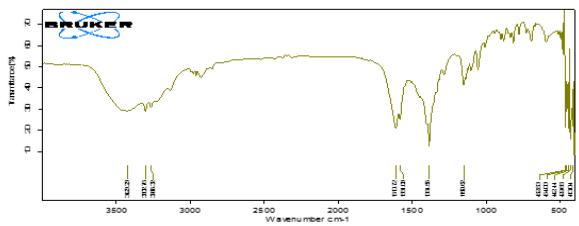


Fig-1(b) :IR Spectra of $[Cu(iso)_2]COOCH_3$

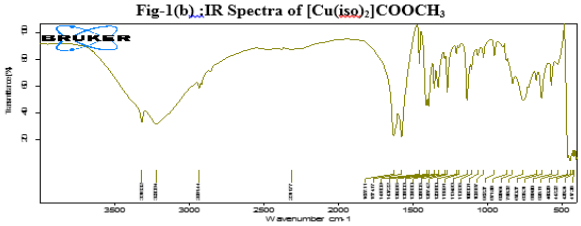


Fig-1(c) :IR Spectra of $[Cu(ser)_2]2SO_4$

Table-2 :IR-frequencies (in cm^{-1}) for copper complexes with amino acids

SN	Complex	Band cm^{-1}	Group
1	$[Cu(leu)_2]$ $2SO_4$	1586	N-H (bending) bounded with metal
		3246-3318	N-H (stretching)
		1620	C=O bounded with metal
	$[Cu(leu)_2]$ 2Cl	1589	N-H (bending) bounded with metal
		2964-3306	N-H (stretching)
		1621	C=O bounded with metal
	$[Cu(leu)_2]$ $2NO_3$	1589	N-H (bending) bounded with metal
		2964-3306	N-H (stretching)
		1621	C=O bounded with metal
	$[Cu(leu)_2]$ $2CH_3COO$	1589	N-H (bending) bounded with metal
		2964-3307	N-H (stretching)
		1622	C=O bounded with metal
2	$[Cu(iso)_2]$ $2SO_4$	1581	N-H (bending) bounded with metal
		3266-3302	N-H (stretching)
		1610	C=O bounded with metal
	$[Cu(iso)_2]$ 2Cl	1581	N-H (bending) bounded with metal
		3266-3302	N-H (stretching)
		1609	C=O bounded with metal
	$[Cu(iso)_2]$ $2NO_3$	1581	N-H (bending) bounded with metal
		3266-3302	N-H (stretching)

		1609	C=O bounded with metal
	[Cu(iso)$_2$] 2CH$_3$COO	1581	N-H (bending) bounded with metal
		3266-3302	N-H (stretching)
		1610	C=O bounded with metal
3	[Cu(ser)$_2$] 2SO$_4$	1574	N-H (bending) bounded with metal
		3220-3315	N-H (stretching)
		1627	C=O bounded with metal
	[Cu(ser)$_2$] 2Cl	-	N-H (bending) bounded with metal
		3246-3318	N-H (stretching)
		-	C=O bounded with metal
	[Cu(ser)$_2$] 2NO$_3$	1589	N-H (bending) bounded with metal
		2964-3307	N-H (stretching)
		1622	C=O bounded with metal
	[Cu(ser)$_2$] 2CH$_3$COO	1589	N-H (bending) bounded with metal
		2964-3307	N-H (stretching)
		1621	C=O bounded with metal

UV-visible spectroscopy The UV-VIS spectra of Cu(II) complexes with the amino acids show absorption bands assigned to intra ligands transitions and a large band around 620 nm (≈16000 cm^{-1}). The Cu(II) ion have the ground term arising from the $t^6 2g\ e^3 g$ configuration in an octahedral field is ^2E_g. The presence of the later band supports an octahedral stereochemistry for these complexes. There were two bands observed in the electronic spectrum of all the complex, at about 270 nm which can be assigned to $^2B_1g \rightarrow {}^2B_2g$ and $^2B_1g \rightarrow {}^2E_1g$ transitions(Tuncay et al 2010 and Benzite et al 2012). The absorption bands of the complexes corresponded to the n→σ*, n→π* and π*→π* transitions of -NH$_2$ and -COO–, Shifts in these bands and the observed d-dtransitions of the compounds, as presented, indicated coordination. Characteristic π→π* transitions are observed in all the spectrum of these complexes.The spectral graphs are represented as Fig-2(a), 2(b) and 2(c)absorptions and assignments related to the complexes are listed in Table-3.

