B-KETO ESTER AS A NOVEL, EFFICIENT, AND VERSATILE LIGAND FOR Pd(II)-COORDINA - TION COMPOUND SHOWING ANTI-BACTERIAL ACTIVITY

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Abstract

Pd(II)-coordination chemistry with cyclic β-ketoester was studied byforming coordination complex with bidentate ligand α-Acetyl-γ-butyrolactone, which is versatile reactive reagent having no. of applications in synthetic organic chemistry. We are first timereporting Pd(II)-coordination chemistry with this ligand. The synthesized complex was well characterized by using IR spectrum, elemental analysis, mass spectrum, molar conductance, TGA and DTA analysis techniques and was evaluated for antimicrobial activity.

Keywords

α-Acetyl-γ-butyrolactone,tetrakistriphenyl phosphine palladium (0), Structural analysis, Biological activity.

1 INTRODUCTION

The 1, 3-dicarbonyl compounds are important class of organic compound used as intermediate for the synthesis of core heterocycles such as pyrazole[1-3], isoxazole[3-6] and triazole [7]. They find extensive applications in CVD (Chemical vapour deposition), processes which are used in wide range of industrial components and various items of chemical plant [8]. They are also used as chelating ligands for various lanthanide and transition metals in material chemistry [9-11]. Owing to such an enormous applications, the synthesis of 1, 3-diketones has gained considerable interest in recent years. The applications of 1,3-dicarbonyl compounds as ligand is well explored and are found to showedgood catalytic activities [12-17]. On contrary the chemistry of 1,3-keto ester is not much explored and there is

huge scope to explore to Pd(II) coordination chemistry with b-ketoester. There are only few reports available on use of cyclic 1,3-keto ester as a ligand for organic transformation which utilizes *in situ*in the preparation of metal complex [18]. There is tremendous scope in exploration of premade metal 1,3-keto ester complexes. The premade 1, 3-keto ester metal complex will be stable, less-sensitive to water, easy to handle and having ability to exhibit catalytic and show medicinal properties. Considering all the above facts, we have prepared metal complex of α-acetyl-γ-butyrolactone with Pd (II), characterized and study its biological and catalytic activities discussed in current research.

α-Acetyl-γ-butyrolactone a cyclic β-ketoester is potentially reactive bis-electrophile, having ability to co-ordinate with metals due to presence of oxygen lone pair. The literature reports revealed that α-acetyl-γ-butyrolactone was used for the synthesis of various heterocyclic compounds having good biological activity.

γ-Butyrolactone (GBL) is a hygroscopic colourless liquid with weak characteristic odour, soluble in water, commonly used as solvent and reagent in chemistry also used as used as a flavouring agent and cleaning solvent, as a superglue remover, and as a solvent in some wet aluminium electrolytic capacitors. In humans it acts as a pro-drug for γ-hydroxybutyric acid and used as recreational intoxicant with effects similar to alcohol.

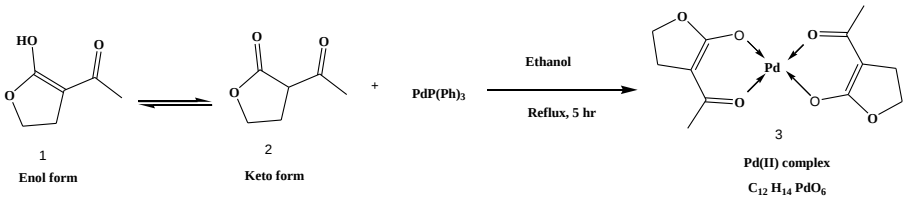
2. Experimental

2.1 Material and methodology

All reagents were purchased commercially and were used directly without any further purification. The complex was characterized by IR, UV, HRMS, CHN analysis and physical constant. IR spectra were recorded as KBr pellets on a Shimadzu FTIR-408 instrument. UV-visible spectra were recorded on Shimadzu 2450 UV-visible spectrophotometer. Mass spectra were recorded on a Shimadzu LC-MS: EI QP 2010A mass spectrometer with an ionization potential of 70eV. Elemental analyses were performed on Quest flash 1112 Series EA Analyzer at SAIF, Punjab University, Chandigarh. Molar conductivity of complexes was recorded using $1x10^{-3}$ M solutions in DMSO on Toshniwal TSM 15 conductivity metre. Melting points were determined on a Gallenkamp melting point apparatus. Reactions were monitored by thin layer chromatography (TLC), carried out on 0.2 mm silica gel 60 F254 Merck plates using UV light (254 and 366 nm) for detection.

