

# Design, Synthesis, Characterization and Biological Evaluation of a Cu (II)–Cd (II) Heterobimetallic Schiff Base Complex

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**Abstract**—The Schiff base reaction is used in the current study to create the reagent *N, N*-1,2-Phenylenebis (2-Hydroxybenzaldehyde). The reagent is characterized by FT-IR and NMR. This reagent is coupled with Cu (II) to get a monometallic complex. Another monometallic complex has been prepared by using 1-Nitroso-2-Naphthol as a reagent with Cd (II). The combination of these two will give a Hetero-bimetallic complex. This prepared complex is characterized by using FT-IR, powder XRD, and Elemental analysis. The said hetero-bimetallic complex shows better antibacterial, and antifungal activity than the mono-metallic complex.

**Index Terms**—Schiff base, Hetero-bimetallic, Powder XRD, Cu (II)

## I. INTRODUCTION

The chemistry of coordination compounds is an area ranging from the theoretical studies of bonding to the production of organometallic compounds.<sup>[1]</sup>

Bimetallic complexes are crucial objectives for numerous applications due to their advantageous functional characteristics. For example, two metallic centers can enable collaborative multi-electron reactions involving transition metal ions. The promise of bimetallic complexes in augmenting reactivity instills optimism and enthusiasm among scholars in the discipline.<sup>[2,3]</sup>

The formulation of a new Schiff base mixed ligand was likely the most pivotal phase in the creation of metal complexes that demonstrate distinctive characteristics and innovative reactivity. The characteristics of the ligand as an electron donor and acceptor, its structural-functional groups, and its placement within the coordination sphere, along with the reactivity of coordination compounds, could influence several research.<sup>[4]</sup>

The ligands in the bimetallic complexes are polydentate and can bind to a minimum of two metals. In contrast to mono-metallic complexes, bimetallic complexes have an accelerated reaction rate and enhanced selectivity. Bimetallic complexes represent a rapidly expanding domain due to their potential bioactivities. Bimetallic complexes may be homobimetallic, featuring two identical metal centres, or heterobimetallic, comprising different metal centres. Bimetallic complexes offer a unique array of characteristics that can be ascribed to the presence of two identical or distinct metals. The distinct central metals in heterobimetallic complexes may collectively contribute qualities from both metals, hence improving the complex's characteristics relative to homobimetallic complexes.<sup>[5]</sup> The metallic centres of the bimetallic complexes often collaborate by altering the reactivity and/or physical characteristics of the complexes involved. Each metal can individually or together influence the overall characteristics of the complexes.<sup>[6]</sup> Heterobimetallic complexes can be synthesized through the sequential introduction of several metal ions.<sup>[7-9]</sup>

The therapeutic characteristics of metal complexes are contingent upon the attributes of both the metal and the ligand. In some instances, the incorporation of metal ions into an organic ligand might enhance its biological efficiency. A review of the literature indicates that numerous bimetallic compounds have been developed, with investigations into their applicability across various domains, including agriculture<sup>[11]</sup>, industry<sup>[12]</sup>, and medicine<sup>[13]</sup>.

The synthesis, characterization, and investigation of the antimicrobial properties of homometallic complexes with transition metals such as Cu, Co, Mn, and Zn have been the subject of more research papers

in recent years <sup>[14]</sup>. The bimetallic complexes of Ru(II)-Fe(III) have been utilized for the detection of signal amplification and the degradation of oxalate, an industrial pollutant. <sup>[15]</sup> The electronic effect of aluminum-containing ligands in Ir-Al and Rh-Al was also studied in several hetero-bimetallic complexes. <sup>[16]</sup> It was discovered that copper complexes in oxidation state +2 had strong antioxidant and anti-free radical action. <sup>[17,18]</sup> Bimetallic copper complexes have been thoroughly researched and may serve as models for a number of significant biological systems with a few sites. <sup>[19–25]</sup> Coordination chemistry is further advanced by the synthesis of a novel copper (II) complex with a multidentate Schiff base ligand. <sup>[26–28]</sup>

The major ligand used in this work is *N*, *N*-1,2-phenylenebis (2-hydroxybenzaldehyde), which is produced by the Schiff base reaction. When this reagent and Cu (II) combine, a monometallic complex is produced. The heterobimetallic complex is produced by coupling it with another Ag (I) monometallic complex (1-nitroso-2-naphthol). Compared to the monometallic complex, the heterobimetallic complex has more promising biological activities.

