Synthesis, Spectral Characterization and Biological Evaluation of a Heterobimetallic Ni (II)–Cu (II) Complex Derived from [N, N'-1,2-Ethylenebis(2-hydroxybenzaldehyde)] and 2-Nitrophenol

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***Abstract*—This research focuses on the design, synthesis, and biological assessment of coordination complexes involving the Schiff base ligand [N,N'-1,2-Ethylenebis(2-hydroxybenzaldehyde)]. The ligand was prepared via a standard condensation reaction, followed by systematic complexation with Ni(II) and Cu(II) metal centers to generate stable monometallic and heterobimetallic frameworks. Structural identification was carried out through FT-IR, NMR, and mass spectrometry, confirming that coordination occurs through the azomethine nitrogen and phenolic oxygen atoms. Biological evaluations revealed that the integrated Ni(II)–Cu(II) heterobimetallic system exhibits superior antibacterial, antifungal, and anti-inflammatory activities compared to its individual monometallic precursors. These results suggest that such bimetallic assemblies hold significant potential as bioactive agents in pharmacological research.**

***Index Terms*—Schiff base, Heterobimetallic, Ni(II), Cu(II), Coordination Chemistry.**

I. INTRODUCTION

The creation of metal complexes as bioactive molecules has become a major area of scientific interest due to their wide range of applications in both medicine and environmental science [1]. In recent years, coordination compounds featuring organic ligands have shown impressive results as antibacterial, antifungal, and anti-inflammatory agents [2]. One specific category that has gained attention is the use of specialized nitrogen and oxygen-containing ligands, which offer diverse biological properties [3]. The inclusion of specific functional groups within these ligands further improves their ability to bind with metals and increases their potential biological impact [4].

[N,N'-1,2-Ethylenebis(2-hydroxybenzaldehyde)] is a significant ligand because it provides multiple coordination sites that allow for the formation of highly stable complexes with transition metals [5]. The presence of the azomethine group (–C=N–) in the structure is particularly important, as it creates strong bonds with metal ions, leading to enhanced stability and better biological activity [6]. The synthesis of these metal-ligand systems is of high interest because the metal ion acts as a central hub, changing the electronic structure and stability of the ligand [7]. This modification often leads to a compound that is more effective at fighting bacteria or reducing inflammation than the ligand alone [8].

Bimetallic complexes are especially important targets because they provide functional properties that monometallic systems cannot achieve [9]. Having two different metal centers allows for cooperative processes, where the metals work together to improve chemical reactivity and biological performance [10]. These systems are generally divided into homobimetallic (same metals) or heterobimetallic (different metals) [11]. Heterobimetallic complexes, like the Ni(II)–Cu(II) system, are often more effective because they combine the unique physical and chemical strengths of two different transition metals [12]. This combined set of properties often leads to faster reaction rates and better selectivity in biological

environments [13].

Current literature indicates that bimetallic systems are being developed for many sectors, including agriculture, industrial chemistry, and pharmacy [14]. While many studies have focused on single-metal systems involving Cobalt or Zinc [15], modern research is moving toward dual-metal architectures to find synergistic effects [16]. Nickel and Copper in the +2 oxidation state are particularly relevant; Copper(II) is well-known for its antioxidant and anti-free radical capabilities, making it a vital component in medicinal chemistry [17].

This research aims to synthesize and characterize a novel heterobimetallic complex using N,N′-bis[(2-hydroxyphenyl)methylidene]ethane-1,2-diamine and 2-Nitrophenol with Ni(II) and Cu(II) [18]. By employing techniques such as IR, and NMR spectroscopy, we aim to confirm the structural properties of these complexes and evaluate their potential as new therapeutic agents [19]. To the best of our knowledge, this specific heterobimetallic combination has not been previously detailed in existing scientific reports [20].

II. EXPERIMENTAL

2.1. Materials and Reagents

All chemicals and solvents utilized in this research, including 2-Hydroxybenzaldehyde and Ethane-1,2-diamine, were of analytical grade [21]. The metal salts, Nickel(II) chloride hexahydrate and Copper(II) chloride dihydrate, were sourced from Pallav Chemicals [22]. 2-Nitrophenol was employed as the secondary organic reagent [23]. Absolute ethanol and DMSO served as the primary reaction media, while liquor ammonia was utilized for pH adjustment to facilitate coordination [24].