2.2Synthesis of Palladium (II) complex with α-acetyl-γ-butyrolactone

A solution of α-acetyl-γ-butyrolactone(Merk) (2.15 mL; 20 mmol)**1** in ethanol (30 mL) was stirred for 10 min. To this solution oftetrakistriphenyl phosphine palladium (0)(1.156 gm,10mmol) **2** in ethanol was added slowly at room temperature. The reaction mixture was stirred at reflux temperature for 5 hrs. After cooling to room temperature, dark brown coloured complexprecipitated was filtered off, washed thoroughly with ethanol followed by diethyl ether. The compound obtained was recrystallizedfrom methanol and was finally dried under vacuum. (Scheme 1)



Scheme 1: Synthesis of Pd (II) complex of α-acetyl-γ-butyrolactone

3.0 Results and discussion

3.1 Elemental Analysis

This complex is hygroscopic having dark brown colour and stable to atmospheric conditions, soluble in DMSO and methanol. The elemental analysis data for percentage of carbon and hydrogen and metal were determined, was in aggregate with the calculated based on proposed formula. (Table 1)

Table No. 1: Analytical, physical data of lactone complex

Complex	Colour % yield	Melting point ^{o}C	Elemental analysis found (Calculated %)			
			C	H	M	P
C_{12} H_{14} PdO_6	Dark brown (85%)	230^{o}C	68.88 (66.28)	6.40 (5.30)	13.59 (13.98)	10.26 (8.14)

3.2 Infrared Spectra

IR spectra of complex and ligand showed change in stretching, and bending frequenciesindicatingcoordination occurs between lactone with Pd(II). Table No 2.Ligand α–acetyl-γ-butyrolactone showed band at 1779 $cm^{-1}$for cyclic esterand stretching frequency at 1726 cm^{-1} is for carbonyl group, while IR of this complex showed lowered value at 1739cm^{-1} and 1587 cm^{-1} for cyclic ester and carbonyl respectively, indicating the coordination with ester and carbonyl oxygen with metal ions. In Palladium complex the IR stretching frequency at 690 cm^{-1} is attributed to

presence of pph_3 group. New M-O stretching at 450 cm^{-1}in spectra of Pd(II) complex confirms the co-ordination between metal and oxygen. (Fig.1,2; Table 2)

Table No. 2: IR spectra data of lactone and its complexes

Compound	υC=0 ester	υC=O carbonyl	M - O	PPh_3
α–Acetyl-γ-butyrolactone	1779 cm^{-1}	1726 cm^{-1}	--	--
$C_{12}H_{14}PdO_6$	1739 cm^{-1}	1587 cm^{-1}	450 cm^{-1}	690 cm^{-1}