To the best of our knowledge, the Cu (II)-Ag (I) bimetallic combination utilizing the reagents (*N*, *N*-1,2-phenylenebis (2-hydroxybenzaldehyde)) and (1-nitroso-2-naphthol) has not been documented thus far.

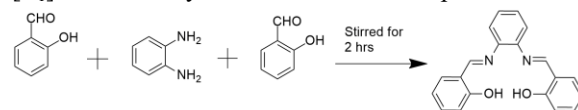
### Experimental

All chemicals are purchased from commercial sources and used without further purification. *O*-phenylenediamine, Salicylaldehyde, copper chloride, cadmium chloride, and 1-nitroso-2-naphthol were purchased from Sigma Aldrich. Infrared spectral studies were recorded in the KBr phase on a Shimadzu 8201 FTIR spectrophotometer. Elemental analyses for C, H, and N were performed on a Perkin Elmer 240 C elemental analyser.

#### A) Synthesis of [*N*, *N*-1,2-Phenylenebis (2-Hydroxybenzaldehyde)]

An ethanolic solution of *O*-phenylenediamine was stirred with Salicylaldehyde to obtain the final product

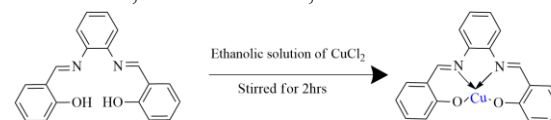
[L<sub>1</sub>]. L<sub>1</sub> is recrystallized with the help of ethanol.



Scheme I : Synthesis of [*N*, *N*-1,2-Phenylenebis (2-Hydroxybenzaldehyde)]

#### B) Synthesis of [*N*, *N*-1,2-Phenylenebis (2-Hydroxybenzaldehyde)] Cu (II) Complex

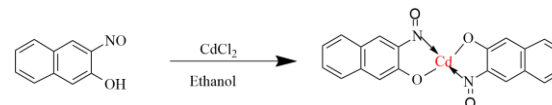
An ethanolic solution of copper chloride was added to an ethanolic solution of (L<sub>1</sub>) while stirring. A green precipitate formed upon stirring at room temperature. Stirring continued for one hour to ensure the reaction was complete. The product was filtered, washed with hot ethanol, then cold water, and dried.



Scheme II : Synthesis of [*N*, *N*-1,2-Phenylenebis (2-Hydroxybenzaldehyde)] Cu (II) Complex

#### C) Synthesis of 1-Nitroso-2-Naphthol Cd (II) complex

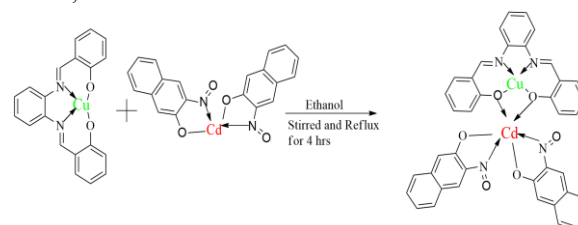
The hot ethanolic solution of (L<sub>2</sub>) was added to the ethanolic solution of Cadmium Chloride with stirring. The red precipitate (L<sub>2</sub>M<sub>2</sub>) was formed upon stirring at room temperature. Stirring was continued for one hour to ensure the reaction was completed. The product was filtered off, washed with hot ethanol, followed by cold water, and then dried.



Scheme III: Synthesis of C<sub>20</sub>H<sub>10</sub>CdN<sub>2</sub>O<sub>4</sub> Complex

#### D) Synthesis of Heterobimetallic Cu(II)-Cd(II) Complex

The ethanolic solution of (L<sub>1</sub>M<sub>1</sub>) was added to an ethanolic solution of (L<sub>2</sub>M<sub>2</sub>) with stirring. This mixture was refluxed for 4 hours. The product was filtered off, washed with hot ethanol, followed by water, and dried.