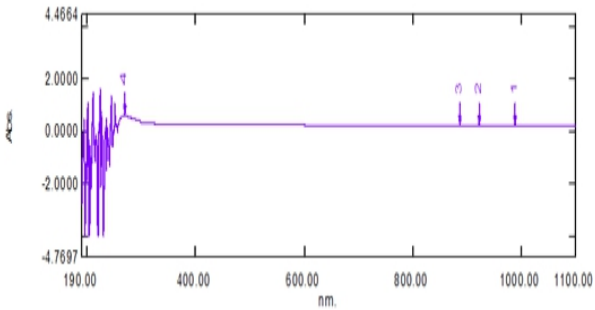


Fig. -1(a) :Uv-vis Spectra of [Cu(leu)$_2$]2Cl

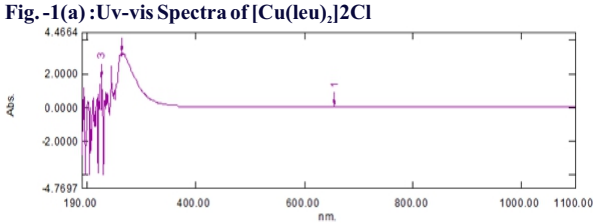


Fig. -2(b) :Uv-vis Spectra of [Cu(iso)$_2$]2NO$_3$

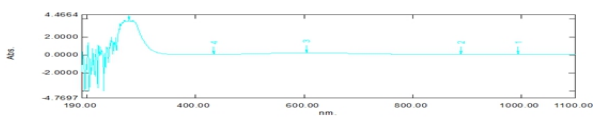


Fig. -2© :Uv-vis Spectra of [Cu(ser)$_2$]2SO$_4$

Table-2: λmax(nm) values (in 100% DMSO solution) for copper complexes with amino acids.

SN	Complex	λmax(nm)
1	[Cu (leu)$_2$]2SO$_4$	263
	[Cu (leu)$_2$]2Cl	262
	[Cu (leu)$_2$]2NO$_3$	269
	[Cu (leu) $_2$]2COOCH$_3$	272
2	[Cu (iso)$_2$]2SO$_4$	249
	[Cu (iso)$_2$]2Cl	264
	[Cu (iso)$_2$]2NO$_3$	245
	[Cu (iso) $_2$]2COOCH$_3$	273
3	[Cu (ser)$_2$]2SO$_4$	604
	[Cu (ser)$_2$]2Cl	278
	[Cu (ser)$_2$]2NO$_3$	249
	[Cu (ser)$_2$]2COOCH$_3$	272

Cyclic voltammetry The determination of the number of electronsinvolved in the electron transfer process for the Cu(II) amino acid complexes were determined. Cyclic voltammogram (CV) scanned cathodicallyin the potential region between +0.00 and -0.750 V vs Ag/AgCl in 0.1M sodium perchlorate solutionat different pH (isoelectric point of amino acids). In thisscan range, the CVs show a single reduction peak approximateat -400 mV (B1) in the forward sweep only one oxidation waves. All the voltamogram clearly represented that reduced moiety of Cu(II) doesn't fullyoxidized in further sweep (Przemyslaw et al 2004).Electrochemical studies of complexes were performed at a Pt as a working electrode shows a single step, one electron-transfer, (Fig-3(a), 3(b) and 3(c)and CV results (in mV) for these complexes are given in Table-4

