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Antibacterial and Antifungal Studies on Some Schiff Base Complexes of Zinc(II)

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Two Schiff base ligands L_1 and L_2 were obtained by the condensation of glycylglycine respectively with imidazole-2-carboxaldehyde and indole-3-carboxaldehyde and their complexes with Zn(II) were prepared and characterized by microanalytical, conductivity measurement, IR, UV-Vis., XRD and SEM. The molar conductance measurement indicates that the Zn(II) complexes are 1 : 1 electrolytes. The IR data demonstrate the tetradentate binding of L_1 and tridentate binding of L_2. The XRD data show that Zn(II) complexes with L_1 and L_2 have the crystallite sizes of 53 and 61 nm respectively. The surface morphology of the complexes was studied using SEM. The *in vitro* biological screening effects of the investigated compounds were tested against the bacterial species *Staphylococcus aureus, Escherichia coli, Klebsiella pneumaniae, Proteus vulgaris* and *Pseudomonas aeruginosa* and fungal species *Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus, Rhizoctonia bataicola* and *Candida albicans* by the disc diffusion method. A comparative study of inhibition values of the Schiff base ligands and their complexes indicates that the complexes exhibit higher antimicrobial activity than the free ligands. Zinc ions are proven to be essential for the growth-inhibitor effect. The extent of inhibition appeared to be strongly dependent on the initial cell density and on the growth medium.

KEYWORDS : Antibacterial, Antifungal, IR, Schiff base, SEM, XRD

Many metal complexes have powerful antimicrobial activities and are already in common day-to-day use in medicinal field such as silver bandages for treatment of burns, zinc antiseptic creams, bismuth drugs for the treatment of ulcers and metal clusters as anti-HIV drugs. The potential for further development of metal-based drugs and treatments as an antimicrobial agent (Scozzafava *et al.*, 2001; Scozzafana and Supuran, 2000) is enormous and also of great importance with the evolution of drug-resistant bacteria and threats from a range of viral diseases. The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. Metal-based drugs represent a novel group of antifungal agents with potential applications for the control of fungal infections. This inspires synthetic chemists to search for new metal complexes for bioactive compounds and zinc in particular has attracted the researchers. The field of macrocylic chemistry of metals is developing very rapidly because of its applications and importance in the area of coordination chemistry (Ashu *et al.*, 2003). The finding that the tetrahedral zinc complexes in its cavities generally represent the optimal, least strained structures among various zinc polyhedra may explain why four-coordinate zinc is chosen to play a structural role in zinc fingers and enzymes. Recently, we have synthesized and characterized some Schiff base metal complexes and their *in vitro* antimicrobial activities have been investigated (Nair *et al.*, 2007). In the present paper, we report the results on the synthesis, characterization and antimicrobial activities of Zn(II) complexes of Schiff base ligands L_1 and L_2 derived from glycylglycine and imidazole-2-carboxaldehyde and indole-3-carboxaldehyde respectively.

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Materials and Methods

Reagents and apparatus. The dipeptide, glycylglycine was purchased from Fluka (LTD) and used without further purification. Imidazole-2-carboxaldehyde and indole-3-carboxaldehyde were obtained from Lancaster (LTD) and Zn(II) nitrate was obtained from Merck (LTD). All other reagents and solvents were purchased from commercial sources and were of analytical grade. Solvents were purified and dried by standard methods. The metal contents of the complexes were determined by EDTA titration. Elemental analysis was done using a Perkin-Elmer elemental analyzer. IR spectra were recorded in KBr discs on a JASCO FT/IR-410 spectrometer in the 4000~400 cm^{-1} region. The electronic spectra were recorded on a Perkin Elmer Lambda-25 UV/VIS spectrometer. Molar conductance of the complexes was measured in DMSO (10^{-3} M) solutions using a coronation digital conductivity meter. XRD was recorded on a Rigaku Dmax X-ray diffractometer with CuKα radiation ($\lambda = 1.5404$ A°). SEM images were recorded in a Hitachi SEM analyzer.

