TEMPLATE SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF MACROCYCLIC COMPLEXES

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Abstract
The macrocyclic complexes of transistion metalas of [TATPD Zn (II)] [TAHMBD Ni (II)], [TAHMDD Cu (II)], [TAHMD Co (II)] , [TAHMDD Zn (II)], [TATMDD Ni (II)], [TAHMDD Ni (II)], [TAHMDD Co (II)] were synthesized by template method. The complexes were characterized by elemental analysis UV Vis and Infrared spectroscopy. These macrocyclic complexs were showed broad spectrum activity ie inhibit the growth of gram positive and gram negative bacteria**.** These complexes were tested against *Staphylococus aureus*, *Bacillus subtillus* ,*Pseudomonas aureginosa* and *E.coli* **.**

Key words: Template synthesis, antibacterial activity, tetraaza macrocyclic complexes

Introduction

The macrocyclic transition metal complexes has become a fascinating subject in the field of coordination chemistry, biology and materials science.[1-6] There is a significant interest in the synthesis and properties of polyazamacrocycles. These molecules and their transition metal complexes has focused on application to catalysis, ion selective and their use as radio immunotherapy agents.[7-14]

Macrocyclic compounds have been extensively used to tune metal ion selectivity. The fit between the size of the cation and the cavity provided by the macrocycle is the crucial key for the design of the right host with the alkaline or alkaline earth metal ions, such as crown ethers, spherands or calixarenes. [15-17] The field of macrocyclic chemistry of metal is developing very rapidly because of its applications and importance in the area of coordination chemistry. Structural factors such as ligand rigidity, the types of donor atoms and their disposition have shown to play significant roles in determining the binding features of macrocyclic ligands towards metal cations. The development of the field of bioinorganic chemistry has been another important factor in spurring the growth of macrocyclic compounds. The chemical properties of macrocyclic compounds can be tuned to force metal ions to adopt unusual geometry. Transition metal macrocyclic complexes have received much attention as an active part of metalloenzymes and as biominic modal compounds.[18-24]

The antimicrobial activity of ligand and its complexes as growth inhibiting agents have been screened in vitro against bacteria and plant pathogenic fungi.The complexes showed varying degrees of inhibitory effect on the growth of the bacterial strains tested. It was observed from the result that the activity of complexes is high. This enhancement of the activity can be rationalized on the basis of their structures possessing an addition C=N bond. Furthermore, coordination reduces the polarity of metal ion mainly because of

partial sharing of its positive charge with these donor groups and possibly the π-electron delocalization within the whole chelate ring system. Thus, the chelation increases the lipophilic nature of the central metal atom, which, in turn , favors its permeation through the lipid layer of the membrane of the microorganism cell wall more effectively thus raising the activity of drug.[25-30]

Materials and methods

All macrocyclic complexes were synthesized by the template method (Rafat *et. al*., 2004), by condensation of Ethylenediamine / 3,5- diamino benzoic acid / O- phenylenediamine / acetone / acetyl acetone / benzil and metal salt (chloride, sulphate) in the ratio of 2:2:1 in a round bottom flask followed by a methanolic solvent (~50 ml). Shake well and refluxing was carried out for 6-8 hours with constant stirring till their change in color. After this round bottom flask kept aside to cool. The complexes were filtered, washed by methanol and dried in vacuum.

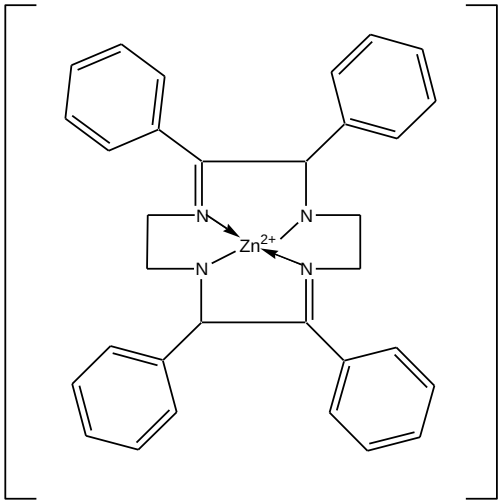
The present investigation deals with the synthesis and charecterization of some macrocyclic complexes of transition metal viz. Ni, Cu, Zn, Co, etc. These complexes were crystalline and non-hygroscopic solids. The UV-Visible identification of macrocyclic complexes has been obtained from Systronics UV-Vis spectrophotometer using DMSO, metanol and dichloromethane. The absorption bands in UV-VIS region shows the geometry of complexes, π-π* and n-π* transition in ligand part which falls in between 200-500 nm. The preliminary elemental identification of macrocyclic complexs has been obtained from Elemental analysis system Vario EL III. Infrared spectra were obtained with KBr pellets on PerkinElmer Spectrum Version 10.03.06 and were recorded in cm^{-1}. The IR spectrum showed the absence of uncondenced functional groups of amines, it showed the formation of proposed macrocyclic.

