

Synthesis And Comprehensive Characterization of a Palladium (II) Schiff Base Complex Derived from 2'-Hydroxy-3-phenylpropiophenone and Ethylenediamine: Structural Elucidation and Biological Activity Assessment.

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Abstract

A palladium (II) metal complex was synthesized using the Schiff base ligand Derived from condensation of 2'-Hydroxy-3-phenylpropiophenone and Ethylenediamine (EDABIS(3-PP) D). The complex was prepared by reaction of palladium chloride (PdCl₂) with the Schiff base ligand in ethanol as reaction solvent. The synthesized palladium complex was characterized by various analytical and spectroscopic techniques such as ¹H NMR, FT-IR spectroscopy, elemental analysis, mass spectrometry and inductively coupled plasma optical emission spectroscopy (ICP-OES) for the determination of palladium content. The complex was also studied by powder X-ray diffraction (P-XRD) analysis for its structural and crystalline properties. Also, synthesized palladium complex was screened for antibacterial and antifungal activities against selected bacterial and fungal strains. The complex showed good antimicrobial activity, which indicates that coordination of the Schiff base ligand with palladium may improve its biological properties. The results indicate that the prepared palladium (II) complex may be a good candidate for further studies in medicinal chemistry and pharmaceutical applications particularly in the development of new antimicrobial agents. Further studies including a wider range of microorganisms, toxicity evaluation and mechanistic studies are needed to prove its suitability for pharmaceutical applications.

Keywords: palladium chloride, ethanol, Schiff base ligand Derived from 2'-Hydroxy-3-phenylpropiophenone and Ethylenediamine (EDABIS(3-PP) D), antibacterial activity & antifungal activity.

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1. Introduction

Schiff bases are an important class of organic compounds containing an imine or azomethine functional group (–C=N–) which are usually formed by the condensation of primary amines with aldehydes or ketones. Since their introduction by Hugo Schiff, Schiff bases have attracted considerable attention in coordination chemistry due to their relatively facile synthesis, structural flexibility and ability to coordinate with a wide variety of metal ions [1-5]. These compounds can act as mono-, bi- or polydentate ligands and form stable coordination compounds with metal ions due to the azomethine nitrogen and other donor

atoms such as oxygen or nitrogen. The versatile coordination behavior of Schiff-bases and their structural diversity have resulted in their extensive investigation in inorganic, bioinorganic, catalytic, materials and medicinal chemistry [6-8].

Coordination of Schiff-base ligands to transition-metal ions can considerably change the physicochemical and biological properties of the resulting complexes compared to those of the free ligands. Coordination of metal may influence the electronic structure, geometry, stability, lipophilicity and reactivity of the ligand

framework thus providing opportunities for the development of compounds with desired chemical and biological properties [9]. Therefore Schiff-base metal complexes have been extensively studied for many applications such as catalysis, molecular materials, sensing, DNA interactions and biological processes. In particular, their potential antibacterial, antifungal, antioxidant, anticancer, antiviral and other biologically important activities have received significant attention [10-13]. The azomethine functionality is an important structural feature in the design of biologically active compounds and the presence of azomethine functionality is also responsible for Schiff bases antimicrobial potential [14].

Transition metal complexes are of special importance in bioinorganic chemistry, since the inclusion of a metal center into an organic ligand framework can result in chemical properties not necessarily present in the individual ligand or metal salt. The biological behavior of the complex formed can be affected by the nature of the metal ion, its oxidation state, its coordination environment, the ligand donor atoms and the overall molecular geometry. Chelation of a ligand with a metal ion may alter the polarity and lipophilicity of the complex and can consequently affect its interaction with biological membranes and intracellular targets. Such changes have been proposed as important factors contributing to the antimicrobial activity of several Schiff-base metal complexes [15-16]. Moreover, the increasing emergence of microorganisms resistant to traditional antimicrobial agents has driven the search for new antimicrobial classes and promoted the study of metal-based coordination compounds as potential alternative or complementary agents.