***In vitro* antimicrobial activity.** Antibacterial activity of the ligands and their complexes were tested against the

bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumaniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* by Kirby Bauer Disc diffusion method (Bayer *et al.*, 1996). The ligands and complexes were also tested against the fungal species *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans*, cultured on potato dextrose agar medium and also performed by the disc diffusion method. *Amikacin*, *Ofloxacin* and *Ciprofloxacin* were used as the standard antibacterial agents whereas Nystatin was used in the technique as the standard antifungal agent. The test organisms were grown on nutrient agar medium in petri plates. The compounds were prepared in DMSO and soaked in filter paper disc of 5 mm diameter and 1 mm thickness. The discs were placed on the previously seeded plates and incubated at 37°C and the diameter of inhibition zone (Ferrari *et al.*, 1999) around each disc was measured after 24 h for bacteria and 72 h for fungi.

Synthesis of Schiff base ligands. Glycylglycine (5 mmol) was dissolved in 40 cm^3 of methanol containing KOH (5 mmol). A solution of imidazole-2-carboxaldehyde (5 mmol) and indole-3-carboxaldehyde (5 mmol) in 20 cm^3 absolute methanol was added dropwise with stirring and refluxed at 50°C for 2 h. The volume of the yellow solution was reduced *in vacuo* using a rotary evaporator. Anhydrous ether was added to deposit a yellowish precipitate, which was then recrystallized from ethanol.

Synthesis of Zn(II) Schiff base complexes. Zn(II) nitrate (1 mmol) was dissolved in 10 cm^3 of methanol. The solution was filtered and added dropwise into 20 cm^3 methanol solution of the Schiff base ligands (L_1 and L_2) (1 mmol). The resulting mixture was stirred for 2 h. After allowing it to stand in air at room temperature, the precipitated complex was filtered off, washed several times with cold ethanol, ether and then dried *in vacuo* over anhydrous $CaCl_2$.

Results and Discussion

Zn(II) complexes are stable at room temperature, insoluble in water but soluble in DMF and DMSO. The physical properties and analytical data of the ligands and their complexes are given in Table 1. Elemental analysis data of the complexes are in good agreement with theoretical values. The analytical data (Table 1) indicate that the metal to ligand ratio is 1 : 1 in all the complex systems and it can be represented as $[ZnL_1](NO_3)(H_2O)$ and $[ZnL_2(H_2O)](NO_3)$, where L_1 and L_2 are Schiff base ligands obtained by the condensation of glycylglycine respectively with imidazole-2-carboxaldehyde and indole-3-carboxaldehyde. The Zn(II) complexes have higher molar conductance values (69 and 76 Scm^2mol^{-1}) indicating that the above complexes are 1 : 1 electrolytes (Geary, 1971) as evidenced by the non-involvement of the nitrate group in coordination.

IR spectra. The Schiff base ligands L_1 and L_2 show ν(C=N) azomethine bands observed at 1631 and 1618 cm^{-1}. On complexation, this band was shifted to 1621 and 1609 cm^{-1} regions (Nakamoto, 1978) due to the coordination of azomethine nitrogen to the Zn(II) ion. In the Schiff base ligand (L_1), the imidazole nitrogen band appeared at 1612 cm^{-1}. This band was shifted to lower frequency at 1602 cm^{-1}, indicating that the imidazole nitrogen is coordinated to Zn(II) ion. In the spectra of Schiff base ligands, the peptide bands are observed at 1532 and 1542 cm^{-1}. On complexation, this band was shifted to 1525 and 1528 cm^{-1} region, indicating the linkage between metal ion and the peptide nitrogen atoms. The asymmetric carboxyl stretching $\nu_{asym}(COO^-)$ was shifted to higher frequency in the 1585 and 1589 cm^{-1} range and the symmetric carboxyl stretching $\nu_{sym}(COO^-)$ was shifted to lower frequency in the 1379 and 1384 cm^{-1} range, indicating the linkage between the metal ion and carboxylato oxygen atom. The asymmetric and symmetric stretching vibration of the carboxylato group in the complexes shows the separation value ($\Delta\nu$) greater than 200 cm^{-1}. This indicates monodentate binding of carboxylato group in Zn(II) complexes. Furthermore, the presence of coordinated and lattice water molecules appeared respectively at 3419 and 3397 cm^{-1} in ZnL_1 and ZnL_2 complexes may be attributed to O-H stretching vibration. The IR spectra of the complexes show the bands at 1363 and 1374 cm^{-1}, which can be ascribed to the presence of free NO_3 group. The appearance of two bands at 520 and 534 cm^{-1} corresponds to ν(M-O) and the bands at 444 and 449 cm^{-1} corresponds to