The appearance of absorption bands in the region 1600-1650 cm^{-1} correspond to (C=N), the absorption bands in the region of 3100-3240 cm^{-1} correspond to (N-H) and the absorption bands in the region 1360-1380 cm^{-1} correspond to $>C(CH_3)$.

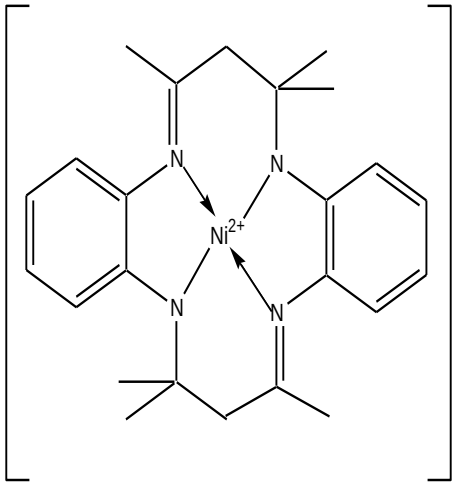
Macrocyclic complexes

1. 1,4,7,10 – tetraazacyclododeca – 5,6,11,12 – tetraphenyl – 4,10 – diene Zn(II) macrocyclic complex. [TATPD Zn (II)]
2. 1,4,8,11 - tetraazacyclotetradeca – 5,7,7,12,14,14 – hexamethyl – biphenyl – 4,11 – diene Ni(II) macrocyclic complex. [TAHMBD Ni (II)]
3. 1,5,9,13 - tetraazacyclohexadeca – 6,8,814,16,16 – hexamethyl - diacido – 5,13 – diene Cu(II) macrocyclic complex. [TAHMDD Cu (II)]
4. 1,4,8,11 – tetraazacyclotetradeca – 5,7,7,12,14,14 – hexamethyl – 4,11 – diene Co(II) macrocyclic complex. [TAHMD Co (II)]
5. 1,5,9,13 - tetraazacyclohexadeca – 6,8,814,16,16 – hexamethyl - diacido – 5,13 – diene Zn(II) macrocyclic complex. [TAHMDD Zn (II)]
6. 1,5,9,13 - tetraazacyclohexadeca – 6,8,14,16 – tetramethyl - diacido – 5,13 – diene Ni(II) macrocyclic complex. [TATMDD Ni (II)]
7. 1,5,9,13 - tetraazacyclohexadeca – 6,8,814,16,16 – hexamethyl - diacido – 5,13 – diene Ni(II) macrocyclic complex. [TAHMDD Ni (II)]

8. 1,5,9,13 - tetraazacyclohexadeca – 6,8,814,16,16 – hexamethyl - diacido – 5,13 – diene Co(II) macrocyclic complex. [TAHMDD Co (II)]