Transition metals have been the focus of much attention and palladium is one of the most studied. This stems from its extensive coordination chemistry and ability to form stable complexes with a range of donor atoms, most notably nitrogen, oxygen and Sulphur. Palladium (II) shows a strong tendency to form well-defined coordination compounds with Schiff base ligands having suitable donor sites. Thus, the presence of Pd (II) in a Schiff-base framework can afford complexes with unique structural, electronic and biological characteristics. Palladium-containing Schiff-base complexes have been the subject of research in coordination and medicinal chemistry. Their molecular frameworks and metal–ligand interactions offer a useful basis for the study of biological activity and structure–activity relationship [17-20].

The biological properties of Schiff-base metal complexes depend on several factors like the nature and denticity of the ligand, the metal ion and its oxidation state, the coordination geometry and the electronic environment around the metal center. Therefore, the synthesis of new Schiff-base complexes and their detailed structural and physicochemical characterization is of great importance if one is to establish the relation between the coordination

behavior and biological properties. Complementary information on composition, functional groups, coordination environment and solid-state aspects of such complexes can be obtained by spectroscopic and analytical techniques such as infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectrometry, elemental analysis, metal-content analysis and powder X-ray diffraction.

In this respect, the design of new palladium (II) complexes with suitably functionalized Schiff-base ligands is an attractive field of research for coordination and bioinorganic chemistry. Metal coordination is particularly suitable for Schiff-base ligands with phenolic oxygen and azomethine nitrogen donor sites because they can provide a favorable chelating environment around the metal center [21-23]. The present work therefore concerns the study of a palladium (II) complex obtained from the Schiff-base ligand Derived from 2'-Hydroxy-3-phenylpropiophenone and Ethylenediamine, named EDABIS(3-PP) D. The study is directed toward investigation of the coordination chemistry and structural features of the formed palladium complex and assessment of its possible biological significance thru antibacterial and antifungal studies.

2. Material And Method

material

Reagents and solvents used in the present research work were of analytical grade and were used without further purification. The Schiff base ligand EDABIS(3-PP) D was synthesized in the laboratory by condensation reaction between 2'-Hydroxy-3-phenylpropiophenone and ethylenediamine in ethanol medium. Purification of the synthesized Schiff base was done by recrystallisation from ethanol. 2-Hydroxy-3-phenylpropiophenone, ethylenediamine and ethanol were purchased from Sigma-Aldrich, St. Louis, MO, USA. Palladium chloride (PdCl₂) was purchased from Vinith Precursors Pvt. Ltd. All chemicals were used as received without further purification unless otherwise stated.

Instruments

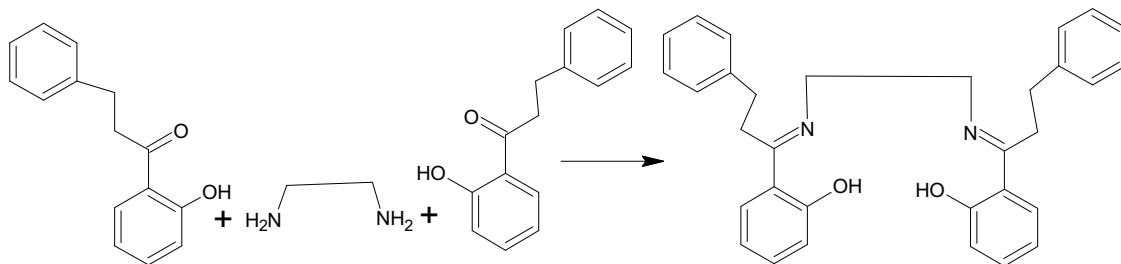
The melting point of the synthesized Schiff base ligand was measured using a Stuart SMP20 melting point apparatus. The FT-IR spectra of synthesized Schiff base ligand and its metal complexes were recorded at 400-4000 cm⁻¹ using 8400 FT-IR Shimadzu spectrometers. The samples were prepared as KBr pellets. The ¹H NMR spectra were recorded on Bruker model no ACEND-EVO-400 NMR spectrometer operating at 400 MHz's. The synthesized compounds were dissolved in DMSO-d₆ and analyzed by NMR measurements under standard instrumental conditions. For metal determination used ICP OES. Thermo Fisher Scientific FLASH EA 1112 Series Elemental Analyzer for elemental analysis.

