Synthesis and antimicrobial activity of Co(II) &Cu(II) metal complexes of Schiff base ligands derived from amino acids with citral

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Abstract

The Schiff base ligands, 2-(5,9-dimethyl-4,8-diene-3-ylidene)aminopropanoic acid (L_1), 2-(5,9-dimethyl-4,8-diene-3-ylidene)amino-3-methylpentanoic acid (L_2) and 2-(5,9-dimethyl-4,8-diene-3-yildene)amino-3-methylbutanoic acid (L_3) were prepared by condensation of L-alanine, L-isoleucine, L-valine with citral. Co(II) and Cu(II) complexes of above ligands were synthesised as well. The synthesised ligands and its complexes have been tested for their antimicrobial activity against bacterial species *staphylococcus aureus*, *Bacillus substills(gram positive)*, *Escherichia coli*, *Klebsiella pneumonia*, *pseudomonas aeroginosa(gram negative)* and fungal species *Aspergillus niger*, *Rhizopus*, *saccharomyces and Candida albicans* and the results concluded that the metal complexes exhibits effective antimicrobial activity against the tested strains as compared to the free Schiff base ligands.

Keywords: Transition metal complex, Amino acids, Antimicrobial activity, Schiff base.

1. INRODUCTION

Schiff bases derived from an amino and carbonyl compound are an important class of ligands that coordinate to metal ions via azomethine nitrogen and have been studied extensively [2], [7],[15]. The presence of a lone pair of electrons in sp2 hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance. Because of the relative easiness of preparation, synthetic flexibility, and the special property of C=N group, Schiff bases generally act as excellent chelating agents [1]. They have also other hetero-elements like oxygen or sulphur which provides binding sites to the metal ion may form ring structure making the complex more stable and biologically more active. [7]. Schiff bases are widely employed in stabilizing rare earth, inner-transition and transition metals in various oxidation states .The coordination between –NH2 group of the amino acid and carbonyl group of the aldehyde or ketone is very difficult because of the Zwitter ion effect and the reaction needs special conditions. It has been observed that pH plays an important role in the process of condensation [2].Several Schiff bases and their metal complexes have been reported to possess remarkable antibacterial, antifungal, anticancer, DNA cleavage and antimalarial activities [1], [7-18]. The coordination behaviour of Schiff base ligand with transition and rare earth metals plays a vital role in the recent days. Especially, transition metal complexes derived from amino acid Schiff bases have received much attention because of possible biological and pharmacological activities [2].

In view of diversified roles of Schiff base transition metal complexes, in this paper the metal complexes of Cu(II) and Co (II) with the Schiff base derived from L-alanine, L-isoleucine, L-valine and Citral have been synthesized. The ligand and their metal chelates have been screened for their antimicrobial activities using the disc diffusion method against the selected bacteria and fungi.

2. EXPERIMENTAL

2.1 Materials

All the chemicals and solvents used in the present work were of analytical grade. L-alanine, L-isoleucine, L-valine and Citral were purchased from spectrochem. The solvents like DMSO, diethyl ether and metal salts were purchased from Merck. The metal salts used were in their hydrated form.

2.2 Synthesis of Schiff base ligand 2-(5,9-dimethyl-4,8-diene-3-ylidene)aminopropanoic acid (L_1)

A mixture of citral (0.02mol, 25 ml) and aqueous solution of L- alanine (0.02 mol, 25 ml) was heated under reflux for 8-9 h at 60-65^{0}c. The completion of the reaction was monitored by TLC. After completion of the reaction, brownish red solution was obtained. The volume of the obtained solution was reduced to half at 60-65^{0}c. The reduced solution was allowed to cool for overnight without shaking. To the reduced solution 15 ml of ethanol was added and refluxed for 1h under mild heating condition. Yellow precipitate was obtained, filtered, repeatedly washed with hot ethanol followed by diethyl ether and dried.

2.3 Synthesis of Schiff base ligand 2-(5,9-dimethyl-4,8-diene-3-ylidene)amino-3-methylpentanoic acid (L_2)

To a solution of citral (0.02mol,) in ethanol (20 ml), aqueous solution of L-isoleucine (0.02mol, 20 ml) was added. The obtained solution was then refluxed for 4-5 h at 60^{0}c in water bath. The completion of the reaction was monitored by TLC. After completion of the reaction, brownish red solution was obtained. The volume of the obtained solution was reduced to half. On cooling brownish red oily precipitate was formed, filtered using Buckner funnel, washed with ethanol followed by diethyl ether and dried.