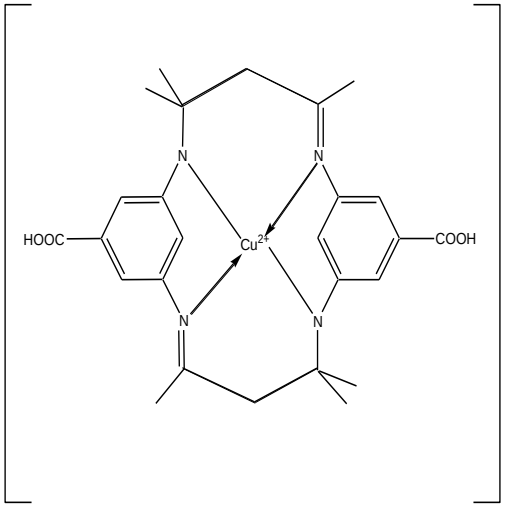


SO_4^{2-}



$2Cl^-$

1 2



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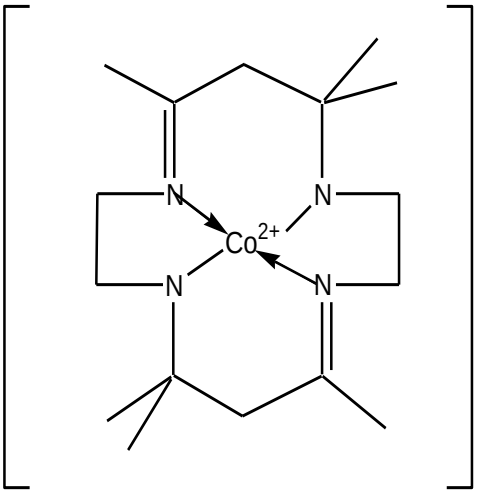
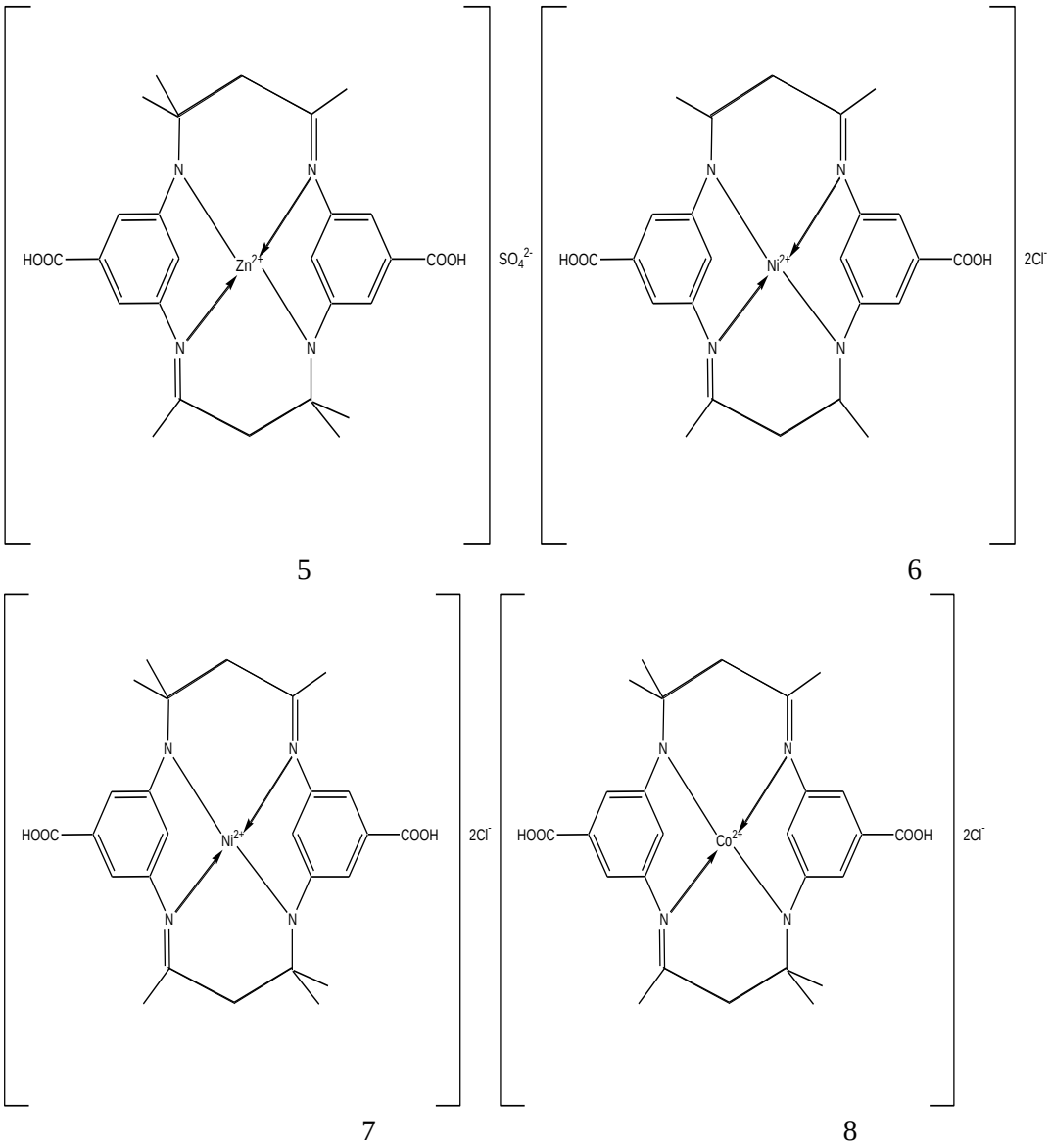


