



ANTIMICROBIAL STUDIES ON KNOEVENAGEL CONDENSATE β -DIKETONE COMPLEXES: SYNTHESIS AND CHARACTERIZATION

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ABSTRACT

Few novel Knoevenagel based metal (II) complexes were prepared by using Knoevenagel condensate β -diketone ligand (obtained by the condensation of acetylacetone and cinnamaldehyde) as primary ligand (L_1) and 1,10-phenanthroline as co-ligand (L_2). The proposed structure of these metal chelates have been characterized by elemental analysis, magnetic susceptibility, molar conductance measurements, UV-Vis., FT IR and ¹³NMR spectral studies. These complexes show higher molar conductance values, supporting their electrolytic nature. Spectroscopic and other analytical data of the complexes suggest square planar geometry around the central metal ion. The analytical data of the complexes accord well with the formula $[ML_1L_2]Cl_2$; where M= Cu(II), Co(II), Zn(II) and Ni(II) ions. The antimicrobial activity of synthesized ligand and their complexes are tested against perceptive organisms such as two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and three Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*) and five fungi (*Aspergillus niger*, *Fusarium solani*, *Curvularia lunata*, *Rhizoctonia bataticola* and *Candida albicans*). The complexes exhibit higher antimicrobial activities than free ligand and it has been investigated by Minimum Inhibition Concentration (MIC) method. Among the metal complexes, Cu (II) complex exhibit higher efficacy against all the pathogens.

Keywords: Knoevenagel based metal (II) complexes; Gram-positive bacteria; Gram-negative bacteria; antimicrobial activity; Minimum Inhibition Concentration method

1. INTRODUCTION

Several ligands derived from β -diketones are also known to form metal complexes. These ligands derived from β -diketones have been employed for the preparation of new complexes. The β -diketone ligands are considered as potential ligands due to their enolising ability. β -diketone and its metal complexes have been widely used in diverse areas because of their unique structural features, chemical functionalities, and toughness for light and heat as electroluminescence materials [1]. β -diketone derivatives possess a broad spectrum of biological effects such as anti-inflammatory and antimicrobial activity effects [2,3]. But being incapable of enolisation, the condensates have not perhaps been considered earlier as potential ligands towards transition metal ions. Nowadays, special attention has been paid for the synthesis of effective conjugative and versatile chelating systems with metal ions due to their novel structural features, unusual redox behaviour and relevance to

biological processes [4]. Amongst various systems, the compounds derived from Knoevenagel condensate ligand have gained much interest due to their delocalized π -orbitals, flexible behavior, multifunctional ligand sites etc. In order to form the higher degree of conjugated versatile ligand system, Knoevenagel condensate ligand is designed for the formation of stable complexes. Studies of these structure, spectral and redox properties would be optimum models for the metalloproteins, which are essential to address the structure-redox relationship [5]. Keeping the facts in mind, herein the synthesis and characterization of few metal complexes containing Knoevenagel condensate ligand and 1,10-phenanthroline (heteroleptic ligand complexes) are described. These complexes have been characterized by physicochemical and various spectral techniques. All the synthesized compounds were screened for their *in vitro* antimicrobial activity against various bacterial and fungal strains.

2. EXPERIMENTAL

2.1. Material

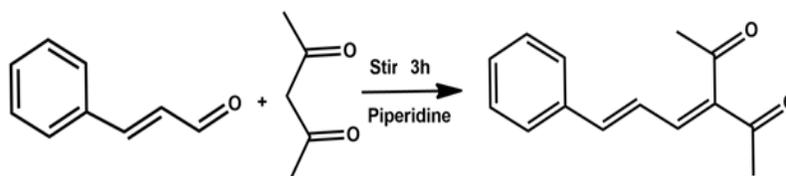
The chemicals involved in this work were of AnalaR grade and were used without further purification. However, the solvents were purified by the standard procedure. Acetylacetone, cinnamaldehyde and 1,10-phenanthroline were SD fine products and used as supplied. All the metal salts were received from E.Merck. Elemental analysis (C, H and N) data were obtained using a Perkin-Elmer 240 elemental analyzer. Vibrational spectra were performed on FT IR–Shimadzu model IR-Affinity-1 spectrophotometer using KBr discs. ^{13}C NMR of ligand and its Zn (II) complex were recorded on a Bruker 400MHz Avance III HD Nanobay NMR spectrometer using DMSO- d_6 as the internal standard. The room temperature molar conductivity of the complexes in DMSO solution (10^{-3} M) was measured using a deep vision 601 model digital conductometer. The absorption spectra were recorded by using Shimadzu

model UV-1601 spectrophotometer at room temperature in DMSO solution.