2.4 Synthesis of Schiff base ligand 2-(5,9-dimethyl-4,8-diene-3-yildene)amino-3-methylbutanoic acid (L_3)

To a solution of citral (0.02 mol) in ethonal (25 ml),

aqueous solution of L-valine(0.02 mol, 25 ml) was added. The obtained solution was then refluxed for 8 h at 65^0 c in water bath. The completion of the reaction was monitored by TLC. After completion of the reaction, brownish red solution was obtained. The volume of the obtained solution was reduced to half. On cooling brownish red oily precipitate was formed, filtered using Buckner funnel, washed with ethanol followed by diethyl ether and dried.

2.5 Preparation of complexes

To a hot magnetically stirred ethanolic solution of Schiff base ligands (L1-L3,0.01 mol), an aqueous solution of metal ions [$Co(NO_3)_2.6H_2O$ and $Cu(NO_3)_2.6H_2O$ (0.01 mol)] was added drop by drop at 60-65^0 c in 1:2 (metal: ligand) molar ratio. The mixture was then refluxed for 1h the intensity of the color becomes translucent. The pH of the mixture was slowly raised to obtain the appropriate pH for the formation of the complex by the drop wise addition of 0.01 mol sodium hydroxide solution [3], [19]. The resulting mixture was stirred for 1/2 h and concentrated to half its initial volume, allowed to stand for 24 h. The colored precipitates were obtained. Later, the precipitates were filtered out, washed repeatedly with distilled water, ethanol followed by diethyl ether and dried.

2.6 Antimicrobial activity:

2.6.1 .Test organisms:

Bacterial species *staphylococcus aureus, Bacillus substills, Escherichia coli, Klebsiella pneumonia, pseudomonas aeroginosa* and fungal species *Aspergillus niger, Rhizopus, saccharomyces* and *Candida albicans* were used as test organisms.

The Schiff base ligand (L_1) and its Co and Cu complexes were screened aganist bacterial species *staphylococcus aureus, Bacillus substills (gram positive), Escherichia coli and Klebsiella pneumonia (gram negative)* and fungal species *Aspergillus niger, Rhizopus and saccharomyces.* The Schiff base ligands (L_2 and L_3) and its Co and Cu complexes were screened aganist bacterial species *Escherichia coli and pseudomonas aeroginosa(gram negative)* and fungal species *Aspergillus niger and Candida albicans* in Mueller Hinton Agar medium(MHA) and potato-dextrose Agar medium(PDA). Solvent used for dissolving the synthesised compounds were DMSO and ethanol.

2.6.2 .Assay of antimicrobial activity:

Agar diffusion assay was carried out to evaluate the antimicrobial activity of some synthesized compounds. The plates were incubated at 37°C for 24 h during which activity was evidenced by the presence of a zone of inhibition surrounding the well and antibacterial and antifungal activity was expressed as mean of diameter of inhibition zones (mm) produced by the synthesized compounds when compared to controls.

2.6.3 . Experimental Methods:

MHA and PDA were prepared with lawn culture using desired test organisms. The inoculated plates were kept aside for few minutes. Using well cutter four wells were made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol. Using sterilized micropipette, 0.1mL of compound extract was added into one well and to another well the same volume of corresponding control were added. After diffusion, the plates were incubated at 37°C for 24 h. After incubation, the inhibition of growth was analyzed and the results were recorded.

3. RESULTS AND DISCUSSION

The condensation of L-alanine, isoleucine and valine with citral promptly give corresponding 1st, 2ndand 3rd Schiff base ligands respectively 2-(5,9-dimethyl-4,8-diene-3-ylidene)aminopropanoic acid(L_1), 2-(5,9-dimethyl-4,8-diene-3-ylidene)amino-3-methylpentanoic acid (L_2) and 2-(5,9-dimethyl-4,8-diene-3-yildene)amino-3-methylbutanoic acid (L_3). Corresponding ligands (L_1-L_3) were coordinated with Co^{2+} and Cu^{2+} metal ion separately to yield colored complexes respectively Co (L_1), Cu(L_1), Co(L_2) , Cu(L_2) , Co(L_3) and Cu(L_3). All the metal complexes are insoluble in water and organic solvents but are soluble in DMSO and DMF

3.1 Antimicrobial activity:

The antibacterial and antifungal activity results of the Schiff bases (L_1-L_3) and their Co(II) and Cu(II) complexes were given in table1-3.The presence of clear zones around the wells indicated that the compounds were active. The diameter of the zone inhibition was deducted in millimetres. The data revealed that the activity of the ligand enhanced on complexation but less than the control (Amilkacin and Flucanazol) used. The schiff base ligand (L_1) and its Co and Cu complexes (dissolved in DMSO) were screened aganist bacterial species *staphylococcus aureus, Bacillus substills(gram positive), Escherichia coli and Klebsiella pneumonia(gram negative)* and fungal species *Aspergillus niger, Rhizopus and saccharomyces*. Co(L_1) complex showed good activity against both gram positive and gram negative bacteria's especially against *Bacillus substills (gram positive).* Whereas Cu(L_1) complex showed only activity against *Klebsiella pneumonia(gram negative)* and no activity against gram positive bacteria. The reason may be due to the morphology of the cell membrane may be a main issue that affects the activity of antimicrobial agents. The cell membrane of the bacteria consists of peptidoglycan which is thicker in the gram positive bacteria and is usually posses a barrier to the degree of diffusion of antimicrobial agents into the enzyme [6]. Co(L_1) complex showed good antifungal activity against *Rhizopus.* Whereas Cu(L_1) showed activity against *saccharomyces*. The ligand L_1exhibits no antifungal activity against any species screened. But ligand L_1exhibits antibacterial activity for all the species screened. From the result (Table: 1) it was revealed that Co(L_1) complex showed greater antimicrobial activity than Cu(L_1) complex. In the present study low activity of the Cu metal complex may be due to the low lipophilicity of the complex, because of which