$2Cl^-$

3 4



5 6

7 8

Fig: 1. Proposed structures of macrocyclic complexes

Preparation of samples: samples were prepared by dissolving macrocyclic complexes in appropriate solvent (water or methanol) in the ratio of 100mg/ml.

Culture media: Muller Hinton agar media M-173 (Hi media Pvt. Ltd., Mumbai, India) was used for conducting antibacterial activities of macrocyclic complexes.

Microorganisms used: 4 diffrent pathogenic bactria such as Bacillus Subtillus, Pseudomonas Aeroginosa, Staphylococcus Aureus, E. Coli were used. All the strains were grown and maintained on nutrient agar slants at 4^oC.

Antimicrobial assay:

The cup- plate method was used to evaluate the antibacterial activity (Prabhat *et. al.*, 2005). This method depends upon the

diffusion of the tested material to such an extent that growth of added microorganisms is prevented entirely in a zone around the hole containing a solution of tested material. One hundred microlitres of diluted inoculums of 105CFU/ml of 24hours old cultures of test organisms were mixed in Muller Hinton agar medium and shaken. Then media was poured (25-30ml) in sterilized Petri dishes (20 × 90 mm). Wells of 6 mm diameter were punched into the agar medium and filled with 45µl of synthesized complexes (100mg/ml). All the solvents served as negative control. Antibiotic (ampicilline concentration 100mg/ml.) was simultaneously used as positive control. Each sample was assayed in triplicate and the mean values were observed. The plates were then incubated at 370C for 24 h. The antimicrobial activity was interpreted from the size of the diameter of zone of inhibition measured in millimeters (mm), it was observed as the clear zones surrounding the hole evaluated by measuring the inhibition zone diameter. The inhibition of the bacterial growth expressed in percentage terms was determined from the growth in the test plate relative to the respective control plate as given below:

Percentage of potency = (C-T) 100/C

Where, C=diameter of the bacterial growth in the control

T= diameter of the bacterial growth in the test

Results and Discussion

The formulae for these macrocyclic complexes can be assigned on the basis of analytical data (Table 1).Analytical and spectroscopic data (Table 2) enable us to predict the possible structure of the synthesized complexes.

The I.R. spectral data indicates some important assignments. The complexes exhibit a C=N absorption in the range of 1600-1617cm^{-1}, which together with the absence of C=O absorption around 1700 cm^{-1}. It shows that amino group of ethylenediamine was condensed with the carbonyl group of acetylacetone / acetone to give a cyclic structure. In the complexes, N-H band were observed at 3220-3250 cm^{-1} indicates the coordination to the metal through nitrogen of NH group. .

Antibacterial activity

The antibacterial activity of macrocyclic complexes against pathogens at concentration 100mg/ml are given in Table 3. Inhibition of solvent (water or methanol) was found to be very negligible and taken as zero mm. All these synthesized macrocyclic complexes showed good antibacterial activity (figure2).The antibacterial activity of complexes were observed in increasing order ***1 > Macrocyclic complex 5 > Macrocyclic complex 2 > Macrocyclic complex 4 > Macrocyclic complex 6 > Macrocyclic complex 8 > Macrocyclic complex 7 > macrocyclic complex 3***

The maximum zone of inhibition against *S. aureus* i.e. 31mm was observed in complex no. 7 and 8 and the minimum zone of inhibition in complex 1 and complexe 5 against *E. coli* and *P. aureginosa* i.e. 8 mm. Similarly complex 6 showed maximum antibacterial activity against E. coli (30 mm) and minimum against *E. coli* (8mm).

The present study has shown that these macrocyclic complexes are potentially rich source of antibacterial agents. This demonstrates their importance in traditional remedies in the population. Each macrocyclic complexes (100 mg/ml) were used for determination of their potency against tested pathogens and compared with antibiotic (Table.4). All the complexes have

good antibacterial activity but complex 8 showed strong activity.