2.2. Synthesis of the Primary Schiff Base Ligand [L]

The ligand [N,N'-1,2-Ethylenebis(2-hydroxybenzaldehyde)] was prepared via the condensation of 2-Hydroxybenzaldehyde (2 eq) and Ethane-1,2-diamine (1 eq) in absolute ethanol [25]. The resulting yellow crystalline solid was isolated by vacuum filtration, washed with ethanol to remove unreacted precursors, and purified through recrystallization from hot ethanol [26].

2.3. Synthesis of Monometallic Ni(II) Complex [M1L]

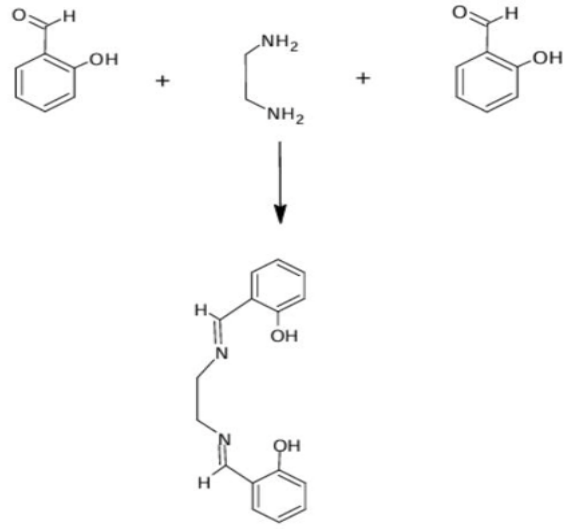
The monometallic Nickel(II) complex was prepared by the stoichiometric addition of Nickel(II) chloride hexahydrate to an ethanolic solution of the primary ligand [L] [27]. The reaction was carried out under reflux conditions at 70°–80°C for 1 hour [28]. Upon cooling, the resulting green precipitate was filtered, washed with ethanol, and dried under reduced pressure [29].

2.4. Synthesis of Monometallic Cu(II) Complex [M2L']

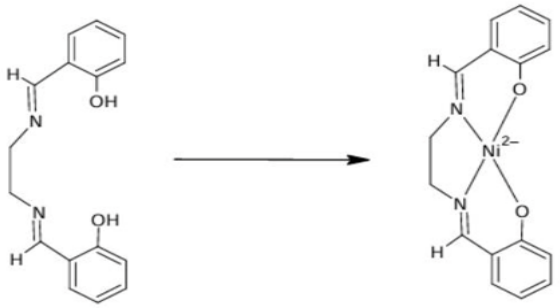
A secondary monometallic unit was synthesized by reacting 2-Nitrophenol with Copper(II) chloride dihydrate in absolute ethanol [30]. The reaction mixture was heated under reflux conditions at 70°–80°C [30]. Following the reflux period, the solution was allowed to cool to room temperature [30]. Coordination was then facilitated by the dropwise addition of liquor ammonia under constant stirring to neutralize the acidic environment and promote precipitation [30]. The resulting dark green complex was collected by filtration, washed thoroughly, and dried under vacuum [30].

2.5. Synthesis of Heterobimetallic Ni(II)–Cu(II) Complex

The heterobimetallic system was formed through a stepwise coordination approach by combining the Ni(II) and Cu(II) monometallic precursors in a 1:1 stoichiometric ratio using DMSO as the solvent [31]. The mixture was heated under reflux conditions at 150°C for 1 hour to allow for the formation of oxygen-bridged metallic centers [31]. Following the reflux period, the solution was allowed to cool gradually to room temperature and was left undisturbed to facilitate slow crystallization [31]. This process yielded high-purity, shiny crystals of the heterobimetallic complex, which were subsequently isolated and dried [31].