2.2. Syntheses

2.2.1. Synthesis of Knoevenagel Condensate β -diketone Ligand (L_1)

The Knoevenagel condensate β -diketone ligand (3-(cinnamyl)-pentane-2,4-dione) was synthesized by acetylacetone (1.0 g, 10 mmol) was mixed with cinnamaldehyde (1.3 g, 10mmol) and piperidine (0.05 mL) in ethanol (50 mL). The reaction mixture was stirred thoroughly for a period of 3 h with occasional cooling. Yellow colored crystalline solid was obtained after kept in refrigerator for two days, which was filtered and washed with ethanol followed by an excess of petroleum ether to remove any unreacted reagents. Washing was repeated for three times and the compound was recrystallized from ethanol. Schematic route for the synthesis of (L_1) is given in Scheme 1.



Scheme 1: Schematic route for the synthesis of Knoevenagel condensate β -diketone ligand (L_1)

2.2.2. Synthesis of mixed ligand complexes

The complexes were prepared by mixing the appropriate molar quantity of ligand and the corresponding metal salts using the following procedure. A solution of metal (II) chloride in ethanol (2 mmol) was stirred with an ethanolic solution of cinnamidene-4-aminoantipyrine (L) (2 mmol), for 30 min on a magnetic stirrer at room temperature. To the above stirring solution 1,10-phenanthroline (2 mmol) was added slowly and refluxed for ca 3 h. Then the mixture was reduced to one-third on a water bath and cooled. The solid product formed was filtered and then recrystallized from ethanol and dried in *vacuo*.

3. RESULTS AND DISCUSSION

The Cu (II) and Zn (II) complexes have been synthesized from mixed ligands having cinnamidene-4-aminoantipyrine and 1,10-phenanthroline. They are found to be stable in air. The Knoevenagel condensate ligand (L_1) is soluble in common organic solvents, but the complexes are soluble only in DMF and DMSO. The complexes have been characterized by the microanalytical data, IR and UV-Vis. spectra. Physical characterization,

microanalytical and molar conductance data of the complexes are given in Table 1. The analytical data of the complexes accord well with the formula $[\text{ML}_1\text{L}_2]\text{Cl}_2$; where M= Cu(II), Co(II), Zn(II) and Ni(II). The high conductance of the chelates supports their electrolytic nature.

3.1. FT-IR spectroscopy

The functionalities of free ligands and their coordinating capability to form metal complexes were investigated by FT-IR spectroscopy. The IR spectra for the ligands and metal complexes have been recorded in the region 400-4000 cm^{-1} . The spectrum of L_1 shows absorption band in the region 1652 cm^{-1} , a characteristic feature of the $\nu(\text{C}=\text{O})$ stretching mode. This band is shifted towards lower frequencies in the spectra of metal complexes 1620-1640 cm^{-1} indicating the role of the carbonyl oxygen in coordination with metal ion [6]. The coordination of the carbonyl oxygen was further supported by the appearance of new bands around 510-540 cm^{-1} and 450-490 cm^{-1} which are due to $\nu_{(\text{M}-\text{O})}$ and $\nu_{(\text{M}-\text{N})}$ respectively. These bands were not identified in the spectrum of L_1 [7].

Table 1: Physical characterization, analytical and molar conductance data of the synthesized compounds

Compound	Yield (%)	Color	Calc (Found) %				Formula weight	Λ_m^{\wedge} (ohm ⁻¹ cm ² mol ⁻¹)
			M	C	H	N		
L ₁ [C ₁₄ H ₁₄ O ₂]	78	yellow	-----	78.48 (77.14)	6.59 (5.58)	----	214	-----
[CuL ₁ L ₂]Cl ₂ [C ₂₆ H ₂₂ CuN ₂ O ₂]Cl ₂	70	Green	12.01 (11.58)	59.04 (58.66)	4.19 (4.02)	5.31 (5.11)	528	106
[CoL ₁ L ₂]Cl ₂ [C ₂₆ H ₂₂ CoN ₂ O ₂]Cl ₂	65	Greenish yellow	11.24 (11.02)	59.56 (57.66)	4.23 (4.12)	5.34 (5.11)	524	112
[ZnL ₁ L ₂]Cl ₂ [C ₂₆ H ₂₂ ZnN ₂ O ₂]Cl ₂	72	Bluish green	12.22 (11.54)	58.84 (57.09)	4.18 (4.01)	5.28 (4.99)	528	110
[NiL ₁ L ₂]Cl ₂ [C ₂₆ H ₂₂ NiN ₂ O ₂]Cl ₂	68	Pale green	11.20 (10.54)	59.59 (68.09)	4.24 (4.01)	5.35 (5.29)	524	110

3.2. Electronic Spectroscopy

The geometry of these metal complexes has been deduced from electronic spectral data of the complexes. Electronic spectra of Schiff base ligand (L₁) and its metal (II) complexes were recorded at room temperature in DMSO medium in the range of 200-1100 nm. The absorption spectrum of the L₁ exhibits two characteristic bands at 35,842 and 29,412 cm⁻¹ which correspond to the intraligand charge transfer transitions of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ respectively. These two transitions are shifted due to the complex formation, either bathochromic or hypsochromic shift.