Preparation of a Schiff Base Ligand through the Condensation of 2'-Hydroxy-3-

phenylpropioiphenone with Ethylenediamine [EDABIS(3-PP) D]

In a round bottom flask, an appropriate amount of 2-hydroxy-3-phenylpropioiphenone was charged and fresh ethanol was added as a reaction solvent. The reaction mixture was heated to 80 °C and a pre-dissolved solution of ethylenediamine in ethanol was added slowly to the ethanolic solution of 2-hydroxy-3-phenylpropioiphenone during 1.0 h maintaining the

temperature at 80 °C. The reaction mixture was stirred continuously at 80 °C for 4.0 h after complete addition to promote the formation of the Schiff base ligand. The reaction mixture was allowed to crystallize at room temperature overnight. The yellow crystals were collected and recrystallized from hot ethanol. The purified Schiff base ligand was then dried and characterized with various spectroscopic and analytical techniques with M.P 141-144°C. [24].

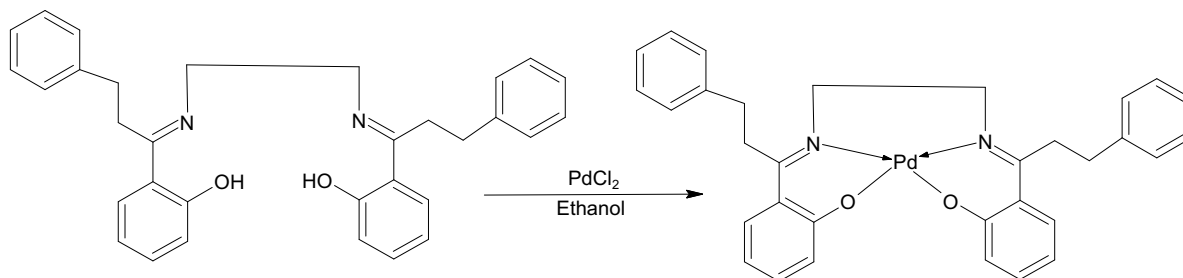


Scheme 01. Preparation Schiff base compound [EDABIS(3-PP) D]

Synthesis of a Palladium (II) Schiff Base Complex Derived from 2-Hydroxy-3-phenylpropioiphenone and Ethylenediamine. [EDABIS(3-PP) D]- Pd

For the synthesis of palladium (II) metal complex, 2.0 g of Schiff base ligand Derived from Condensation 2'-Hydroxy-3-phenylpropioiphenone and Ethylenediamine (EDABIS(3-PP) D) was charged into a round-bottom flask and heated to 80 °C. Then, a solution of 0.78 g of palladium chloride (PdCl₂) was added slowly to the Schiff base reaction mixture at 80 °C. The formation of the palladium complex was evidenced by the formation of the brown precipitate after about 5–10 min. The reaction mixture was kept at

80 °C until the formation of precipitate was complete and the Schiff base was converted to corresponding palladium complex. Upon completion of the reaction, the reaction mass was cooled to room temperature and then cooled to 10 °C to facilitate complete precipitation of the product. The precipitated solid was filtered and washed repeatedly with fresh ethanol to remove unreacted Schiff base and other soluble impurities. The product obtained was dried to give approximately 1.8 g of the palladium complex (74% yield). Different spectroscopic and analytical techniques characterized the synthesized Palladium (II) complex.



Scheme 02. Preparation of Metal complex of Palladium [EDABIS(3-PP) D]- Pd



Fig .01 Dry solid of [EDABIS(3-PP) D]- Pd

Results And Discussion InfraRed Spectroscopy

The FT-IR spectra of the free ligand and its metal complex (L1M1) were used to study the coordination behavior of

the Schiff base ligand (L1) to the Palladium (II) ion.