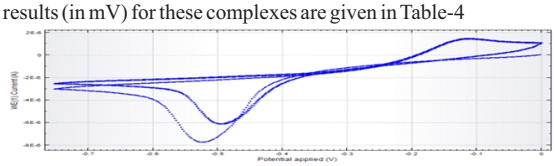


Fig – 3(a): Cyclic volatammogram of [Cu(leu)$_2$]2NO$_3$

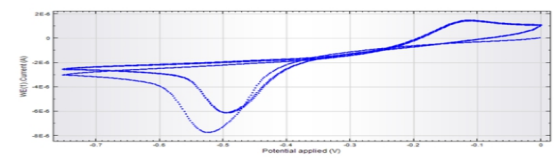


Fig –3(b): Cyclic volatammogram of [Cu(iso)$_2$]2SO$_4$

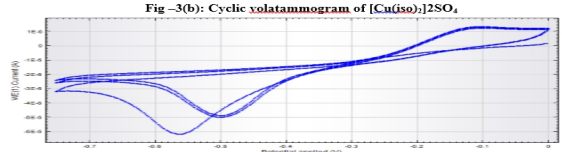


Fig- 3©: Cyclic volatammogram of [Cu(ser)$_2$]2Cl

Table-4: CV results (in mV) forcopper complexes with amino acids

SN	Complex	Reduction Peak(B1)	Oxidation Peak(A1)	Peak (1/2)	Peak width (1/2) V
1	[Cu (leu)$_2$]2SO$_4$	0.12403	-0.48543	0.062349	0.12365
	[Cu (leu)$_2$]2Cl	-0.12765	-0.47654	-0.069843	0.13408
	[Cu (leu)$_2$]2NO$_3$	-0.12613	-0.45644	0.056746	0.12667
	[Cu (leu)$_2$]2COOCH$_3$	-0.12934	-0.47689	0.073421	0.14596
2	[Cu (iso)$_2$]2SO$_4$	0.12348	-0.46766	0.064335	0.12611
	[Cu (iso)$_2$]2Cl	-0.12308	-0.48546	-0.06675	0.13563
	[Cu (iso)$_2$]2NO$_3$	-0.12564	-0.45367	0.054387	0.12632
	[Cu (iso)$_2$]2COOCH$_3$	-0.12332	-0.47498	0.073344	0.14545
3	[Cu (ser)$_2$]2SO$_4$	0.12233	-0.45645	0.064498	0.14216
	[Cu (ser)$_2$]2Cl	-0.12548	-0.43786	-0.06325	0.12134
	[Cu (ser)$_2$]2NO$_3$	-0.12576	-0.45632	0.064558	0.12653
	[Cu (ser)$_2$]2COOCH$_3$	-0.12354	-0.45989	0.059821	0.13425

Biological screeningThe metal complexes are suitable for mimicking the role of metal ions, detoxification mechanism and drug designing. These complexes play a decisive role in the activation of enzymes and also in the storage and transport of active substances. The transition metal complexes have shown catalytic activity and have also shown biological activity. In transition metal series there are numerous biological active metals are present. These metals have an esteemed place in various biological systems and also in medicinal chemistry. Research has shown significant progress in utilization of transition metal complexes as anti-inflammatory agents and free radical quenchers, anti-diabetic agents, anticancer agents, anti-infective agents, anti-proliferative effects in human ovarian cancers, antitumor

activity and as neurological drugs. The metal based drugs are also being used for the treatment of a variety of ailments viz. diabetes, arthritis, inflammatory and cardiovascular diseases as well as diagnostic agents.Therefore, there is a need for asearch of an alternative agent possessing hypoglycemic effect on T_2DM.

α-Glucosidase Inhibition Attempts to identify alternative antidiabetic compounds havebeen reported where metal ions such as vanadium, zinc, manganese,copper, chromium, and tungsten exhibited in-vitro as well as in-vivo antidiabetic activity (Kitture et al 2015, Patil et al 2012, 2013, Sakurai 2002, Munoz et al 2001, Fonteles et al 2000 and Coulston et al 1980). α-glucosidase is promising drug candidates in the treatment and prevention of T2DMas they are involved in sugar absorption (Chiasson et al 1998).Table-6demonstrates the IC50 of acarbose and metal complexes. Method for determination ofα-Glucosidase was adopted from Tripathi et al (2013). A perusal of the data reveals that these complexes of Cu(II) with L-Leucine shows effective alpha-glucosidase activity exhibits good activity. The remaining complexes of Cu(II) with other L-amino acids show less activity than the ligand. The Cu(II)-amino acid complexes can be ranked in the order acarbose>[Cu(leu)2] >[Cu(iso)2]>[Cu(ser)2]show effective α-glucosidase inhibition activity (Sakurai et al 2002) compared to the standard compound (acarbose) and corresponding ligand (Wang et al 2004). Fig-4 demonstrate the graphical representation of % inhibition of α-Glucosidase for complexes.