penetration of the complex through the lipid membrane was decreased and hence, they could neither block nor inhibit the growth of the microorganisms. This is confirming that antibacterial activity is dependent on the molecular structure of the complex [3]. The Schiff base ligands (L_2 and L_3) and its Co and Cu complexes (dissolved in ethanol) were screened against only gram negative bacterial species *Escherichia coli and pseudomonas aeroginosa* and fungal species *Aspergillus niger and Candida albicans*. Ligand (L_2) showed activity against only bacteria and not against fungi. Co(L_2) complex showed(table:2)the potent antimicrobial activity especially against *E.coli (gram negative), Aspergillus niger and Candida albicans(fungi)* and low activity against *Pseudomonas aeruginosa (gram negative)*. Cu(L_2) complex showed the best antibacterial activity specially against *Pseudomonas aeruginosa* and next to *Escherichia coli (gram negative*) than antifungal activity. The ligand L_3 showed medium activity against screened species. Co(L_3) showed the(table:3) remarkable antibacterial activity against *E.coli (gram negative)* compared to standard drug and Cu(L_3) showed better antifungal activity against *Candida albicans*. It is observed that metal complexes have higher antimicrobial activity. This is because of an increase in cell permeability. The lipid membrane which surrounded the cell favours the passage of only lipid soluble materials and it is known that liposolubility is an important factor controlling antimicrobial activity [3],[15]. The increase in biological activity of the metal chelates may be explained in the light of Tweedy's chelation theory. Chelation considerably reduce the polarity of the metal ion because of partial sharing of its positive charge with the donor group and possible π electron delocalization within the whole chelate ring system that is formed during coordination. Such chelation could enhance the lipophilic character of the central metal atom and hence increasing the hydrophobic character and lipo solubility of the complex favouring its permeation through the lipid layers of the cell membrane thereby deactivates respiration process in the microorganisms.[2],[4]. From the results it was concluded that cobalt complex [Co(L_1)]in DMSO solvent exhibits a higher antibacterial activity than copper complex [Cu(L_1)]. Similarly cobalt complex [Co(L_2)] in ethanol act as best antimicrobial agent than copper [Cu(L_2)]complex. The cobalt complex [Co(L_3)] in ethanol act as potent antibacterial agent whereas copper [Cu(L_2)] complex act as good antifungal agent. Thus the metal complexes showed differential effect on the species screened. The antimicrobial activity dependent on the molecular structure of the compound, the solvent used [5] and the species screened under consideration. [3]