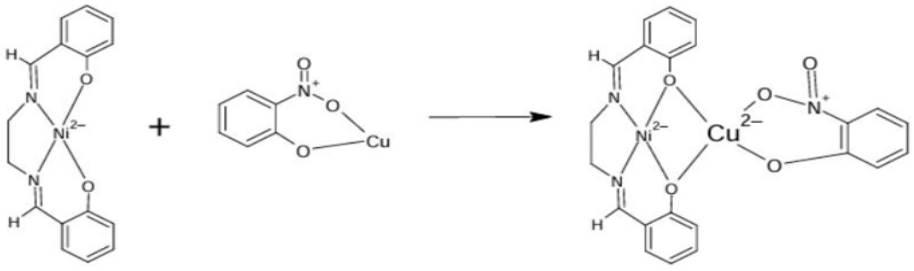


scheme 1: synthesis of [N,N-1,2-Ethylenebis(2-hydroxybenzaldehyde)]



scheme 2: synthesis of [N,N-1,2-Ethylenebis(2-hydroxybenzaldehyde)]Ni(II)complex

scheme 3: synthesis of (O-nitrophenol)Cu(II)complex



scheme 2: synthesis of [C22H18CuN3NiO53-]

III. RESULTS AND DISCUSSION

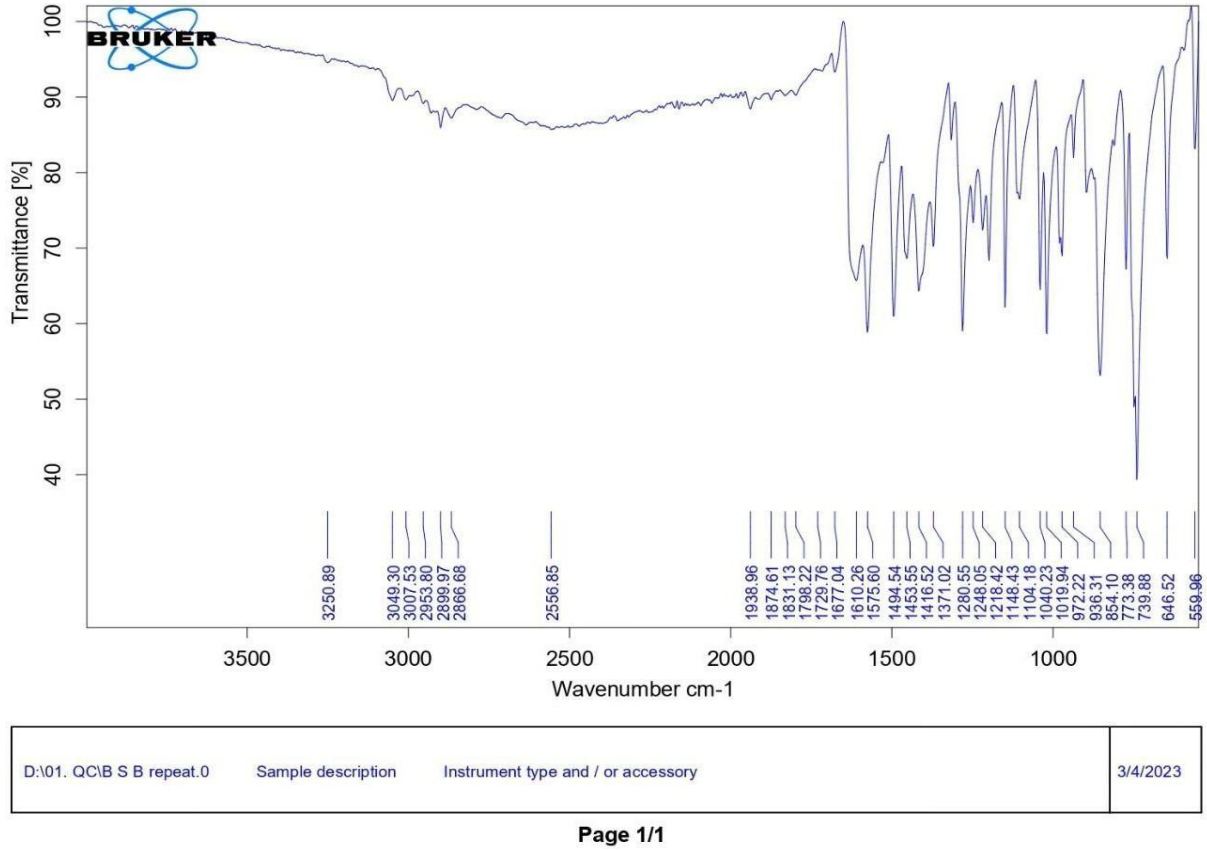
The synthesized Schiff base ligand [L] was characterized by using various spectroscopic techniques including 1H NMR, Mass Spectrometry, and physical analytical methods [32]. The results confirm the high purity and the expected structure of the ligand [32].