FT-IR spectra of [EDABIS(3-PP) D]- Pd (II) were assigned to bands at (1456), (3055) & (1416) These can be assigned to (ν Ar C=C), (ν Ar C-H) & (ν C-O) respectively. considerable changes were observed in the FT-IR spectrum. The azomethine ν (C=N) and ν (CH₂-N) stretching bands shifted to lower frequencies suggesting the involvement of the azomethine nitrogen atom in coordination with the metal ion. Moreover, the characteristic phenolic O-H band was absent in the spectrum of the complex indicating that the hydroxyl

group was deprotonated and coordinated via the phenolic oxygen atom. The ν (C-O) stretching vibration of phenolic group was shifted from 1455 cm⁻¹ in the free ligand to 1416 cm⁻¹ in the pallidum complex complex as another evidence of the metal-oxygen bond formation. Besides, two new absorption bands appearing at 510–600cm⁻¹ and 400–460cm⁻¹ regions could be assigned to ν (M-O) and ν (M-N) vibrations, respectively. The spectral changes observed are consistent with the coordination of the Schiff base ligand to the Pd (II) ion thru the azomethine nitrogen and the deprotonated phenolic oxygen atoms leading to the formation of a stable metal complex.

Table 1. IR Interpretation [EDABIS(3-PP) D]- Pd (II)

Complex	ν Ar C=C	ν Ar C-H	ν C-O	ν M-O	ν M-N	ν O-H
[EDABIS(3-PP) D]	1405 (s)	3010 (m)	1455 (s)	-	-	3250 (m)
[EDABIS(3-PP) D]- Pd (II)	1456 (S)	3055 (S)	1416 (S)	510-600 (S)	400-460 (S)	-

NMR

The Pd complex NMR recorded on make BRUKER model no ACEND-EVO-400

¹H NMR (400 MHz, DMSO-d₆, δ ppm): δ 7.76 (d, J $\approx$ 8 Hz, 1H), 7.36–7.27 (m, Ar-H), 7.25 (d, J $\approx$ 8 Hz, Ar-H 2H), 4.88–3.27 (t, Ar-CH₂ 4H), 2.67–2.53 (t, Ar-CH₂-CH₂ 4H), 2.50-2.33 (t, N=CH₂-CH₂, 4H). The ¹H NMR spectrum of the synthesized compound recorded in DMSO-d₆ exhibited characteristic resonances consistent with the proposed molecular structure. The aromatic protons appeared in the region of δ 7.76–7.25 ppm, with a doublet at δ 7.76 ppm and multiple signals in the δ 7.36–7.27 ppm region. The resonance at δ 4.88–3.27 ppm was assigned to the protons of the aliphatic methylene groups adjacent to aromatic environments. The methylene protons further appeared as a triplet at δ 2.67–2.53 ppm, while the triplet at δ 2.50-2.33 ppm was attributed to the N-CH₂ protons.

Mass spectroscopy

For liquid chromatography-mass spectrometry (LC-MS) analysis of the synthesized compound, an Agilent Technologies instrument (Model G6545B) was used. The mass spectrum revealed a clear molecular ion/adduct peak at the closely predicted molecular mass of the compound prepared. The observed mass to charge ratio (m/z) was in good agreement with the calculated molecular mass thus supporting the proposed molecular composition of the compound. The fragmentation pattern in the LC-MS spectrum showed unique and characteristic fragment ions that can be attributed to the cleavage of particular structural moieties with in the molecule. Molecular element composition is Composition: C (66.15%) H (5.20%) N (4.82%) O (5.51%) Pd (18.32%) for this molecular weight is 581.02.