Scheme IV: Synthesis of C<sub>40</sub>H<sub>26</sub>CuCdN<sub>4</sub>O<sub>6</sub> Complex

## II. RESULT AND DISCUSSION

## IR Spectrum

The infrared spectra for the metal complex ligand  $C_{20}H_{12}CuN_2O_2$  were recorded. A spectral band at  $1274.23\text{ cm}^{-1}$  originated, which is attributed to phenolic C–O str. Band. When the ligand  $C_{20}H_{12}CuN_2O_2$  formed heterobimetallic complexes with the Cu (II) metal chelates of 1-Nitroso-2-naphthol, shifting and splitting of the phenolic C–O str. band was observed. Therefore, in  $C_{40}H_{26}CuCdN_4O_6$ , spectral bands emerge at 1274 and  $1230\text{ cm}^{-1}$ . The consequent shifting and splitting of phenolic C–O str. bands in the heterobimetallic complexes justifies the coordination through phenolic oxygen.

The FT-IR spectra for the metal complex ligands  $C_{20}H_{12}CuN_2O_2$  and  $C_{20}H_6NO_2Ag$  arise at  $435.11$  and  $440.21\text{ cm}^{-1}$  due to M–O(str) modes, respectively. There is a shift in the position of these bands towards the higher energy side after the formation of heterobimetallic complexes, which is observed at  $570.45\text{ cm}^{-1}$

|                            | $C_{20}H_{12}CuN_2O_2$ | $C_{20}H_{12}N_2O_4Cd$ | $C_{40}H_{26}CuCdN_4O_6$ |
|----------------------------|------------------------|------------------------|--------------------------|
| $\nu(\text{ArC}=\text{C})$ | 1434.42                | 1405.47                | 1445.89                  |
| $\nu(\text{ArC}-\text{H})$ | 908.12                 | 971.74                 | 3053.10                  |
| $\nu(\text{CH}=\text{N})$  | 1608.53                | -----<br>--            | 1605.93                  |
| $\nu(\text{N}=\text{O})$   | -----                  | 1342.96                | 1304.12                  |
| $\nu(\text{C}-\text{O})$   | 1274.23                | 1230.12                | 1025.02                  |
| $\nu(\text{M}-\text{O})$   | 435.11                 | 440.21                 | 570.45                   |
| $\nu(\text{M}-\text{N})$   | 525.14                 | -----                  | 542.31                   |
| $\nu(\text{M}-\text{Cl})$  | 482.14                 | 465.31                 | -----                    |

## NMR Spectroscopy:

The  $^1\text{H}$  NMR spectra of the Schiff base ligands demonstrated characteristic azomethine ( $\text{CH}=\text{N}$ ) protons at 4.89 ppm as a singlet, respectively. The ( $\text{CH}_2$ ) protons present in all the ligands were observed at 3.05–3.98 ppm as a singlet.

## Physical parameters of the Metal complexes

| Complex                          |         | $C_{20}H_{12}CuN_2O_2$ | $C_{20}H_{12}N_2O_4Cd$ | $C_{40}H_{26}CuCdN_4O_6$ |
|----------------------------------|---------|------------------------|------------------------|--------------------------|
| Molecular Weight                 |         | 375.54                 | 456.41                 | 833.95                   |
| Colour                           |         | Dark green             | Red                    | Brown                    |
| Analysis (%), Found (Calculated) | C       | 63.75 (63.90)          | 52.24 (52.62)          | 54.31 (57.55)            |
|                                  | H       | 3.01 (3.19)            | 2.21 (2.62)            | 2.92 (3.11)              |
|                                  | N       | 7.05 (7.45)            | 5.04 (5.25)            | 6.24 (6.71)              |
|                                  | Cu (II) | 16.12 (16.91)          | -----                  | 7.21 (7.61)              |
|                                  | Cd (II) | -----                  | 24.43 (24.62)          | 13.12 (13.47)            |
| Yield (%)                        |         |                        |                        |                          |

## Powder XRD

The powder XRD pattern of the Cu-Cd bimetallic complex recorded in the range ( $2\theta = 0-80^\circ$ ). The XRD pattern of the bimetallic complex shows the sharp crystalline peaks indicating its crystalline phase. The average crystallite size (dXRD) of the complex was calculated using Scherer's formula. The bimetallic complex has an average crystallite size of 8.5 nm.