In case of Cu (II) complex, d-d band is observed at 19,145 cm⁻¹, assigned to ${}^2B_{1g} \rightarrow {}^2A_{1g}$ transition, strongly supports the square planar geometry around the Cu(II) ion. Besides, d-d band at 18,856 cm⁻¹ is detected in Co (II) complex, with respect to ${}^1A_{1g} \rightarrow {}^1B_{1g}$ transition, which indicates the square planar geometry. The magnetic moment value (2.65 BM) of this complex for the square planar geometry, reveals that the four unpaired electrons with the low spin four coordinated square planar environment is observed around the Co(II) ion [8] Ni(II) complex shows d-d band at 17,458 cm⁻¹ corresponds to ${}^1A_{1g} \rightarrow {}^1B_{1g}$ transition. The square planar geometry is established by the observed diamagnetism of Ni (II) complex. Similarly, the Zn (II) complex is also being noticed to exhibit diamagnetism, which has not shown any d-d transition in the visible region. Altogether, based on absorption spectra, magnetism and elemental analysis, the stoichiometry of this complex is concluded to reveal tetra coordinate, which could be square planar geometry.

3.3. ¹³C NMR spectra

The NMR spectrum is exploiting to determine the identity of prepared ligand and its diamagnetic metal complexes. The ¹³C NMR spectra of Schiff base ligand (L₁) and its Zn (II) complex have been recorded in DMSO-d₆ using TMS (tetramethylsilane) as the internal standard. The ¹³C NMR spectrum of L₁ shows multi signals within the range of 130-140 ppm (m) and 125 ppm (s), concord to the phenyl multiplet and (-CH=CH) of cinnamaldehyde moiety. Moreover, the carbonyl (C=O) carbons are appeared at 196 ppm. In Zn (II) complex, it is identified that carbonyl peaks are shifted to upfield region, representing the coordination of (C=O) groups towards metal center. There is no such significant difference noticed with other signals in this complex.

Based on the above spectral data, the Knoevenagel condensate mixed ligand metal complex is given in Fig.

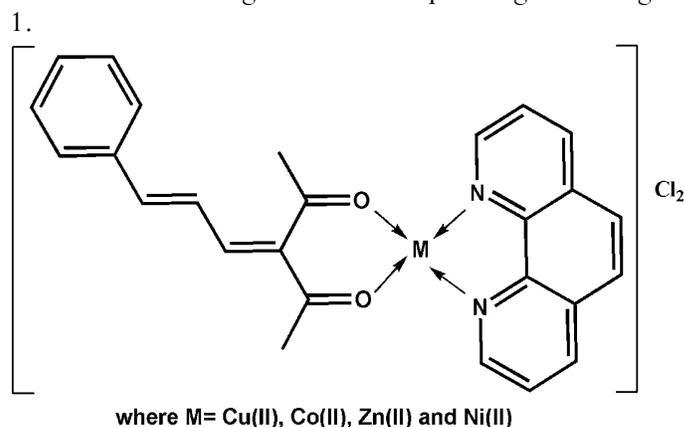


Fig. 1: Proposed structure of the Knoevenagel condensate mixed ligand complex

3.4. Antimicrobial Screening

The *in vitro* antimicrobial activity of the synthesized ligands and its metal complexes was tested against perceptive organisms such as two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and three Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*) and five fungi (*Aspergillus niger*, *Fusarium solani*, *Curvularia lunata*,

Rhizoctonia bataticola and *Candida albicans*) by Minimum Inhibition Concentration (MIC) method. Kanamycin and Fluconazole were used as standards for bacteria and fungi respectively. The minimum inhibitory concentration (MIC) values, measured in antibacterial and antifungal studies of the complexes are given in the following Table 2 and Table 3.