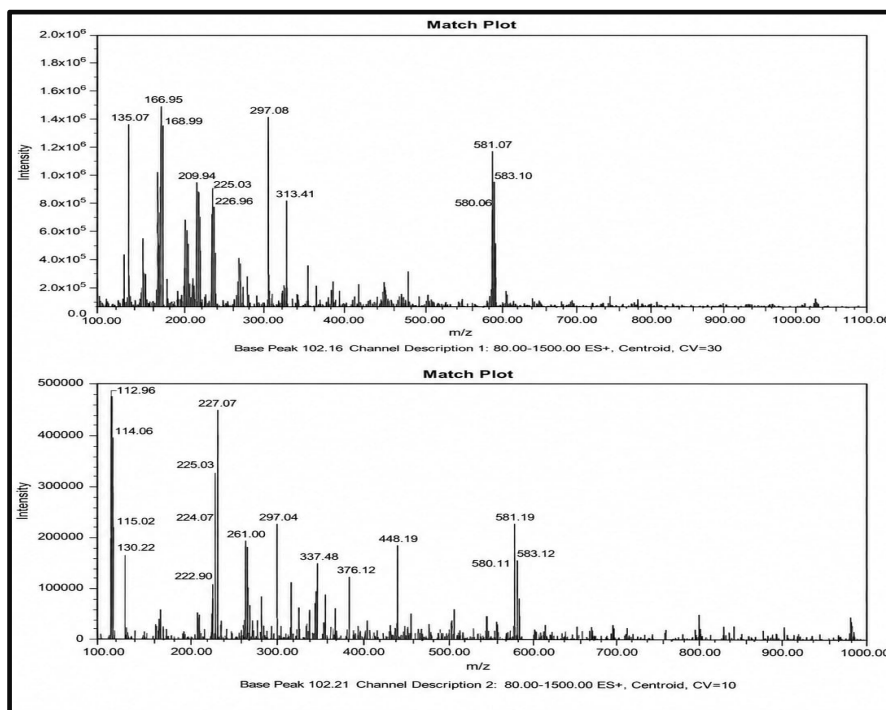


Fig .2 Mass fragmentation of [EDABIS(3-PP) D]- Pd

Powder X-Ray Diffraction (PXRD)

The complex 1 was subjected to powder X-ray diffraction (PXRD) studies at room temperature to

check its crystallinity and phase purity. The diffraction data were collected with Cu K α

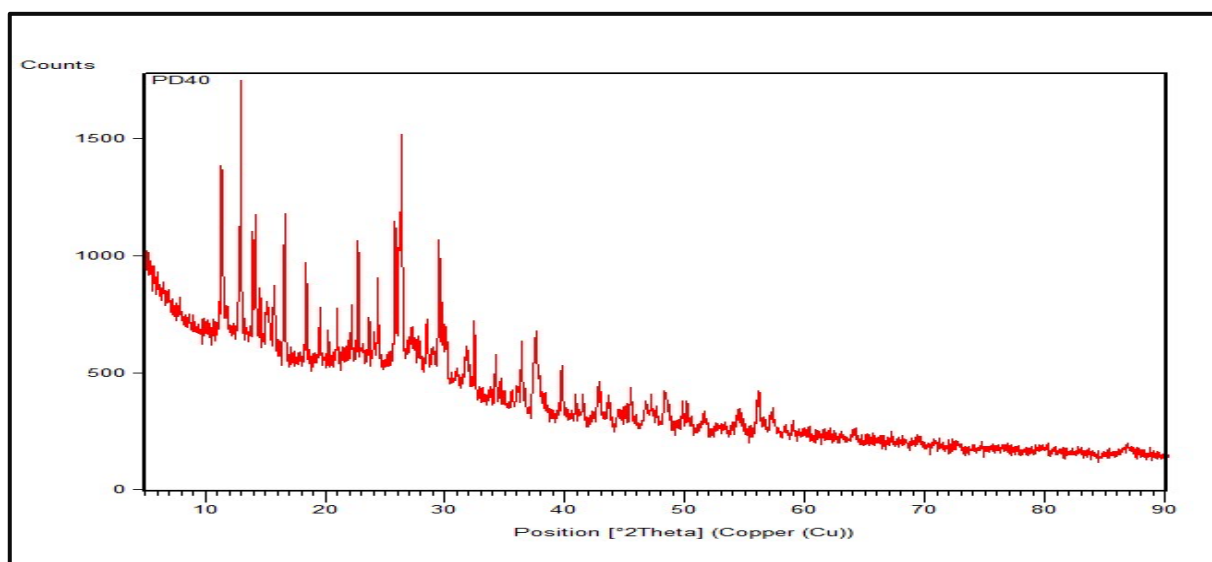


Fig .3 P-XRD Pattern of [EDABIS(3-PP) D]- Pd radiation ($\lambda = 1.5405 \text{ \AA}$) at 45 kV and 40 mA with a scanning speed of $5^\circ (2\theta)$ per minute. The PXRD patterns were recorded on a Shimadzu XR-6100 diffractometer using Cu K α radiation ($\lambda = 1.54 \text{ \AA}$) at a scanning rate of 2° per minute with a receiving slit of 0.15 mm. The resulting diffraction pattern showed sharp and well resolved peaks, indicating a highly crystalline material and thus confirming the successful synthesis of the complex in phase-pure form average crystalline is 10 to