Table 1. Physical and analytical data of the Schiff base ligands and their complexes

Compound	Formula	Colour	Found (calc.) (%)			
			C	H	N	M
$K(L_1)$	$C_8H_9N_4O_3K$	yellow	38.32 (38.70)	3.49 (3.65)	22.51 (22.57)	–
$K(L_2)$	$C_{13}H_{12}N_3O_3K$	yellow	52.51 (52.35)	4.07 (4.05)	14.13 (14.08)	–
$[ZnL_1](NO_3)(H_2O)$	$C_8H_{11}N_5O_7Zn$	colourless	27.10 (27.69)	3.13 (3.18)	19.75 (19.76)	18.44 (18.48)
$[ZnL_2(H_2O)](NO_3)$	$C_{13}H_{14}N_4O_7Zn$	colourless	38.68 (38.64)	3.50 (3.16)	13.88 (13.67)	16.20 (15.91)

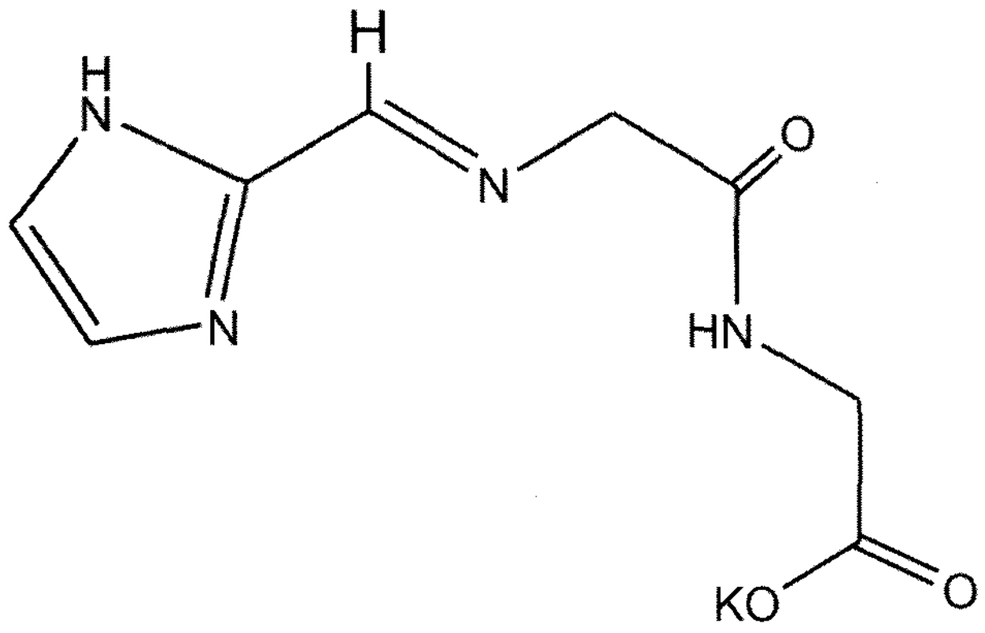

Fig. 1. Proposed structure of Schiff base ligand (L_1).