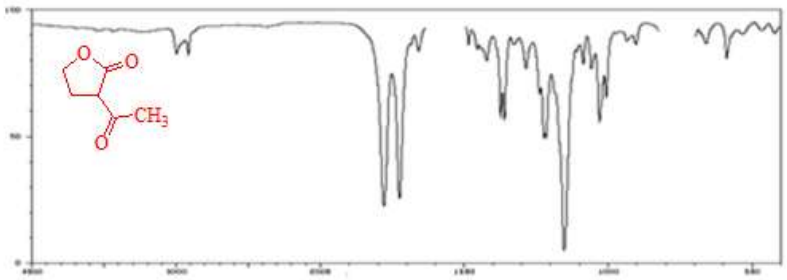


Fig. 1: IR Spectrum of α-acetyl-γ-butyrolactone

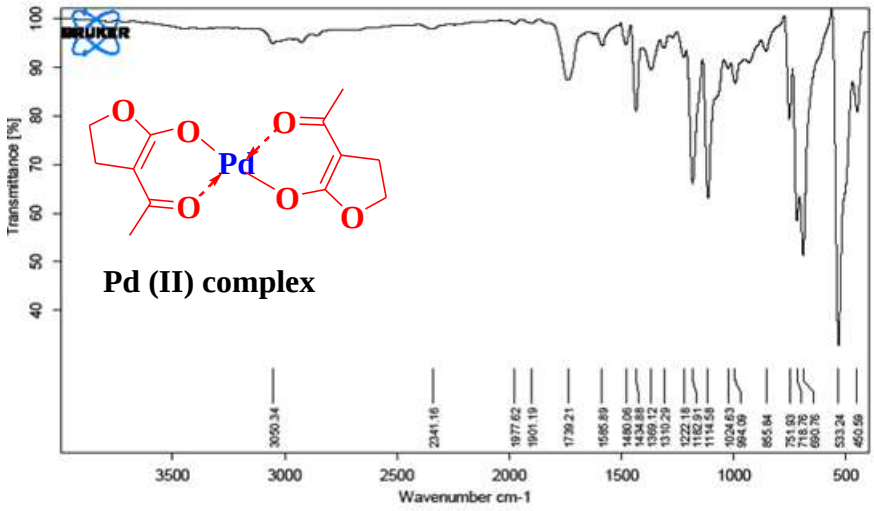


Fig.2: IR Spectrum of Pd (II) complex

3.3Molar conductance Measurements

The conductance of complex $C_{12}H_{14}PdO_6$ when dissolved in DMSO was 30.9 Ω -1 cm^2, which indicate the non-electrolyte nature of the complex.