Further, on the basis of chelation theory, antibacterial activity of the metal chelates can be explained. Chelation may enhance the biochemical potential of bioactive species. Because on chelation, the polarity of the metal ion will be reduced due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Hence macrocyclic complexes become very stable due to delocalization of π-electrons. It enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organisms.

TABLE 1, Analytical Data of Macro cyclic Complexes

Macrocyclic Complex	**C % Found/Cal.**	**H% Found/Cal.**	**N % Found/Cal.**	**M% Found/Cal.**
$ZnC_{32}H_{28}N_4SO_4$	60.20 (60.98)	4.60 (4.44)	10.10 (8.89)	8.28 (10.43)
$NiC_{24}H_{30}N_4Cl_2$	57.15 (57.25)	4.60 (5.95)	10.37 (10.72)	11.0 (11.65)
$CuC_{26}H_{30}N_4O_4SO_4$	46.40 (46.33)	4.75 (4.82)	9.85 (9.01)	9.23 (10.23)
$CoC_{16}H_{30}N_4Cl_2$	47.30 (47.07)	6.48 (7.35)	13.25 (13.72)	13.55 (14.43)
$ZnC_{26}H_{30}N_4O_4SO_4$	50.67 (50.02)	4.50 (4.81)	9.75 (8.97)	12.45 (10.43)
$NiC_{24}H_{24}N_4O_4Cl_2$	51.10.0 (51.27)	4.50 (4.27)	9.90 (9.96)	11.65 (10.45)
$NiC_{26}H_{30}N_4O_4Cl_2$	52.33 (52.72)	5.10 (5.07)	9.35 (9.46)	11.24 (9.92)
$CoC_{26}H_{30}N_4O_4Cl_2$	52.60 (52.72)	6.10 (5.06)	10.23 (9.46)	8.90 (9.95)

TABLE 2, IR DATA OF MACROCYCLIC COMPLEXES

S. No.	**MACROCYCLIC COMPLEXES**	**ν (N-H)**	**ν (C=N)**	**ν >C(CH_3)**
1.	$ZnC_{32}H_{28}N_4SO_4$	3150m	1605m	-
2.	$NiC_{24}H_{30}N_4Cl_2$	3219s	1605s	1360m
3.	$CuC_{26}H_{30}N_4O_4SO_4$	3130m	1611s	1366m
4.	$CoC_{16}H_{30}N_4Cl_2$	3213s	1615vs	1381s
5.	$ZnC_{26}H_{30}N_4O_4SO_4$	3230s	1600m	1379s
6.	$NiC_{24}H_{24}N_4O_4Cl_2$	3235s	1606m	-
7.	$NiC_{26}H_{30}N_4O_4Cl_2$	3237m	1617m	1352s
8.	$CoC_{26}H_{30}N_4O_4Cl_2$	3220s	1616s	1375s

Where s=strong, v=very strong, m=medium

TABLE 3: Antibacterial activities of complexes: (conc. 100 mg/ml)