DPPH free radical scavenging activity The antioxidant properties were expressed as 50% inhibitory concentration (IC_{50}) values are illustrated. The antioxidant activity of these complexes can be attributed to the electron withdrawing effect of the Cu(II) ions which facilitates the release of hydrogen to reduce the DPPH radical. This proton release were very effective in $[Cu(leu)_2]$, with an IC50 value of 150.21 ± 0.37 µM. The DPPH radical scavenging ability of the test samples can thus be ranked in the order Vitamin C (ascorbic acid) > $Cu(leu)_2$> $Cu(iso)_2$>$Cu(ser)_2$. The % free radical scavenging of DPPH for these complexesare given in Table-7and Table-8 shows their IC50 values. Figures-5represents graphical arrangement of % inhibition.

ABTS free radical scavenging activity The antioxidant assay study was carried out using different concentrations of these complexes of Cu(II) with ABTS radicals, while ascorbic acid(vitamin C) was used as standards. The scavenging of the ABTS+ radical by the L-amino acid complexes was found to possess moderate to high activities relative to those of the standard. [Cu(iso)2]2NO3exhibited the highest activity with an IC50 of about 103.76 g/ml amongst the synthesized metal complexes.Figures-6 represents graphical arrangement of % inhibition and the % free radical scavenging of ABTS for these complexesare given in Table-7with evaluated values of IC50 values in Table-8.

Table-5: The % inhibition of α-Glucosidase for complexes

Acarbose	Conc. in µg/ml	Leucine								Isoleucine								Serine							
		Com-1	Error ± SD	Com-2	Error ± SD	Com-3	Error ± SD	Com-4	Error ± SD	Com-5	Error ± SD	Com-6	Error ± SD	Com-7	Error ± SD	Com-8	Error ± SD	Com-9	Error ± SD	Com-10	Error ± SD	Com-11	Error ± SD	Com-12	Error ± SD
17.87	200	4.09	0.87	4.26	0.88	3.89	0.77	5.58	0.75	1.87	0.84	1.22	0.86	2.04	0.79	1.87	0.84	4.43	0.82	4.21	0.88	5.72	0.89	3.21	0.83
26.04	400	9.03	0.8	8.51	0.79	7.34	0.64	7.86	0.67	7.5	0.76	6.7	0.73	8.48	0.71	7.5	0.76	9.37	0.77	8.32	0.75	7.55	0.8	8.24	0.76
48.39	600	18.57	76	14.44	0.67	15.46	0.52	14.32	0.6	15.67	0.68	12.43	0.66	14.82	0.65	15.67	0.68	18.06	0.74	16.52	0.67	16.37	0.74	15.86	0.64
59.15	800	29.98	0.62	27.48	0.58	23.11	0.48	25.41	0.54	22.49	0.59	17.37	0.58	21.56	0.57	22.49	0.59	23.51	0.69	22.48	0.58	20.15	0.64	22.65	0.59
68.23	1000	33.9	0.51	29.46	0.45	30.56	0.36	28.48	0.49	34.58	0.51	32.64	0.47	35.71	0.43	34.58	0.51	36.46	0.54	34.73	0.43	33.71	0.57	32.44	0.45
79.87	1200	39.52	0.37	33.15	0.26	35.64	0.28	36.54	0.37	44.46	0.48	36.15	0.26	43.78	0.34	44.46	0.48	49.23	0.44	44.07	0.36	45.13	0.41	44.95	0.37

Com-1 $[Cu(leu)_2]2SO_4$ Com-2 $[Cu(leu)_2]2Cl$ Com-3 $[Cu(leu)_2]2NO_3$ Com-4 $[Cu(leu)_2]2COOCH_3$ Com-5 $[Cu(iso)_2]2SO_4$ Com-6 $[Cu(iso)_2]2Cl$
Com7 $[Cu(iso)_2]2NO_3$ Com-8 $[Cu(iso)_2]2COOCH_3$ Com-9 $[Cu(ser)_2]2SO_4$ Com-10 $[Cu(ser)_2]2Cl$ Com-11 $[Cu(ser)_2]2NO_3$ Com-12 $[Cu(ser)_2]2COOCH_3$