3.4 Thermogravimetric Analysis; Complex $C_{12}H_{14}PdO_6$

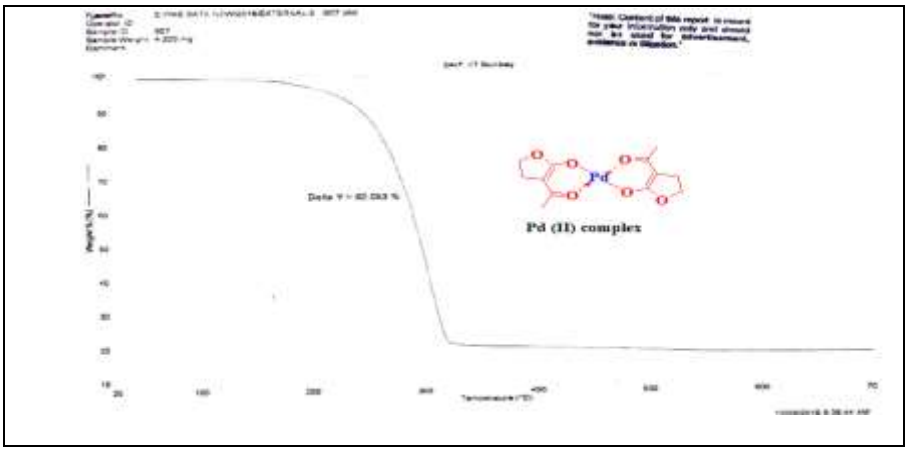


Fig.3: TGA curve complex $C_{12}H_{14}PdO_6$

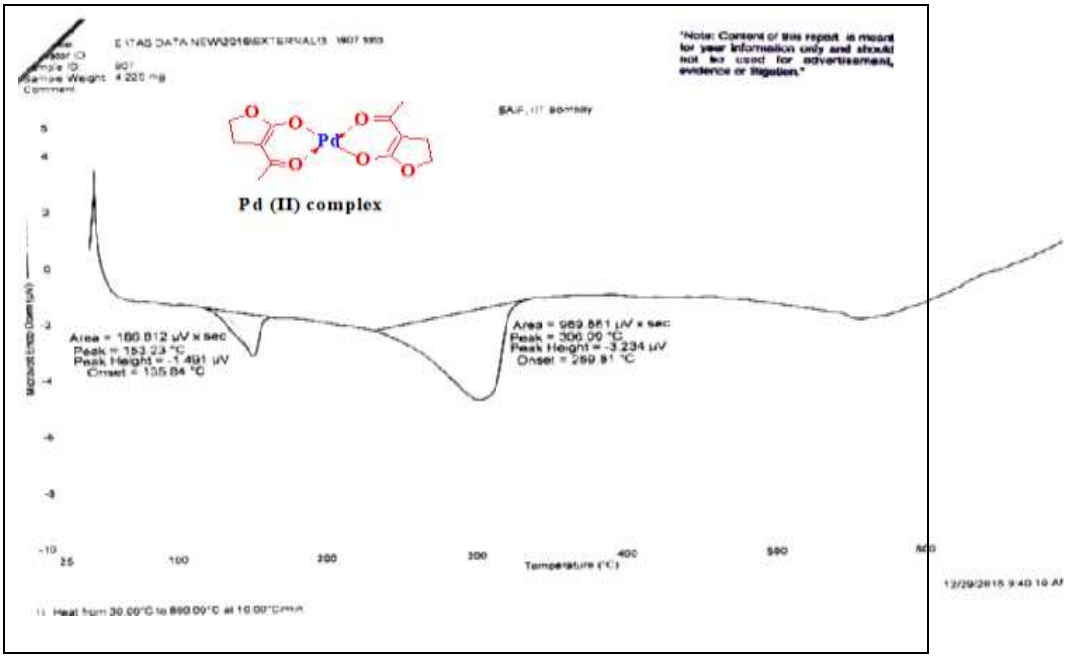


Fig. 4: DTA Curve for complex $C_{12}H_{14}PdO_6$

Thermoanalytical measurement was performed in range the 25°C-700°C. Thermal decomposition takes place in one step. The complex is stable below 250 °C. Gradual decomposition from 180^0C to 350^0C is related to loss of the organic moiety by a 82.093 % weight loss (theoretical 79.454%). The weight loss is due to decomposition of phosphine group and formation of PdO. The weight of the residue is consistent with metal oxide PdO from the 34.31% residual weight (calcd=35.11%). (Fig.3). The complex is stable below 180 ^{o} C and Gradual decomposition from 170-25°C

3.5The x-ray diffractogram of Pd (II) complex

The x-ray diffractogram of Pd (II) complex was scanned in the range 0-60° at wavelength 1.543Å. The diffractogram and associated data depict the 2θ value for each peak, relative intensity and interplanar spacing (d-values). The diffractogram of Pd (II) complex of L had twelve reflections with maxima at 2θ = 16.999° corresponding to d value 5.2118 Å. The x-ray diffraction pattern of these complexes with respect to major peaks of relative intensity greater than 12 % has been indexed by using computer programme. The above indexing method also yields Miller indices (hkl), unit cell parameters and unit cell volume. The unit cell of Pd II) complex of L yielded values of lattice constants, a=19.0293 Å, b=19.8973 Å, c = 20.3938 Å and unit cell volume V=1983.87 $Å^3$. In concurrence with these cell parameters, the condition such as a = b = c and $\alpha = \beta = \gamma = 90^0$ required for sample to be Orthorhombic were tested and found to be satisfactory. Hence it can be concluded that Pd(II) complex has orthorhombic crystal system.

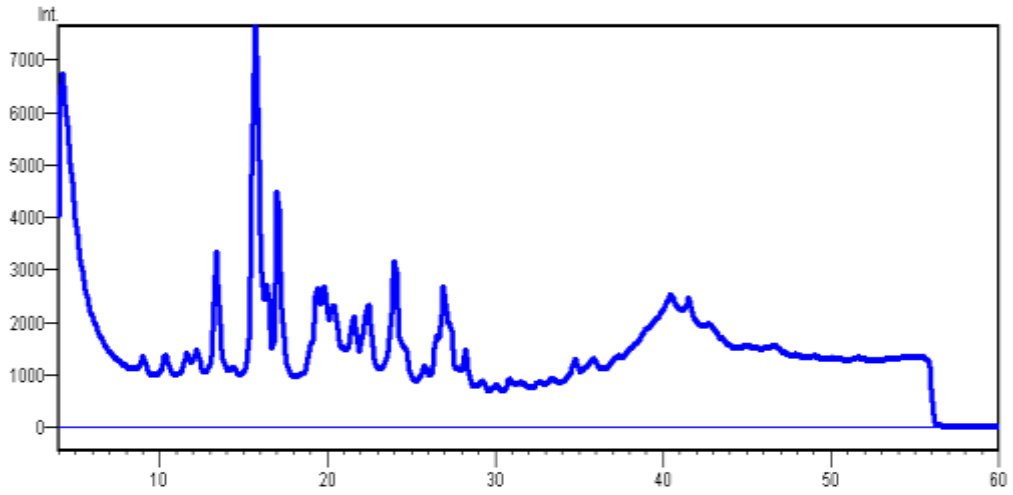


Fig.5: X-ray diffractogram of Pd (II) complex

Table No. 3: The x-ray diffractogram data of Pd (II) complex:-

2Theta	Relative Intensity	d spacing	hkl
10.438	16.19094	8.4679	101
13.354	59.49803	6.6244	111
15.832	96.25984	5.593	101
16.484	33.71063	5.3798	112
16.999	100	5.2118	100
19.38	38.16437	4.5766	212
20.449	29.6752	4.3396	201
20.266	28.10039	4.3912	222
21.566	28.61713	4.1172	202
24.045	42.5935	3.6981	234
26.863	37.94291	3.3162	303
27.349	26.45177	3.2564	311
34.784	26.13189	2.577	300
41.587	29.84744	2.1648	301