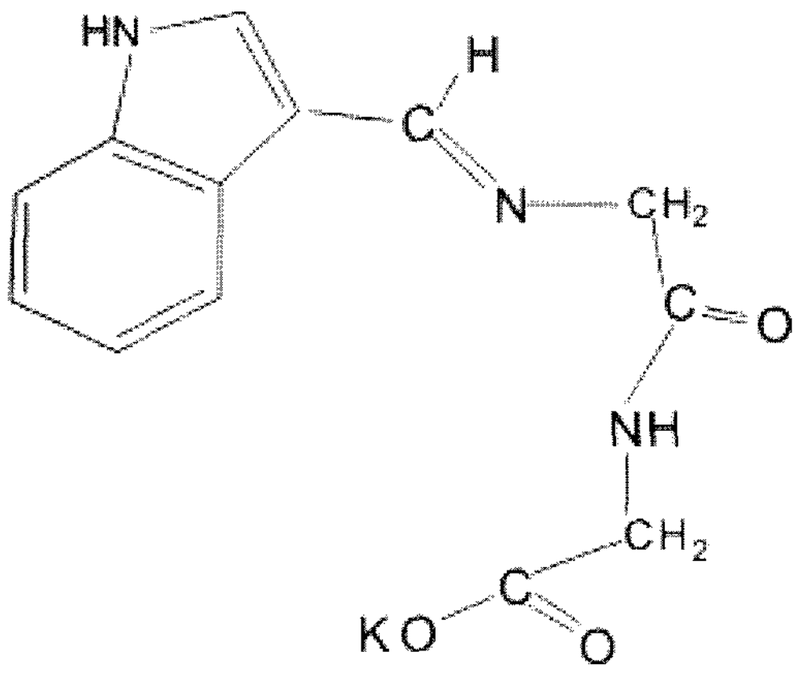

Fig. 2. Proposed structure of Schiff base ligand (L_2).

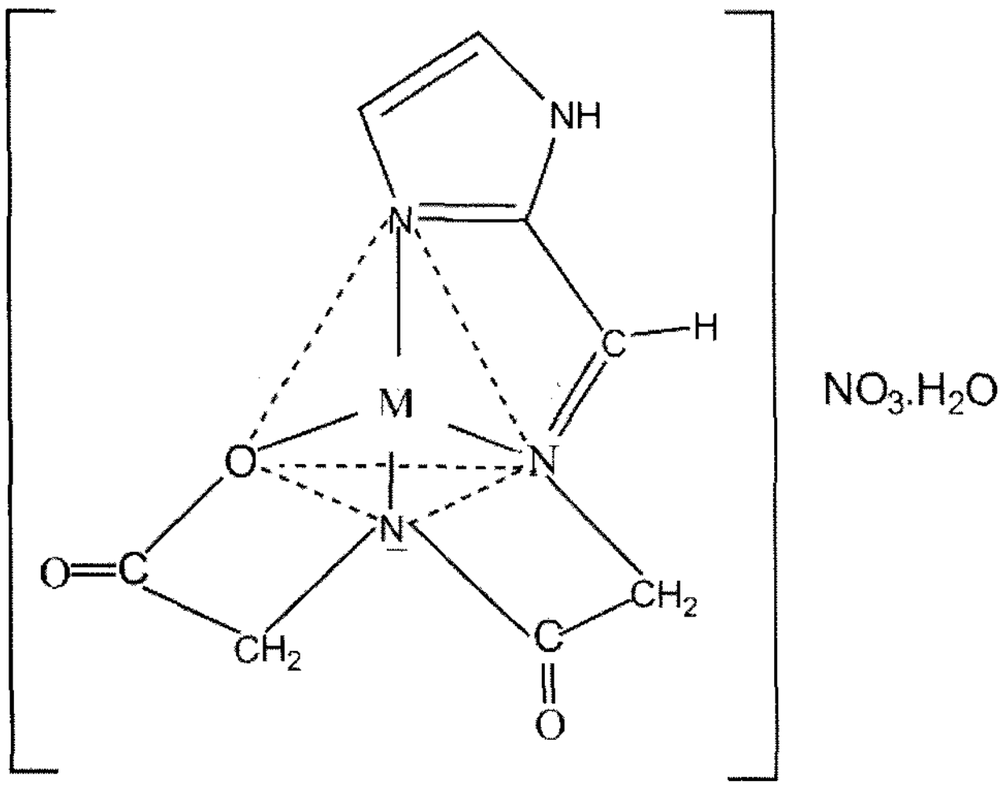

Fig. 3. Proposed structure for ZnL_1 Schiff base complex.

ν(M-N) stretching vibrations respectively. Thus, the IR spectral data indicate that in the ZnL_1 complex, L_1 is tetradentate binding through azomethine nitrogen, imidazole nitrogen, amide nitrogen and carboxylato oxygen atoms, whereas in ZnL_2 complex, L_2 is tridentate binding through azomethine nitrogen, amide nitrogen and carboxylato oxygen atoms (Figs. 1 and 2).