## Biological activity

Procedure: Antibacterial activity against *E. coli*, *Bacillus subtilis*, *S. aureus*, and *P. aeruginosa* bacteria by the well diffusion method. The inoculums of the microorganism were prepared from the bacterial cultures. 15 ml of nutrient agar (Hi Media) medium was poured into clean, sterilized Petri plates and allowed to cool and solidify. 100  $\mu\text{l}$  of broth of the bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly. Once the agar was hardened, the Sample Slides were placed on the plate, and the plates were incubated at  $37^\circ\text{C}$  for 24 hours. Antibacterial activity was evaluated by measuring the diameters of the zones of inhibition (ZI).<sup>[29]</sup>

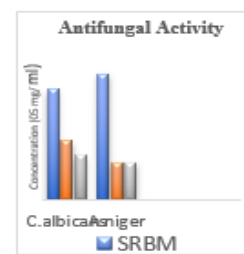
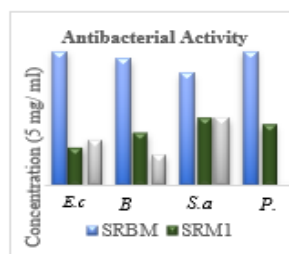
Antibacterial Activity of samples against *E. coli*, *Bacillus subtilis*, *S. Aureus*, *P. aeruginosa*,

| SAMPLES                                            | $C_{40}H_{26}Cu$<br>$CdN_4O_6$<br>Complex<br>(SRBM) | $C_{20}H_{12}Cu$<br>$uN_2O_2$<br>(SM <sub>1</sub> ) | $C_{20}H_{12}N$<br>$_2O_4Cd$<br>(SM <sub>2</sub> ) |
|----------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------|----------------------------------------------------|
| CONCENTRATION (mg/ml)                              | 5 mg                                                | 5 mg                                                | 5 mg                                               |
| ZONE IN DIAMETER (mm) <i>E. coli</i>               | 18                                                  | 05                                                  | 06                                                 |
| ZONE IN DIAMETER (mm) <i>Bacillus subtilis</i>     | 17                                                  | 07                                                  | 04                                                 |
| ZONE IN DIAMETER (mm) <i>Staphylococcus Aureus</i> | 15                                                  | 09                                                  | 09                                                 |
| ZONE IN DIAMETER (mm) <i>Pseudomonas</i>           | 18                                                  | 08                                                  | 07                                                 |

Procedure: Antifungal activity against *Candida Albicans*, *A. Niger* & *A. Barsiliniase* (Agar well plate diffusion Method) WELL DIFFUSION METHOD FOR THE DETERMINATION OF ZONE OF INHIBITION Antifungal activity Stock solution for antifungal activity: For antifungal study sample concentration of 5mg and 10 mg stored in a refrigerator till further used. Antifungal activities of the sample were evaluated using agar well diffusion assay. The assay was carried out according to the method of (Hufford et al., 1975). Sabouraud dextrose agar (Hi Media) was used for the growth of the fungus. Media with acidic pH (pH 5.5 to 5.6) containing a relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose and distilled water and autoclaved at 121°C for 15 minutes. Twenty-five ml of molten (45°C) SDA medium was aseptically transferred into each 100mm×15mm sterile

Petri dish. For counting of spores (fungi) were counted by suspending them in normal saline to make the volume up to 1ml and then counted with the help of a hemocytometer (Neubauer chamber). Once the agar was hardened, 6mm wells were bored using a sterile cork borer. Then 0.1 mL (100µl) from each stock solution of the sample, having a final concentration of 5 mg and 10mg, was placed in each well, and the plates were incubated for 72 hours at 29 °C. The antifungal activity was measured as the diameter (mm) of the clear zone of growth inhibition. [30,31,32]

| SAMPLES                                | Concentration | ZONE IN DIAMETER (mm) against <i>Candida albicans</i> | ZONE IN DIAMETER (mm) against <i>A. Niger</i> |
|----------------------------------------|---------------|-------------------------------------------------------|-----------------------------------------------|
| $C_{30}H_{18}CuCd$<br>$N_3O_4$ Complex | 5 mg          | 15                                                    | 17                                            |
| $C_{20}H_{12}CuN_2$<br>$O_2$ (SRM1)    | 5 mg          | 08                                                    | 05                                            |
| $C_{10}H_6NO_2Cd$ (SRM2)               | 5 mg          | 06                                                    | 09                                            |



### III. CONCLUSION

The synthesized Schiff's base was characterized by TLC, FT-IR. The downward shift of the band in complexes indicates that the  $\nu$  (CH=N) and  $\nu$  (N=O) groups of the Ligand are coordinated to the metal. Biological studies of these complexes reveal that these complexes show antibacterial activity against *E. coli*, *Bacillus subtilis*, *Staphylococcus Aureus* and *Pseudomonas* as well as antifungal activity against *Candida albicans*, *A. Niger*. As compared to monometallic complexes, a bimetallic complex shows promising biological activities. All metal complexes are coloured and thermally stable solids.