4.Biological Activity

The synthesized Palladium (II) complex was tested in vitro against representative Gram-positive/negative bacteria species *E. Coli*and*Staphylococcus aureus*, and two fungal species *Aspergillusniger* and *Candida albicans* by agar well diffusion method. All the bacterial strains was incubated at 37°C for 48hrs by inoculation into nutrient broth and the fungal strains were incubated for 72hrs by inoculation in to potato dextrose broth. The molten media were inoculated with 100µL of the inoculums and poured into the Petri plate. After medium was solidified, a well was made in the plates with the help of cup-borer (0.85cm). Then the test compound was introduced into the well and Petri plates were incubated. Compounds were dissolved in DMSO to prepare stock solution. Commercially available bactericide Gentamicin and antifungal Flucanozole were used as standard (100µg per 100µL of sterilized distilled water) concurrently with the test samples. The diameter of inhibition zones (in mm) was determined and data was statistically evaluated by Turkey's pair-wise comparison test. All the experiments were repeated for three times and the results were confirmed.

The newly synthesized complex was found to reveal considerable antibacterial activity almost equal to the activity of Gentamicin. The complex was also screened for its antifungal activity against *Aspergillusniger* and *Candida albicans* by agar disc diffusion method. The results of the antifungal testing of the complex was compared with the typical broad spectrum of the potent antifungal drug Flucanazole. The antifungal activity data shows that Palladium

(II) had comparable activity for *A. Niger* and had showedexcellent activity against *Candida albicans*, which is found to be better than the standard Flucanazole.The antimicrobial assay evaluation of the synthesized of Palladium (II) complex was done using agar well plate method. The antibacterial and antifungal assays were performed in Muller-Hintonbroth and CrazekDox broth. The standard strains used for the antimicrobial assay was procured from MicrobialCulture Collection, Pune, India.Antimicrobial evaluation was performed using the bacteria reseeded in Muller-Hinton broth for 24 hr at 37ºC andfungi reseeded in CrazekDox broth for 48 hr at 25ºC. The antibacterial activity of tested samples were studied intriplicate against gram positive bacteria *Staphylococcus aureus* (ATCC 29737) and gram negative bacteria*Escherichia coli* (ATCC 25922). The same samples were tested for antifungal activity in triplicate against *Candidaalbicans*(MTCC 277) and *Aspergillusniger*(MCIM 545). The compounds were dissolved in DMSO at desiredconcentrations of 40, 20, 10 µg/ mL. DMSO was loaded as negative control. Gentamicin (10 µg/ mL) andFluconazole (20 µg/ mL) were used as standards for evaluating the antibacterial and antifungal activity. The zone ofinhibition (mm) was determined from the diameter of the zone of inhibition using caliper as per National Committeefor Chemical Laboratory Standards (NCCLS, M7-A5, January 2000). ThePd complex shows good antibacterial as well as good antifungal activity.

Table 4: Antimicrobial screening of compounds 1-12:*Inhibition Zone Diameter (mm)*

Complex	*E. coli* (ATCC25922)	*S. aureus* (ATCC 29737)	*A.Niger* (MCIM 545)	*C.albicans* (MTCC 277)
$C_{12}H_{14}PdO_6$	16 ± 1.1	16 ± 0.6	15 ± 0.4	19 ± 0.7
DMSO	11 ± 0.7	12 ± 0.9	12 ± 0.6	13 ± 0.3
Gentamicin	22 ± 0.4	23 ± 0.7	-	-
Fluconazole	-	-	23 ± 0.8	24± 0.5
Gentamicin (10 µg/ mL) and fluconazole (20 µg/ mL) Inhibition Zone= 9-14 mm: slight activity, 15-19 mm: moderate activity, 20 -24 mm : high activity, >25 mm: excellent activity NT: Not Tested				