Table 2 Minimum inhibitory concentration of the synthesized ligand (HL) and its complexes against the growth of bacteria (μM)

Compound	Minimum inhibitory concentration ($\times 10^4 \mu\text{M}$)				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. typhi</i>
L_1	25.8	2.4	28.6	26.8	30.5
$[\text{Cu}L_1L_2]\text{Cl}_2$	10.4	10.8	11.5	10.2	12.2
$[\text{Co}L_1L_2]\text{Cl}_2$	12.6	14.5	13.4	14.2	12.8
$[\text{Zn}L_1L_2]\text{Cl}_2$	11.8	10.8	12.8	14.8	12.2
$[\text{Ni}L_1L_2]\text{Cl}_2$	10.8	12.2	11.2	11.5	12.8
^a Kanamycin	2.4	2.2	1.8	2.8	3.2

Table 3: Minimum inhibitory concentration of the synthesized ligand (HL) and its complexes against the growth of bacteria (μM)

Compound	Minimum inhibitory concentration ($\times 10^4 \mu\text{M}$)				
	<i>A. niger</i>	<i>F. solani</i>	<i>C. lunata</i>	<i>R. bataticola</i>	<i>C. albicans</i>
L_1	18.8	16.2	15.8	17.5	16.0
$[\text{Cu}L_1L_2]\text{Cl}_2$	7.8	7.6	7.2	8.4	8.2
$[\text{Co}L_1L_2]\text{Cl}_2$	9.2	8.7	8.6	9.0	7.8
$[\text{Zn}L_1L_2]\text{Cl}_2$	8.6	9.4	8.4	8.8	8.2
$[\text{Ni}L_1L_2]\text{Cl}_2$	7.4	8.3	8.8	8.4	8.2
^b Fluconazole	4.2	4.5	3.2	3.8	4.8

^aKanamycin and ^bFluconazole are used as standard

The ligand and all its complexes have inhibitory action against all microorganisms. The metal complexes exhibit higher inhibition against all microorganisms tested compared to the free ligand. The rate of antimicrobial activity of the metal complexes depends on the following five principal factors [9, 10]. They are (i) the chelate effect, (ii) the nature of coordinated ligands, (iii) the total charge of the complex, (iv) the nature of the ion neutralizing the ionic complex and (v) the nuclearity of the metal center in the complex. The increased activity of the metal chelates than the bare ligand can also be explained on the basis of chelation theory [11]. This theory states that chelation reduces the polarity of the metal ion by the partial sharing of its positive charge with donor groups and possible π electron delocalization over

the whole ring. This results in increasing lipophilic character of the complex and favors the penetration of the complex through the lipid layer of cell membrane. The complex may block the binding sites of microorganisms; consequently it disturbs the metabolism pathways and respiration process in the cell, and thus blocks the synthesis of proteins, which restricts further growth of the organism and resulting in the extinction of microorganisms.

4. CONCLUSION

A Knoevenagel condensate ligand has been prepared by the condensation of cinnamaldehyde and acetylacetone. Its mixed ligand complexes of Cu (II), Co (II), Zn (II) and Ni (II) have been synthesized and characterized by micro

analytical data, IR, UV-Vis. and NMR spectra. The data show that they have composition of the type $[ML_1L_2]Cl_2$ where M= Cu (II), Co (II), Zn (II) and Ni(II). The spectral data of the complexes suggest square planar geometry around the central metal ion. They show good electrical conductance which reveals that these chelates are electrolytes. The minimum inhibitory concentration (MIC) values of the compounds against the growth of microorganisms are interesting to observe that in all the cases, the synthesized complexes have higher antimicrobial activity than the free ligand.

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6. REFERENCES

1. Arom G, Gamez P, Reedijk J. *Coord. Chem. Rev.*, 2008; **252**:964.
2. Del Campo R, Criado JJ, Garc E. *J. Inorg. Biochem.*, 2002; **89**: 74.
3. Lv J, Liu T, Cai S, Wang X, Liu L, Wang Y. *J. Inorg. Biochem.*, 2006; **100**:1888.
4. Desai PS, Desai ER. *J. Indian Chem. Soc.*, 1993; **70**:177.
5. Jeewoth T, Bhowon MG Wah HKL. *Transit. Met. Chem.*, 1999; **24**:445.
6. Beinert H. *J. Inorg. Biochem.*, 1991; **44**:17.
7. Ballhausen CJ. *Introduction to Ligand Field Theory*, McGraw-Hill, New York, (1962)
8. Lever ABP. *Inorganic Electronic Spectroscopy*, Elsevier, New York, 1968.
9. Dhanaraj CJ, Johnson J. *Spectrochim. Acta A*, 2014; **118**: 624.
10. Sharaby CM, Mohamed GG, Omar MM. *Spectrochim Acta A*, 2007; **66**:935.
11. Uddin MN, Chowdhury DA, Islam MT, Hoque F. *Orbital Electron. J. Chem.*, 2012; **4**: 273.