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# REFERENCES

- [1] Inorganic-Chemistry-by-James-E-House, ISBN 978-0-12-356786-4.
- [2] D. G. McCollum, G. P. A. Yap, L. Liable Sands and A. L. Rheingold, *Inorg. Chem.*, 1997, 36, 2230–2235.
- [3] C. Incarvito, A. L. Rheingold, A. L. Gavrilova, C. J. Qin and B. Bosnich, *Inorg. Chem.*, 2001, 40, 4101–4108.
- [4] Synthesis, Spectroscopic Characterization and Biological Evaluation Studies of Mixed ligand Schiff base with Metal (II) Complexes Derived from o-phenylenediamine Ekamparam Akila, Markandan Usharani, Sampath Vimala, and Rangappan Rajavel\*
- [5] Brewer KJ, Swavey S, Williams RL, et al. designing mixed-metal supramolecular complexes. *Complex Adapt Struct* 2001; 53–64.
- [6] Iqbal H, Ali S, Shahzadi S, et al. Synthesis and characterization of hetero-bimetallic complexes with 2-mercapto-5-methyl-benzimidazole: theoretical study and biological activities. *J Coord Chem* 2015; 68: 2434–2448.
- [7] A. S. Borovik, L. Que, V. Papaefthymiou, E. Münck, L. F. Taylor and O. P. Anderson, *J. Am. Chem. Soc.*, 1988, 110, 1986–1988.
- [8] A. S. Borovik, V. Papaefthymiou, L. F. Taylor, O. P. Anderson and L. Que, Jr., *J. Am. Chem. Soc.*, 1989, 111, 6183–6195.
- [9] L. K. Blusch, O. Mitevski, V. MartinDiaconescu, K. Pröpper, S. DeBeer, S. Dechert and F. Meyer, *Inorg. Chem.*, 2014, 53, 7876–7885.
- [10] Synthesis and crystal structure of new monometallic Ni(II) and Co(II) complexes with an asymmetrical aroylhydrazone: effects of the complexes on DNA/protein binding property, molecular docking, and in vitro anticancer activity† Yueqin Li, \*ab Zhiwei Yang, a Minya Zhoua and Yun Lia
- [11] Gisela Manuela de Franca Bettencourt ‘green biosynthesis of single and bimetallic nanoparticles of iron and manganese using bacterial auxin complex to act as plant bio-fertilizer’ biocatalysis and Agricultural Biotechnology. 2020; 30: 101822
- [12] Khyle Glainmer N Quiton, Ming- -ChunLu, Yao-Hui Huang ‘Synthesis and catalyst utilization of bimetallic systems for wastewater remediation’ *Chemosphere*. 2021; 0045-6535: 128371
- [13] Frederic Pelletier ‘Development of bimetallic Titanocene-Ruthenium-Arene Complexes AsAnticancer Agents: Relationship between Structural and Biological Properties’ *J.Med.Chem.*2010; 53: 6923-6933
- [14] M. Pervaiz, M. Yousaf, M.Sagir2, M. Mushtaq2, M.Y.Naz3, S. Ullah, and R.Mushtaq ‘Synthesis and Characterization of Bimetallic Post Transition Complexes for Antimicrobial Activity’*Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-metal Chemistry* (2015) 45,546-552.
- [15] Cheuk- Fai Chow, Pui-Yu-Ho, and Cheng-Bin Gong ‘An Ru(II)-Fe(III) bimetallic complex as a multifunctional device for detecting, signal amplifying and degrading oxalate. *Royal Society of Chemistry, Analyst* 2014, 139, 4256-4263.
- [16] R. Malcolm Charles, III Natalie S. Taylor Angel A. Mercado, Connor E. Frost, Timtohy W.Yokley. William T. Eckenhoff, Nathan D. Schley, Nathan J.DeYonker and Timothy P. Brewster Systematic evaluation of the electronic effect of aluminum-containing ligand in iridium-aluminum and rhodium aluminum bimetallic complexes. *Royal Society of Chemistry* 2020.
- [17] R. M.S.Pereira, N. E. D. Andrades, N. Paulino, et al., “Synthesis and characterization of a metal complex containing naringin and Cu, and its antioxidant, antimicrobial, anti-inflammatory and tumor cell cytotoxicity,” *Molecules*, vol. 12, no. 7, pp. 1352–1366, 2007
- [18] I. B. Afanasev, E. A. Ostrskhovitchm, E. V. M. Chik, C. A. Ibraginova, and L. G. Korkina, “Doestheantitumor cyclopropylpyrroloindole antibiotic CC-1065 cross-link DNA in tumor cells?” *Biochemical Pharmacology*, vol. 61, no. 1, pp. 67–72, 2001.
- [19] M. Mohan, N. K. Gupta, and M. Kumar, “Synthesis, magnetic and electrochemical properties of binuclear copper(II) complexes of