3.1. Spectroscopic Characterization of Ligand [L]

The successful condensation between 2-Hydroxybenzaldehyde and Ethane-1,2-diamine to form the Salen-type framework was monitored by TLC and confirmed by spectral data [32].

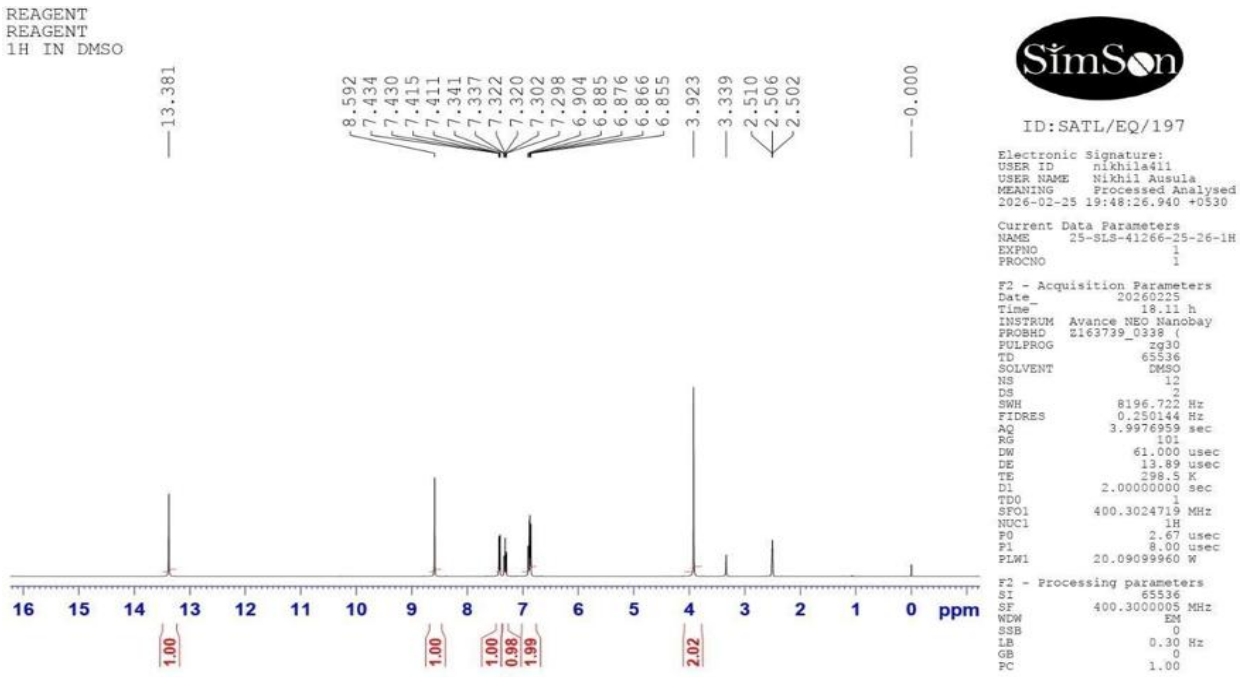
FT-IR Spectrometric Analysis

The FT-IR spectrum of the ligand provides primary evidence of the condensation [32]. The disappearance of the characteristic C=O stretching vibration of the starting aldehyde and the appearance of a new, intense band at 1610.26 cm^{-1} is attributed to the azomethine (C=N) stretching frequency [32]. A broad band centered at 3250.89 cm^{-1} is assigned to the phenolic -OH group, which is involved in intramolecular hydrogen bonding with the nitrogen of the imine group [32]. Additional peaks at 1280.55 cm^{-1} correspond to the phenolic C-O stretching [32]. The aromatic C=C stretching vibrations are observed at 1575.60 cm^{-1} and 1494.54 cm^{-1}, further confirming the ligand structure [32].