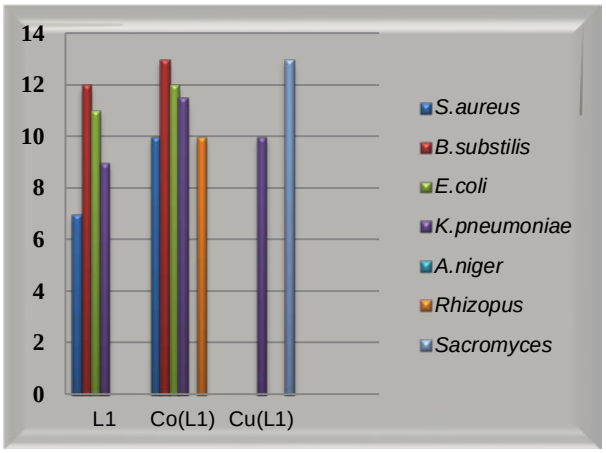

Fig 1: Antimicrobial activities of L1 and their metal complexes

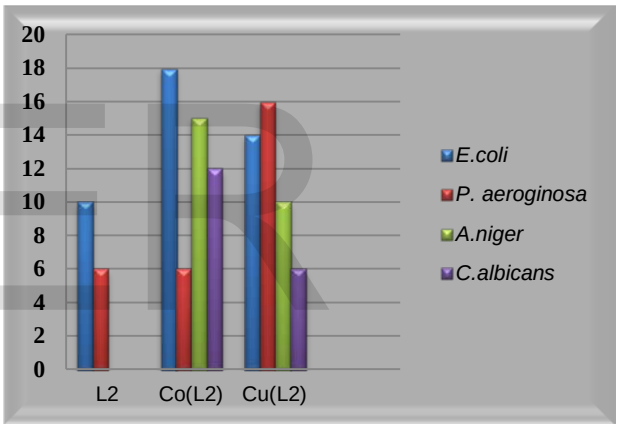

Fig 2: Antimicrobial activities of L2 and their metal complexes

TABLE 1: ANTIMICROBIAL ACTIVITY OF L_1 AND THEIR METAL COMPLEXES

Ligand/ complexes	Antibacterial activity(mm)				Antifungal activity(mm)		
	S. aures	*B.substillis*	*E.coli*	*K. pneumoniae*	*A.niger*	*Rhizopus*	*Saccromyces*
L_1	7	12	11	9	-	-	-
$Co(L_1)$	10	13	12	11.5	-	10	-
$Cu(L_1)$	-	-	-	10	-	-	13
Amikacin	20	22	20	21	-	-	-
Flucanazol	-	-	-	-	15	15	18

TABLE 2: ANTIMICROBIAL ACTIVITY OF L_2 AND THEIR METAL COMPLEXES

Ligand/ Complex	Antibacterial activity (mm)		Antifungal activity (mm)	
	E.coli	*P.aeruginosa*	*A. niger*	*C.albicans*
L_2	10	6	-	-
$Co(L_2)$	18	6	15	12
$Cu(L_2)$	14	16	10	6
Amilkacin	20	20	-	-
Flucanazol	-	-	15	25

TABLE 3: ANTIMICROBIAL ACTIVITY OF L_3 AND THEIR METAL COMPLEXES

Ligand/ Complex	Antibacterial activity (mm)		Antifungal activity (mm)	
	E.coli	*P.aerugi nosa*	*A. niger*	*C.albicans*
L_3	6	10	-	6
$Co(L_3)$	27	12	10	6
$Cu(L_3)$	12	15	6	18
Amilkacin	20	20	-	-
Flucanazol	-	-	15	25

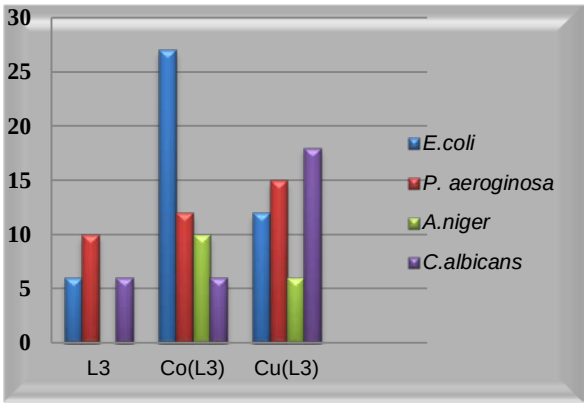

Fig 3: Antimicrobial activities of L3 and their metal complexes

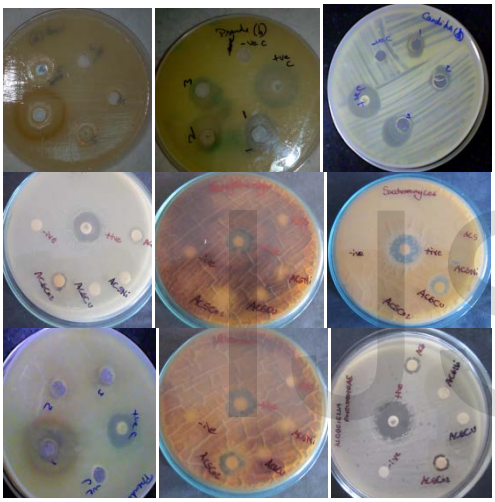

Fig 4: Inhibition zone against screened bacteria and fungi by the synthesized ligands and complexes

4. CONCLUSION.

The Schiff base ligands and its Co(II) and Cu(II) complexes were synthesized and tested for their antimicrobial activity. The outcome of antimicrobial studies showed that the Schiff base ligands possessed mild activity and metal(II) complexes possessed moderate to significant activities against different bacterial and fungal strains. From the results it was concluded that cobalt complex [Co(L_1)]in DMSO solvent exhibits a higher antibacterial activity than copper complex [Cu(L_1)]. Similarly cobalt complex [Co(L_2)] in ethanol act as best antimicrobial agent than copper [Cu(L_2)]complex. The cobalt complex [Co(L_3)] in ethanol act as potent antibacterial agent whereas copper [Cu(L_2)] complex act as good antifungal agent. Thus the metal complexes showed differential effect on the species screened. The biological activity findings exhibited that majority of the Schiff base ligands possessed increased activity upon coordination with different metal ions.

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