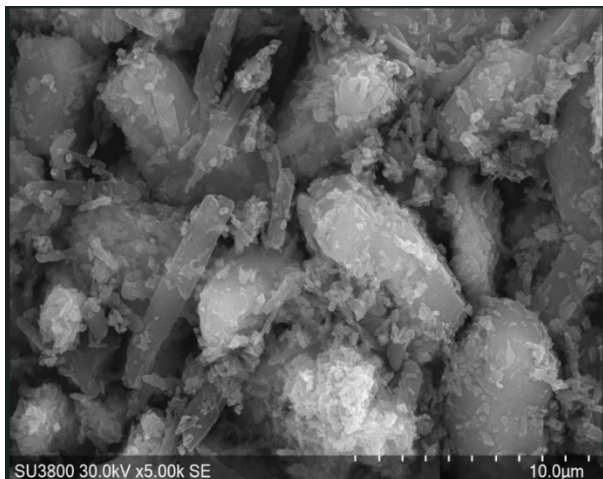


Fig .4 Crystal Image at 100µm

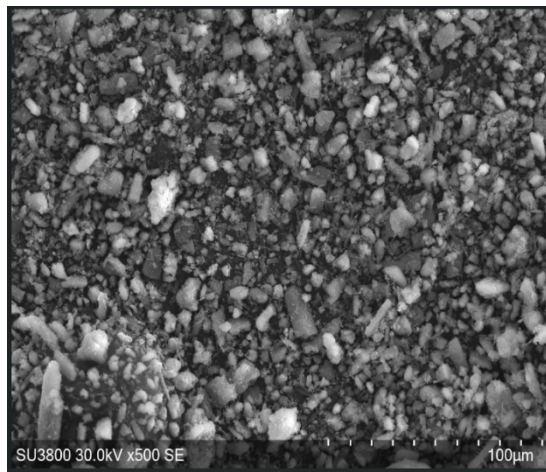


Fig .5 Crystal Image at 10µm

Elemental analysis.

CHNS analysis was performed on a Thermo Fisher Scientific FLASH EA 1112 Series Elemental Analyzer. The metal determination was carried out by ICP-OES

in an Agilent 5110 ICP-OES instrument. The elemental composition determined by CHNS and ICP-OES analysis is given in the table below.

Table 2. Elemental analysis of [EDABIS(3-PP) D]- Pd (II)

Complex	Molecular Weight	Color	Analysis, Found (Calculated)				Yield (%)
			% C	% H	% N	% Pd	
[EDABIS(3-PP) D]-Pd (II)	581.02	Dark Brown	66.10 (66.15)	5.19 (5.20)	4.76 (4.82)	18.30 (18.32)	75%

Antimicrobial nativity.

The synthesized Pd (II) complex was evaluated for its in vitro antibacterial activity against four pathogenic bacterial strains, namely *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*, using the agar diffusion method. Streptomycin (1 mg/mL) was employed as the standard antibacterial agent, whereas dimethyl sulfoxide (DMSO) was used as the negative control. The antibacterial activity of the synthesized Pd (II) complex was investigated at two concentrations, 5 and 10 mg/mL, in order to assess its concentration-dependent inhibitory behavior. The antibacterial efficacy was evaluated by measuring the diameter of the zone of inhibition (ZOI), expressed in millimeters.

The synthesized Pd (II) complex exhibited measurable antibacterial activity against all four tested bacterial strains, although the extent of inhibition varied depending on the bacterial species and concentration of the complex. At a concentration of 5 mg/mL, the complex produced inhibition zones of 12, 10, 6, and 14 mm against *B. subtilis*, *S. aureus*, *S. typhi*, and *E. coli*, respectively. Upon increasing the concentration to 10 mg/mL, a pronounced enhancement in antibacterial activity was observed, with the corresponding inhibition zones increasing to 18, 20, 22, and 18 mm,

respectively.