- pyridoxal hydrazones,” *Inorganica Chimica Acta*, vol.197, no. 1, pp. 39–40, 1992.
- [20] S. K. Mandal and K. Nag, “Dinuclear metal complexes. Part 3. Preparation and properties of hydroxo-bridged dicopper(II) complexes,” *Journal of the Chemical Society, Dalton Transactions*, no. 10, pp. 2141–2149, 1984.
- [21] P. K. Coughlin and S. J. Lipard, “A monohydroxobridged, strongly antiferromagnetically coupled dicopper(II) center in a binucleating macrocycle. Comparisons with binuclear coppersites in biology,” *Journal of the American Chemical Society*, vol.103, no. 11, pp. 3228–3228, 1981.
- [22] R. N. Patel, “Magnetic, epr and SOD studies of some CuII-CuII, CuII-NiII and CuII-ZnII imidazolate bridged complexes,” *Spectrochimica Acta Part A*, vol. 59, no. 4, pp. 713–721, 2003.
- [23] R. R. Gagne, R. P. Kreh, and J. A. Dodge, “Unusual structural and reactivity types for copper(I). Synthesis and structural and redox properties of binuclear copper(I) complexes which are probably three coordinate in solution and experience inter molecular metal-metalinteractions in the solid state,” *Journal of the American Chemical Society*, vol. 101, no. 23, pp. 6917–6927, 1979.
- [24] E. I. Solomon, “Electronic and geometric structure-function correlations of the coupled binuclear copper active site,” *Pure and Applied Chemistry*, vol. 55, no. 7, pp. 1069–1088, 1983.
- [25] R. N. Patel, N. Singh, K. K. Shukla, V. L. N. Gundla, and U.K. Chauhan, “Synthesis, spectra and biomimetic properties of copper(II)-copper(II) and copper(II)-zinc(II) binuclear complexes with CuN5 chromophores,” *Spectrochimica Acta Part A*, vol. 61, no. 11-12, pp. 2603–2610, 2005.
- [26] A. S. Gardberg, P. E. Daon, B. M. Hoffman, and J. A. Ibers, “Singly and doubly oxidized phthalocyanine (pc) Rings: [Cu(pc)(ReO4)] and [Cu(pc)(ReO4)2],” *Angewandte Chemie International Edition*, vol. 40, no. 1, pp. 244–246, 2001.
- [27] J. K. Swearingen and D. X. West, “Structural and spectral studies of di-2-pyridyl ketone N(4)-methyl- and N(4) dimethylthiosemicarbazone and their metal complexes,” *Transition Metal Chemistry*, vol. 26, no. 3, pp. 252–260, 2001.
- [28] G. Wilkinson, R. D. Gillard, and J. A. McCleverty, *Comprehensive Coordination Chemistry*, vol. 2, Elsevier, Oxford, UK, 1987.
- [29] M. Varadarasu, S. Mounisamy, V. Kavimani, S. Balu, and D. Quine, “Evaluation of anti inflammatory and membrane stabilizing properties of ethanol extract of *Cassia sophera* J.Gmelin, (Fabaceae),” *Iranian Journal of Pharmacology & Therapeutics*, pp. 235–237, 2007.
- [30] Hufford CD, Funderburk JM, Morgan JM, Robertson LW (1975). Two antimicrobial alkaloids from the heart wood of *liriodendron tulipifera*. *I.J. pharma. Sci.*, 64:789-792.
- [31] Umadevi S, Mohanta G P, Chelladurai V, Manna PK, Manavalan and antifungal R( activity 2003). *Antibacterial of Andrographis echinodes*. *J. Nat. Remedies.*, 3:185-188.
- [32] Saifullah Khan and Gul Majid Khan, *In vitro Antifungal activity of Rhazya stricta*, *Pak. J. Pharm.Sci.*, 2007, Vol.20(4), 274-279