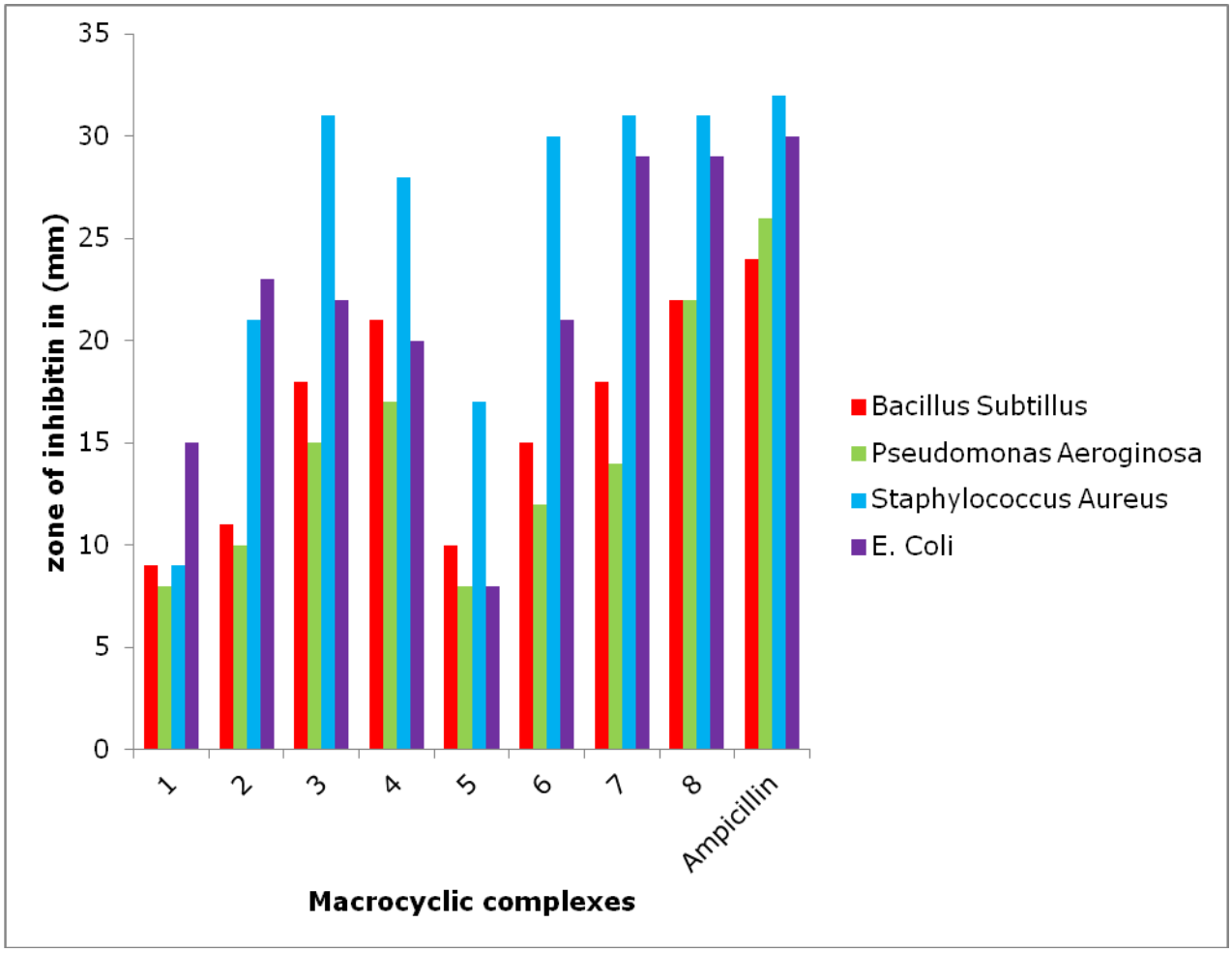
Macrocyclic Complex	**Bacillus Subtillus**	**Pseudomonas Aeroginosa**	**Staphylococcus Aureus**	**E. Coli**
1	9	8	9	15
2	11	10	21	23
3	18	15	31	22
4	21	17	28	20
5	10	8	17	8
6	15	12	30	21
7	18	14	31	29
8	22	22	31	29
Ampicillin	24	26	32	30

Diameter of inhibition zone (mm)

TABLE 4: Percentage of potency of complexes compared with Ampicilline

Macrocyclic Complex	**Bacillus Subtillus**	**Pseudomonas Aeroginosa**	**Staphylococcus Aureus**	**E. Coli**
1	62.50	69.23	71.87	50.0
2	54.10	61.53	34.37	23.33
3	25.0	42.30	3.12	26.66
4	12.50	34.61	12.50	33.33
5	58.30	69.23	46.87	73.33
6	37.50	53.84	6.25	30.0
7	25.0	46.15	3.12	3.33
8	8.33	15.38	3.12	3.33

Antibacterial activity:



Conclusion

The present investigation has shown that all these synthesized macrocyclic complexes rich source of antibacterial agents. The macrocyclic complexes inhibited the growth of all pathogens. The sensitivity of test organism to macrocyclic complexes in *decreasing order* is

Staphylococcus aureus> E.coli> Bacillus subtillus> Pseudomonas aeruginosa

The antibacterial activity of complexes were observed in *increasing order* is

Macrocyclic complex 1 > Macrocyclic complex 5 > Macrocyclic complex 2 > Macrocyclic complex 4 > Macrocyclic complex 6 > Macrocyclic complex 8 > Macrocyclic complex 7 > macrocyclic complex 3

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