A clear concentration-dependent increase in antibacterial activity was observed for all four bacterial strains. Against *B. subtilis*, the inhibition zone increased from 12 mm at 5 mg/mL to 18 mm at 10 mg/mL. Similarly, the inhibition zone against *S. aureus* increased from 10 to 20 mm, while a substantial enhancement was observed against *S. typhi*, with the inhibition zone increasing from 6 to 22 mm. In the case of *E. coli*, the inhibition zone increased from 14 to 18 mm with increasing concentration. These results indicate that the antibacterial efficacy of the Pd (II) complex is strongly influenced by its concentration, with the higher concentration producing considerably greater bacterial growth inhibition.

Among the tested microorganisms, *S. typhi* exhibited the most pronounced concentration-dependent response. The complex also demonstrated considerable inhibitory activity against *S. aureus* and *B. subtilis*, while moderate activity was observed against *E. coli*. The comparatively low inhibition observed against *S. typhi* at 5 mg/mL suggests that a higher concentration of the complex may be required to achieve substantial growth inhibition of this strain.

For comparison, the standard antibiotic streptomycin (1

mg/mL) exhibited considerably higher antibacterial activity against all tested bacterial strains, producing inhibition zones within the range of 25–30 mm. In contrast, DMSO did not produce any detectable inhibition zone, indicating that the observed antibacterial activity was attributable to the synthesized Pd (II) complex rather than to the solvent.

Overall, the antibacterial screening results demonstrate that the synthesized Pd (II) complex possesses significant in vitro antibacterial potential against both Gram-positive and Gram-negative bacterial strains. The enhanced inhibition observed at 10 mg/mL compared with 5 mg/mL clearly indicates a concentration-dependent antibacterial response. The observed activity may be associated with the ability of

the coordinated Pd (II) center and the Schiff-base ligand framework to interact with bacterial cellular components and interfere with essential biological processes. However, the precise mechanism of antibacterial action requires further investigation.

The present findings suggest that the synthesized Pd (II) complex represents a promising candidate for further investigation as a metal-based antimicrobial compound. Further studies involving minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), time-dependent antibacterial assays, and mechanistic investigations would be valuable for establishing its antimicrobial efficacy and determining its potential applications in the development of novel antibacterial agents.

Table 3 . Antibacterial Activities of samples against *S. aureus*, *S. typhi*, *E. coli* & *B. subtilis*.

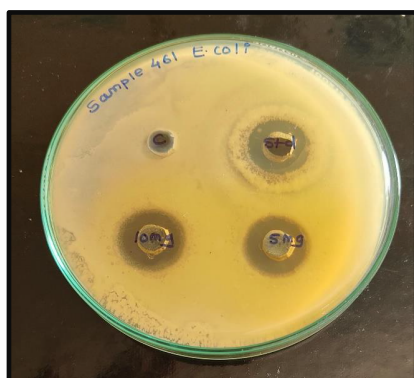
Sr. No	Sample/Concentration	<i>S. aureus</i> (mm)	<i>S. typhi</i> (mm)	<i>E. coli</i> (mm)	<i>B. subtilis</i> (mm)
1	Control (DMSO)	-	-	-	-
2	Streptomycin (1 mg/ml)	22	20	20	28
3	Pd/G-04/26 (5 mg/ml)	10	06	14	12
4	Pd/G-04/26 (10 mg/ml)	20	22	18	18



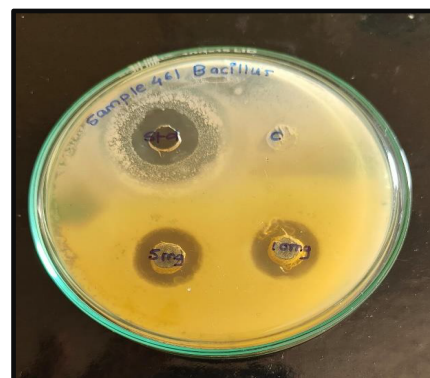
S. aureus



S. typhi



E. coli



B. subtilis

Antifungal activity.