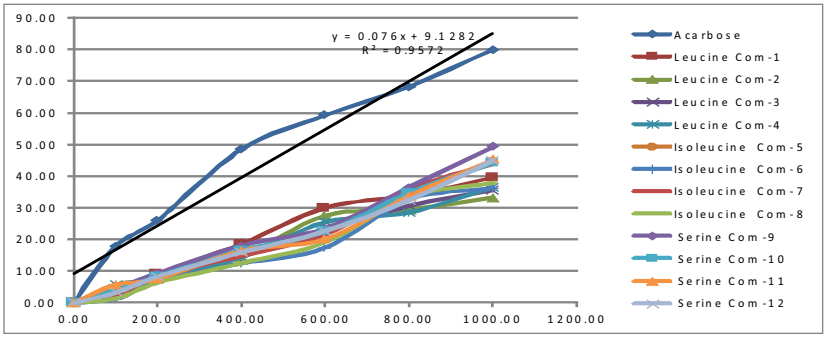


Fig- 4 Graphical representation of % inhibition of α-Glucosidase for complexes

Table-7: % free radical scavenging of DPPH for complexes

Ascorbic acid	Conc. in µg/ml	Leucine								Isoleucine								Serine							
		Com-1	Error ± SD	Com-2	Error ± SD	Com-3	Error ± SD	Com-4	Error ± SD	Com-5	Error ± SD	Com-6	Error ± SD	Com-7	Error ± SD	Com-8	Error ± SD	Com-21	Error ± SD	Com-22	Error ± SD	Com-23	Error ± SD	Com-24	Error ± SD
29.17	200	32.66	0.76	33.78	0.71	35.76	0.74	62.31	0.78	48.38	0.82	63.46	0.84	72.36	0.76	17.7	0.79	13.53	0.78	--	--	29.3	0.76	--	--
41.47	400	19.47	0.62	37.21	0.67	29.54	0.69	55.82	0.61	42.16	0.76	60.14	0.77	77.58	0.66	16.44	0.65	12.37	0.65	--	--	29.72	0.69	--	--
59.38	600	34.65	0.57	41.37	0.58	26.05	0.54	65.29	0.52	42.87	0.66	68.45	0.63	78.33	0.53	18.39	0.54	13.63	0.54	--	--	29.36	0.53	--	--

67.15	800	37.46	0.41	53.6	0.44	29.72	0.42	78.41	0.48	57.62	0.54	71.37	0.57	67.8	0.47	15.3	0.44	13.83	0.47	--	--	36.12	0.42	--	--
76.09	1000	38.04	0.36	57.81	0.32	34.13	0.37	76.93	0.39	59.54	0.43	62.49	0.41	64.56	0.34	14.26	0.31	16.88	0.32	--	--	28.4	0.33	--	--

Com-1 [Cu(leu)2]2SO4 Com-2 [Cu(leu)2]2Cl Com-3 [Cu(leu)2]2NO3 Com-4 [Cu (leu)2]2COOCH3 Com-5 [Cu (iso)2]2SO4 Com-6 [Cu(iso)2]2Cl
Com7 [Cu(iso)2]2NO3 Com-8 [Cu(iso)2]2COOCH3 Com-9 [Cu(ser)2]2SO4 Com-10 [Cu(ser)2]2Cl Com-11 [Cu (ser)2]2NO3 Com-12 [Cu(ser)2]2COOCH3