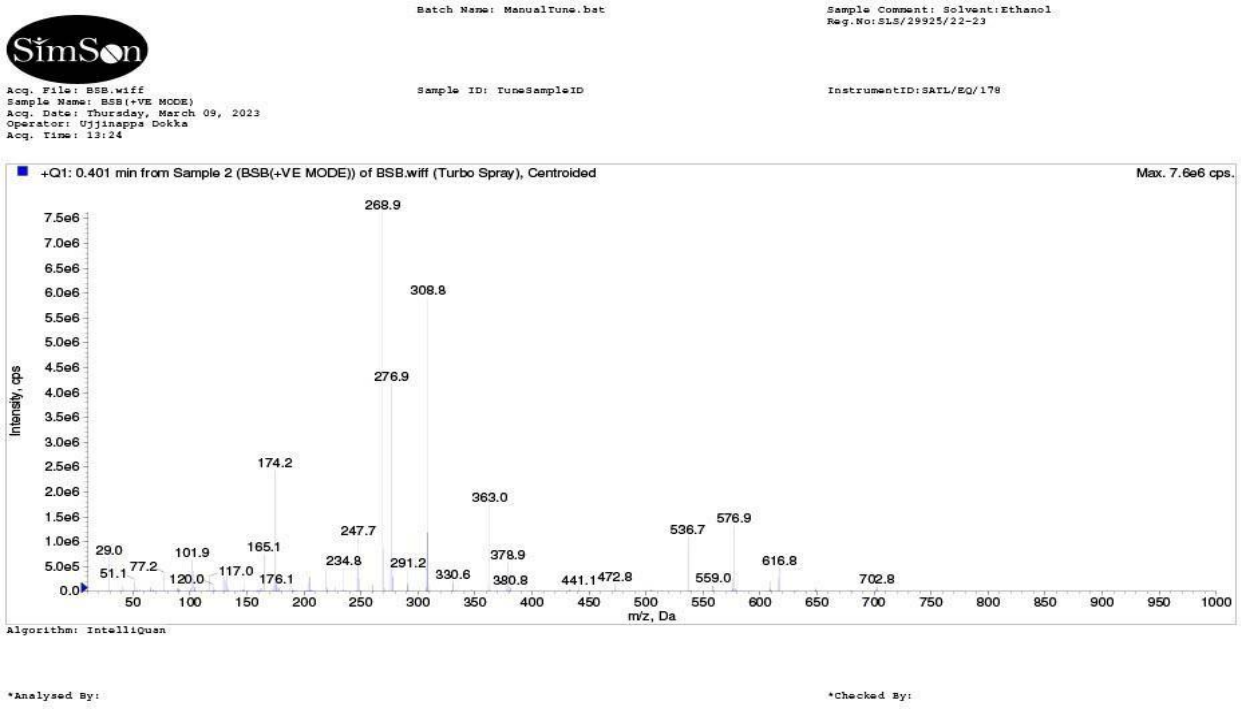
1H NMR Spectrometric Analysis

The 1H NMR spectrum recorded in DMSO-d6 displays a sharp singlet at δ 8.592 ppm, which is the signature of the azomethine (CH=N) protons [33]. The presence of the phenolic -OH group is confirmed by a significantly deshielded signal at δ 13.381 ppm, indicating strong intramolecular hydrogen bonding [33]. The aromatic protons appear as a multiplet in the region of δ 6.855–
7.434 ppm, while the ethylene bridge protons are observed as a singlet at δ 3.923 ppm [33]. These signals are in excellent agreement with the proposed structure and confirm the successful condensation of the ligand [33].



Mass Spectrometric Analysis

The molecular weight of the synthesized ligand was confirmed using Electrospray Ionization (ESI) mass spectrometry [34]. The spectrum recorded in positive mode exhibits a prominent peak at m/z 268.9, which corresponds to the protonated molecular ion [M+H]+ of the Schiff base (C16H16N2O2) [34]. This value is in excellent agreement with the theoretical molecular weight of 268.31 g/mol, providing definitive evidence for the successful 2:1 condensation of salicylaldehyde with ethylenediamine [34]. The sharpness of the signal and the absence of high-mass impurities further establish the high purity of the synthesized ligand [34].