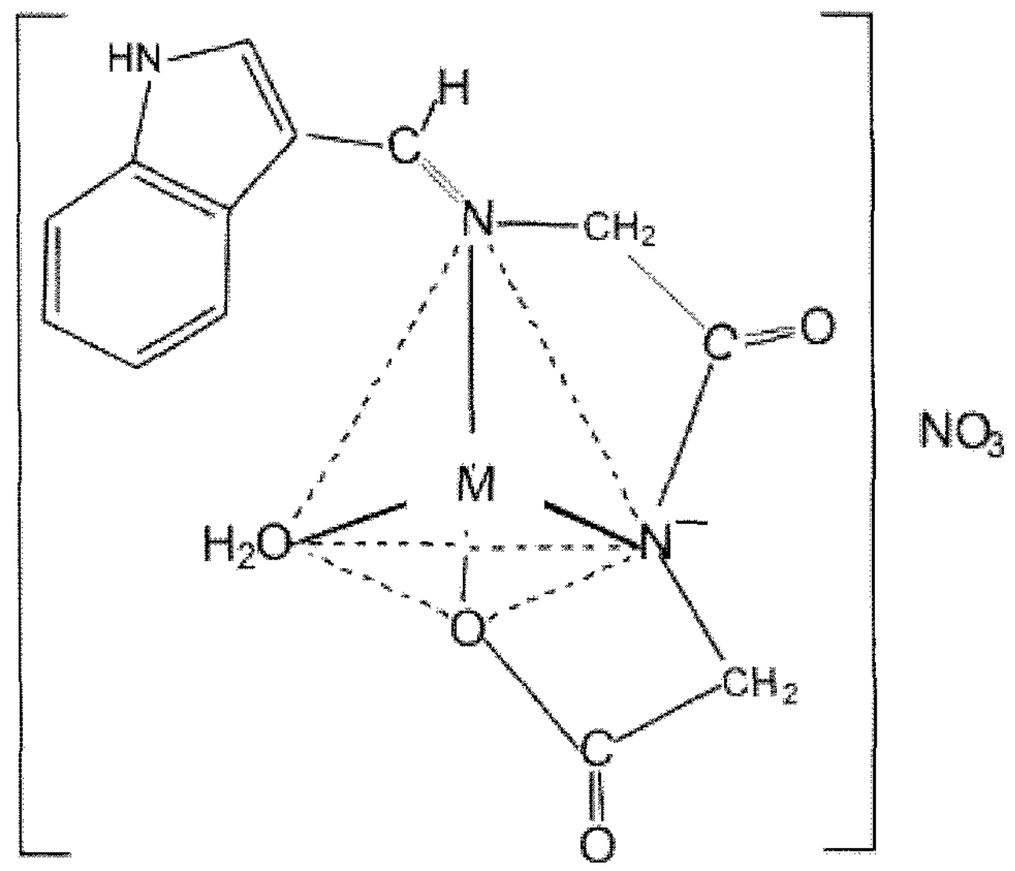

Fig. 4. Proposed structure for ZnL_2 Schiff base complex.