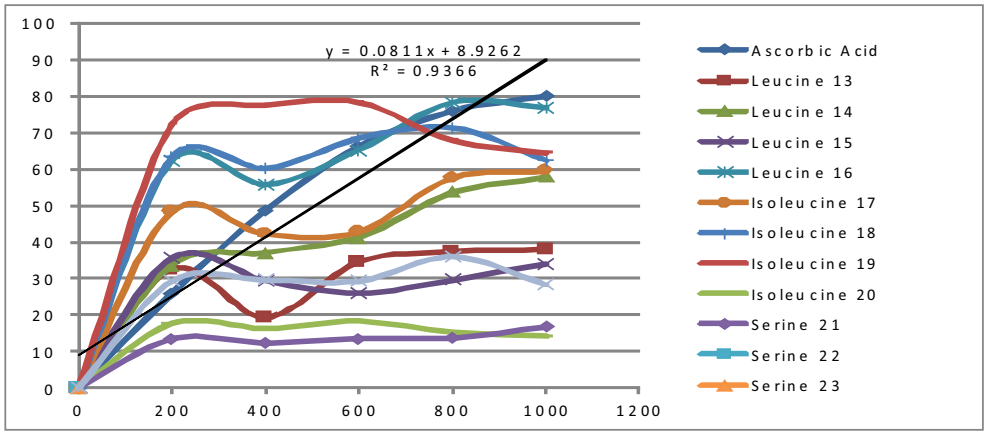


Fig- 5: Graphical representation of % free radical scavenging of DPPH for complexes

Table-7: % free radical scavenging of ABTS for complexes

Ascorbic acid	Conc. in µg/ml	Leucine								Isoleucine								Serine							
		Com-1	Error ± SD	Com-2	Error ± SD	Com-3	Error ± SD	Com-4	Error ± SD	Com-5	Error ± SD	Com-6	Error ± SD	Com-7	Error ± SD	Com-8	Error ± SD	Com-21	Error ± SD	Com-22	Error ±	Com-23	Error ±	Com-24	Error ±
29.17	200	23.34	0.76	22.04	0.85	19.03	0.89	22.17	0.82	8.38	0.31	7.92	0.83	9.29	0.85	12.48	0.89	8.56	0.34	--	--	7.43	0.93	13.75	0.82
41.47	400	21.59	0.74	21.72	0.68	21.55	0.87	22.54	0.71	10.36	0.86	9.38	0.79	9.51	0.76	14.62	0.84	9.02	0.91	--	--	9.75	0.87	14.44	0.79
59.38	600	25.28	0.63	25.82	0.56	25.32	0.73	25.71	0.64	12.03	0.78	11.52	0.7	14.35	0.71	13.72	0.82	10.43	0.85	--	--	11.23	0.76	15.26	0.72
67.15	800	24.92	0.54	26.48	0.47	25.89	0.66	26.62	0.52	11.69	0.56	14.71	0.62	16.06	0.68	15.83	0.64	11.87	0.79	--	--	12.56	0.52	16.8	0.64
76.09	1000	27.56	0.51	26.02	0.42	26.21	0.54	27.63	0.46	15.84	0.55	15.32	0.57	16.57	0.62	15.38	0.51	12.67	0.67	--	--	14.9	0.42	18.49	0.57