The in vitro antifungal activity of the synthesized compound was evaluated against *Candida albicans*, and *Aspergillus Niger*, using agar well diffusion method as described by Hufford et al. (1975) and Umadevi et al. (2003) with appropriate modifications. Sabouraud dextrose agar (SDA; HiMedia) was used as the growth

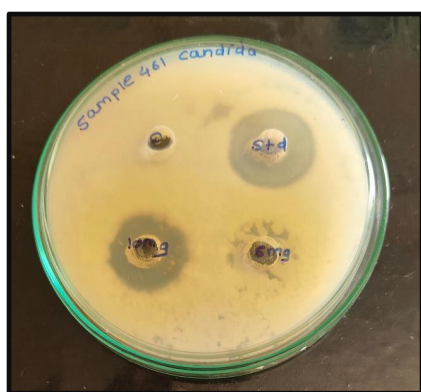
medium and was prepared in distilled water and sterilized in an autoclave at 121 °C for 15 min. Sterile Petri plates (100×15 mm) were filled aseptically with approximately 25 mL of the sterilized molten SDA medium cooled to approximately 45 °C and allowed to solidify. Fungal inocula were prepared in sterile normal saline and the concentration of the fungal spores/cells

was determined with the help of a hemocytometer. Standardized fungal suspensions were uniformly inoculated on the surface of solidified SDA plates and then wells of 6 mm diameter were prepared using sterile cork borer. The test compound was tested at concentrations of 5 and 10 mg/mL and 100 μ L of the respective test solution was carefully added to each well. The inoculated plates were incubated at 29 $^{\circ}$ C for 72 h and examined for clear zones of inhibition of fungal growth. The antifungal activity was expressed as and concentration of the test compound, and higher

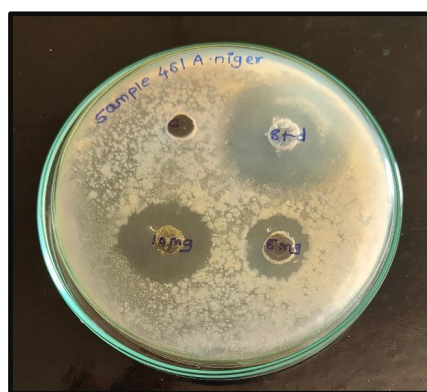
the diameter of the zone of inhibition (ZOI) in mm. The synthesized compound exhibited significant antifungal activity against both *C. albicans* and *A. Niger*. The compound was more effective at the higher concentration of 10 mg/mL. Less inhibitory activity was observed against *C. albicans* at a lower concentration of 5 mg/mL while appreciable inhibition was observed against *A. Niger*. These results indicate that the antifungal activity of the synthesized compound is dependent on fungal species concentration showed higher inhibitory activity.

Table 4. Antifungal Activities of samples against *Candida albicans* & *A. Niger*

Sr. No	Sample/Concentration	<i>Candida albicans</i> (mm)	<i>A. Niger</i> (mm)
1	Control (DMSO)	-	-
2	Streptomycin (1 mg/ml)	25	30
3	Pd/G-04/26 (5 mg/ml)	02	10
4	Pd/G-04/26 (10 mg/ml)	22	18



Candida albicans



***A. Niger* (mm)**

Conclusion

The synthesized Pd (II) Schiff-base complex Derived from 2'-Hydroxy-3-phenylpropiophenone and Ethylenediamine (EDABIS(3-PP) D) was evaluated in vitro for its antibacterial and antifungal activities. The antibacterial activity was assessed against *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* at concentrations of 5 and 10 mg/mL. The complex exhibited concentration-dependent antibacterial activity, with inhibition zones increasing from 12, 10, 6, and 14 mm at 5 mg/mL to 18, 20, 22, and 18 mm at 10 mg/mL, respectively. The highest antibacterial response was observed against *S. aureus* and *S. typhi* at 10 mg/mL. The complex also demonstrated antifungal activity against *Aspergillus Niger* and *Candida albicans*, with enhanced inhibition at the higher concentration. Overall, the results indicate that the Pd (II) Schiff-base complex Derived from 2'-Hydroxy-3-phenylpropiophenone and Ethylenediamine (EDABIS(3-PP) D) possesses promising broad-spectrum antimicrobial activity, particularly at 10 mg/mL. The observed activity may be attributed to the coordinated Pd (II) center and its interaction with essential cellular components of microorganisms. These findings suggest that the synthesized Pd (II) complex could serve as a promising candidate for further investigation in antimicrobial,

medicinal, and functional material applications.

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