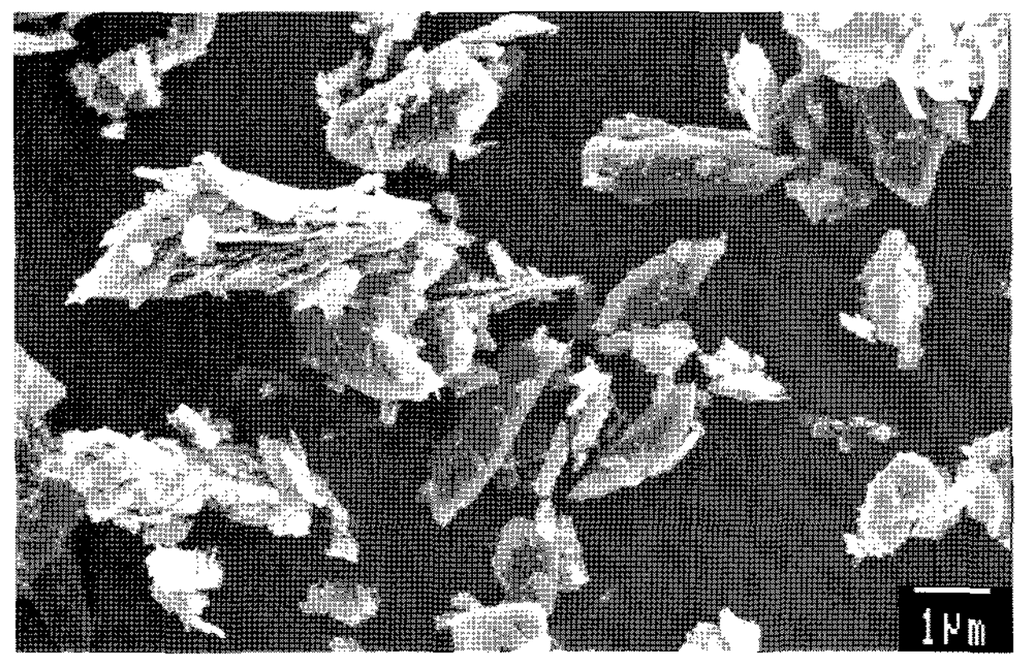

Fig. 5. SEM images of (a) ZnL_1 and (b) ZnL_2 Schiff base complexes.

UV/Vis. spectra. The Schiff base ligands L_1 and L_2 show the absorption bands at 320 and 307 nm, which is assigned to π-π^* transition of the C=N chromophore. On complexation, this band was shifted to lower wavelength region, suggesting the coordination of azomethine nitrogen with Zn(II) ion (Lever, 1984). Zn(II) complex does

not exhibit d-d electronic transition due to the completely filled 'd' orbital. Four coordinate Zn(II) complexes would have tetrahedral geometry (Figs. 3 and 4).

XRD. The XRD pattern of Zn(II) complexes show well defined crystalline peaks indicating that the samples are crystalline in nature. The above complexes have specific 'd' values which can be used for its characterization. The crystallite size of the complexes d_{XRD} could be estimated from XRD patterns by the Scherre's formula (1).

$$d_{XRD} = 0.9\lambda/\beta\,(\mathrm{Cos}\theta) \quad (1)$$

where λ is the wavelength, β is the full width at half maxima and θ is the diffraction angle (Cullity, 1972; Hadi, 2005). The XRD shows that Zn(II) complexes have the average crystallite sizes of 53 and 61 nm respectively, suggesting the complexes to be nanocrystalline.

SEM. The surface morphology of the Zn(II) complexes in this study are shown in Figs. 5(a) and 5(b). The Zn(II) complex with 5(a) has leaflet morphology, while that with 5(b) has cauliflower like morphology. The particle sizes of the Zn(II) complexes were in the diameter range of few microns. However, particles with sizes less than 100 nm were also observed which groups to form agglomerates of larger size. The smaller grain sizes were found from XRD suggesting that these complexes are polycrystalline with nanosized grains.

Antimicrobial activity. The *in vitro* biological screening effects of the investigated compounds were tested against some bacterial and fungal species by the disc diffusion method. The results of the antibacterial and antifungal activities are given in Tables 2 and 3. The results show that both the Schiff base ligands have moderate activity in the antibacterial species. Against all organisms, ZnL_1 and ZnL_2 complexes were found to be highly active in the bacterial species of *S. aureus*, *E. coli* and *P. aeruginosa*. However, ZnL_1 has moderate activity in the species of *K. pneunaniae*, while ZnL_2 is highly active in *K. pneunaniae*. Moreover, the results point out that in *P. vulgaris*, ZnL_1 complex is less active and ZnL_2 is moderately active. Again, the comparison of the above results with amikacin, Ofloxacin and Ciprofloxacin antibacterial standards demonstrates that *S. aureus*, *K. pneunaniae*, *P. vulgaris* and *P. aeruginosa* are moderately active. However, the standards amikacin and Ciprofloxacin show higher activity in the *E. coli* species, while Ofloxacin is less active in the *P. aeruginosa* species.