Table 5 : Antimicrobial screening of complex: MIC in µg / mL values

Complex	*Escherichia coli* (ATCC25922)	Staphylococcus Aureus, (ATCC 29737)	*Aspergillus Niger* (MCIM 545)	*Candida albicans* (MTCC 277)
$C_{12}H_{14}PdO_6$	20	40	10	10
Gentamicin	10	10	-	-
Fluconazole	-	-	20	20
Gentamicin (10 µg/ mL) and Fluconazole (20 µg/ mL), (MIC in µg / mL)=10 µg / mL: excellent activity, 20 µg / mL: moderate activity,40 µg / mL: slight activity				

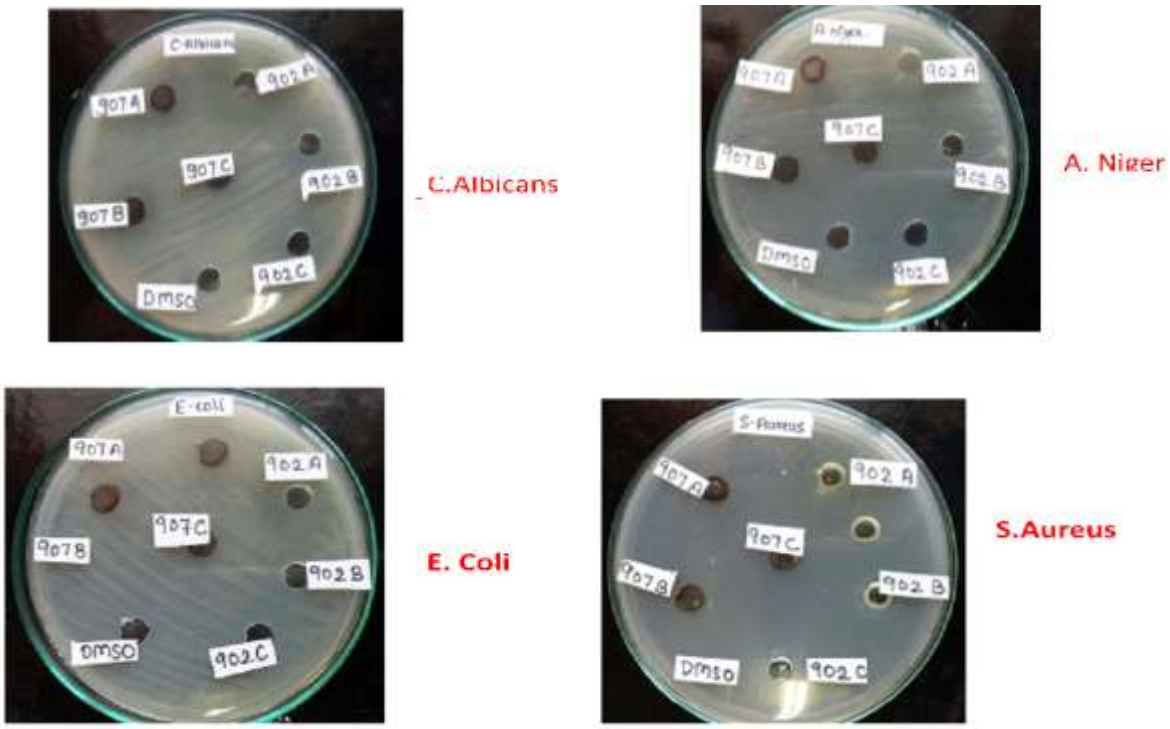


Fig. 6: Biological Activity of 907 ($C_{12}H_{14}PdO_6$)

The complex Palladium (II) $C_{12}H_{14}PdO_6$ showed excellent antibacterial activity against *Escherichia coli*(ATCC25922) w MIC *20 µg/mL* when compared with standard antibacterial drug Gentamicin (*10 µg/mL*). Similarly it also showed excelle anti fungal activities against *Aspergillusniger* (MCIM 545), *Candida albicans* (MTCC 277) with MIC *10 µg/mL* wh compared with standard antifungal drug Fluconazole (*20 µg/mL*). (Fig.6)

5. Conclusion

New Palladium (II) complex with α-acetyl-γ-butyrolactonewas synthesizedandwas well characterized using various analytical tools viz. IR,UV, elemental analysis, conduct meter, physical constant etc. The Pd (II) complexshowed good antibacterial and antifungal activity tested by agar disc diffusion method and showed truly biological activities.

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