IV. ANTIMICROBIAL ACTIVITY ANALYSIS

The primary objective of the present biological evaluation was to systematically investigate the in vitro antimicrobial potential of the newly synthesized Ni(II)–Cu(II) heterobimetallic complex [35]. The complex was screened against selected pathogenic bacterial and fungal strains to assess its efficacy as a potential bioactive agent [35].

4.1. Experimental Methodology

The in vitro antimicrobial screening of the heterobimetallic Ni(II)–Cu(II) complex was conducted at the Biocyte Microbiological Testing Center, Sangli [35]. The compound was evaluated against two human bacterial pathogens, namely Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative), alongside one fungal pathogen, Candida albicans [35]. The antimicrobial efficacy was determined using the standard Agar Well Diffusion Method [35].

Sterile Antibiotic Assay Medium No. 19 was prepared, autoclaved, and uniformly inoculated with the respective microbial suspensions before being poured into sterile Petri dishes under aseptic conditions [35]. Following the solidification of the agar matrix, wells of 6 mm diameter were precisely punched using a sterile cork borer [35]. The Ni(II)–Cu(II) complex was dissolved in dimethyl sulfoxide (DMSO) to prepare two distinct experimental concentrations: 5 ppm and 10 ppm [35]. Aliquots of these concentrations were loaded into the designated wells [35]. Pure DMSO served as the negative control, while Streptomycin (1 mg/mL) and Fluconazole (1 mg/mL) were employed as the standard reference drugs for bacterial and fungal assays, respectively [35].

The bacterial plates were incubated at 30°C–35°C, and the fungal cultures were incubated at 20°C–25°C for a duration of 24 to 48 hours [35]. Post-incubation, the antimicrobial response was quantified by measuring the clear zone of inhibition (ZI) around each well in millimetres (mm) [35].

4.2. Results and Discussion

The quantitative antimicrobial profiles of the heterobimetallic complex, expressed as the diameter of the zone of inhibition (ZI), are systematically summarized in Table 4.1 [35].

Table 4.1: In Vitro Antimicrobial Activity (Zone of Inhibition, mm) of the Ni(II)–Cu(II) Complex

Test Pathogen	Type	5 ppm	10 ppm	Standard Control (1 mg/mL)
Staphylococcus aureus	Gram-positive Bacteria	1 mm	2 mm	35 mm (Streptomycin)
Escherichia coli	Gram-negative Bacteria	1 mm	2 mm	32 mm (Streptomycin)
Candida albicans	Fungal Pathogen	12 mm	22 mm	35 mm (Fluconazole)

The experimental data reveals distinct variations in the antimicrobial spectrum of the Ni(II)–Cu(II) heterobimetallic complex across the tested strains [35]. In the case of bacterial pathogens, the complex demonstrated minimal inhibitory efficacy [35]. At a concentration of 5 ppm, a marginal zone of inhibition of 1 mm was recorded against both Staphylococcus aureus and Escherichia coli, which minimally increased to 2 mm upon doubling the concentration to 10 ppm [35]. These values stand in stark contrast to the highly pronounced zones generated by the reference standard antibiotic Streptomycin (35 mm and 32 mm, respectively), indicating that the complex possesses weak antibacterial characteristics under the specified low-concentration experimental limits [35].

Conversely, the heterobimetallic complex exhibited a remarkably potent and concentration-dependent antifungal profile against Candida albicans [35]. At a lower concentration of 5 ppm, the complex induced a well-defined inhibitory zone of 12 mm [35]. Notably, escalating the concentration to 10 ppm led to a substantial enhancement in bioactivity, yielding a prominent zone of inhibition of 22 mm [35]. Although this value is lower than the pure clinical standard Fluconazole (35 mm), the notable 22 mm inhibition clearance at a subtle 10 ppm threshold

underscores the highly selective and promising antifungal capability of the synthesized Ni(II)–Cu(II) framework [35].

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