The results on antifungal activity of the ligands show moderate activity in *A. niger*, *R. stolonifer*, *A. flavus*, *R. bataicola* and *C. albicans* species, whereas L_1 ligand with *A. niger* shows less activity as compared to the other fungal species. ZnL_1 complex posses higher activity in *A. niger* and *R. stolonifer* species and *A. flavus*, *R. bataicola* and *C. albicans* show less activity. ZnL_2 with *A. niger*, *A. flavus* and *C. albicans* species shows higher activity com-

Table 2. Antibacterial activity of Schiff base ligands and their complexes

Compound	Gram positive bacteria			Gram negative bacteria	
	S. aureus	*K. pneumaniae*	*P. vulgaris*	*E. coli*	*P. aeruginosa*
$K(L_1)$	++	++	++	++	++
$K(L_2)$	++	++	++	++	++
$[ZnL_1](NO_3)(H_2O)$	+++	++	+	+++	+++
$[ZnL_2(H_2O)](NO_3)$	+++	+++	++	+++	+++
Amikacin[a]	++	++	++	+++	++
Ofloxacin[a]	++	++	++	++	+
Ciprofloxacin[a]	++	++	++	+++	++

Inhibition values = 0.1~0.5 cm beyond control = + (less active); inhibition values = 0.6~1.0 cm beyond control = ++ (moderate active); inhibition values = 1.1~1.5 cm beyond control = +++ (highly active).
[a]Standards.

Table 3. Antifungal activity of Schiff base ligands and their complexes

Compound	*A. niger*	*R. stolonifer*	*A. flavus*	*R. bataicola*	*C. albicans*
$K(L_1)$	+	++	++	++	++
$K(L_2)$	++	++	++	++	++
$[ZnL_1](H_2O)(NO_3)$	+++	+++	++	++	++
$[ZnL_2(H_2O)](NO_3)$	+++	++	+++	++	+++
Nystatin[a]	++	+++	++	++	+++

Inhibition values = 0.1~0.5 cm beyond control = + (less active); inhibition values = 0.6~1.0 cm beyond control = ++ (moderate active); inhibition values = 1.1~1.5 cm beyond control = +++ (highly active).
[a]Standards.

pared to those with *R. stolonifer* and *R. bataicola*. The results on antifungal standard nystatin indicate that *R. stolonifer* and *C. albicans* are more active than *A. niger*, *A. flavus* and *R. bataicola*.

Zn(II) ions are adsorbed on the surface of the cell wall of microorganisms and disturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organisms. So, Zn(II) ions are essential for the growth-inhibitor effect. Such increased activity of the complexes can be explained on the basis of Overtone's concept (Anjaneyula *et al.*, 1986) and Tweedy's Chelation theory (Dharmaraj *et al.*, 2001). According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid soluble materials due to which liposolubility is an important factor, which controls the antifungal activity. On chelation, the polarity of Zn(II) ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the zinc ion with donor groups. Further, it increases the delocalization π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and restricts further multiplicity of the microorganisms. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells.

Mode of action. Although the exact mechanism is not understood biochemically, mode of action of antimicrobials may involve various targets in microorganisms.

(i) Interference with the cell wall synthesis, damage as a result of which cell permeability may be altered (or) they may disorganize the lipoprotein leading to the cell death.

(ii) Deactivate various cellular enzymes, which play a vital role in different metabolic pathways of these microorganisms.

(iii) Denaturation of one or more proteins of the cell, as a result of which the normal cellular processes are impaired.

(iv) Formation of a hydrogen bond through the azomethine group with the active centre of cell constituents, resulting in interference with the normal cell process.

Effect of hetero atoms. From the observation (Tables 2 and 3), the higher inhibition of microbial growth is due to uncoordinated hetero atoms and carboxylic moieties. In the complexes, the ligands (L_1 & L_2) have some uncoordinated donor atoms which enhance the activity of the complexes by bonding with trace elements present in microorganisms. This may combine with the uncoordinated site and inhibit the growth of microorganisms.

Effect of azomethine (>C=N) group. The mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group (>C=N-) with the active centers of cell constituents (Malhota *et al.*, 1993; Mishra *et al.*, 1993) resulting in interferences with the normal cell process.

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