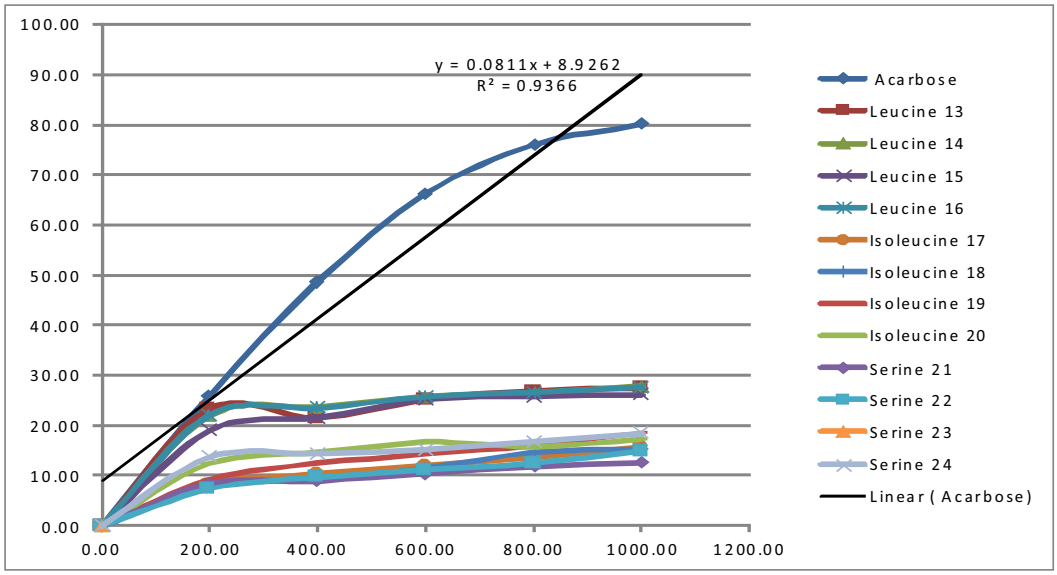


Fig- 6 Graphical representation of % free radical scavenging of ABTS for complexes

Table-6:IC50 values of Cu(II)-Amino acid complexes for % inhibition of α-Glucosidase

S.No.	Complex Name	IC50 value in µg/ml
	Acarbose	674

++++++++++++++++++++++	[Cu (leu)2]2SO4	1095
	[Cu (leu)2]2Cl	1096
	[Cu (leu)2]2NO3	1091
	[Cu (leu) 2]2COOCH3	1098
2	Cu(II)-Isoleucine	
	[Cu (iso)2]2SO4	1134
	[Cu (iso)2]2Cl	1136
	[Cu (iso)2]2NO3	1129
	[Cu (iso) 2]2COOCH3	1133
3	Cu(II)-Serine	
	[Cu (ser)2]2SO4	1631
	[Cu (ser)2]2Cl	1633
	[Cu (ser)2]2NO3	1631
	[Cu (ser) 2]2COOCH3	1634

Table-8: IC50 values of Cu(II)-Amino acid complexes for % free radical scavenging of DPPH

S.No.	Complex Name	IC50 value in µg/ml
	Ascorbic acid	47.58
1	Cu(II)-Leucine	
	[Cu (leu)2]2SO4	150.76
	[Cu (leu)2]2Cl	150.21
	[Cu (leu)2]2NO3	-----
	[Cu (leu)2]2COOCH3	-----
2	Cu(II)-Isoleusine	
	[Cu (iso)2]2SO4	165.28
	[Cu (iso)2]2Cl	-----
	[Cu (iso)2]2NO3	165.77
	[Cu (iso)2]2COOCH3	166.34
3	Cu(II)-Serine	
	[Cu (ser)2]2SO4	178.46
	[Cu (ser)2]2Cl	179.04
	[Cu (ser)2]2NO3	------
	[Cu (ser)2]2COOCH3	177.65

Table-10: IC50 values of Cu(II)-Amino acid complexes for % free radical scavenging of ABTS

S.No.	Complex Name	IC50 value in µg/ml
	Ascorbic acid	19.57
1	[Cu (leu)2]2SO4	103.76
	[Cu (leu)2]2Cl	105.28
	[Cu (leu)2]2NO3	-----
	[Cu (leu)2]2COOCH3	-----
	Cu(II)-Isoleusine	
2	[Cu (iso)2]2SO4	110.65
	[Cu (iso)2]2Cl	110.6
	[Cu (iso)2]2NO3	110.22
	[Cu (iso)2]2COOCH3	110.83
	[Cu (leu)2]2SO4	103.76
3	Cu(II)-Serine	
	[Cu (ser)2]2SO4	147.85
	[Cu (ser)2]2Cl	148.59
	[Cu (ser)2]2NO3	146.81
	[Cu (ser)2]2COOCH3	148.63

Conclusion: In the current study three new Copper-L-amino acid complexes with L-leucine, L-isoleucine and L-serine as ligand has been synthesized and characterized by spectral studies. FTIR spectra proved the bidentate nature of the ligand. Among the synthesized metal complexes Cu(II)-L-leucine complexes exhibited effective α-glucosidase inhibition and also effective on the proton release in DPPH assay with an IC50 value of 150.21 ± 0.37 µM. All the metal complexes showed moderate antioxidant activity. Cu(II)-L-isoleucine exhibited the highest activity in ABTS scavenging with an IC50 of about 103.76 g/ml amongst the synthesized metal complexes.

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