Synthesis and Antimicrobial activity of N-7-Methyl Adenine and its metal complexes.

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ABSTRACT

Copper (II) and silver (I) metal complexesof N-9-alkyladenine are well known in the literature. These studies reveal various important properties of nucleobases in the biological environment. However, N-alkyl-methyl adenine, an unnatural derivative of adenine, is very less studied. The aim of this study is to prepare copper and silver analogs of N-7- methyl adenine and study their properties. The Copper (II) metal salts were reacted with ligand with molar ratio M: L (1:1), (0.5:1) and silver metal salt with ligand molar ratio (1:1). The synthesized compound and prepared metal complexes were characterized and antibacterial activity against gram-negative, gram-positiveandonefungal species carried out.The resultsof these studies are reported here.

Keywords N-7-methyl Adenine, 6-chloro purine,N-7-Methyl adenine:Cu complex,N-7-Methyl adenine:Ag complex,antibacterial,antifungal activity.
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INTRODUCTION

The interaction of nucleic acids/nucleobase and their analogs with metal ions has been extensivelyinvestigated, mostlydue to their importance in the biological environment and, to some extent, their supramolecular and other properties[1–6].

The nucleobase adenine, apart from being a key constituent of nucleic acids, plays an important role as an integral component of many functional biological cofactors such as ATP, NADH, etc. [7]. Therefore, many researchers have studied the interaction of nucleobase with various metals. For example, Lippert[8–10] reviewed the binding modes of nucleobases (including adenine and its derivatives) to various transition metals. More recently, Verma et al. described the emerging use of adenine nucleobase for the design of metal−nucleobase frameworks[11].Also, an unnatural analogue of adenine has been studied. The binding modes of deaza-adenines , aza-adenines, and adenine isomersin transition metal complexes[12], and the intramolecular interligand interactions within the transition metal complexes of the purine nucleobases and N9-derivatives of adenine[13] are summarized by[14]. The understanding of the structure and function of RNA and DNA systems depends partly on the binding sites of metal ions to nucleobases [15, 16].It is essential to understand the interactions between nucleic acids, proteins, and their

constituents with metal ions to follow many biological processes. The complexation of metal ions with adenine has been well studied, and its binding site(s) has also been clearly elucidated[17, 18].

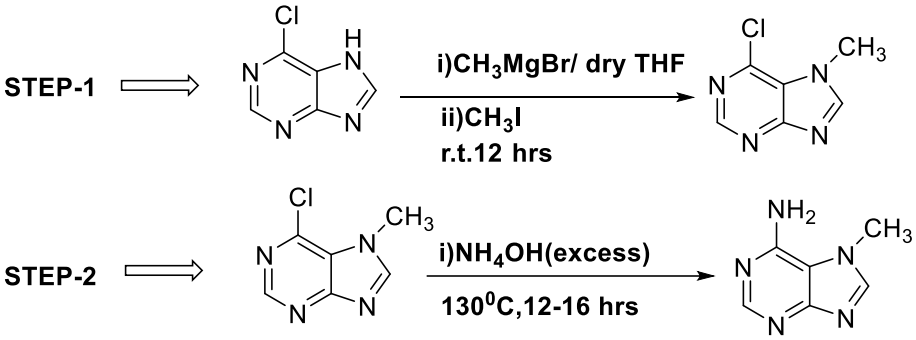
Adenine Metal Complexes and Their Binding Sites- The complexation of metal ions with adenine has been well studied, and its binding sites have also been clarified. The different coordination sites have been observed for adenine in copper complexes by X-ray studies[19-28]. Among the four ring nitrogen atoms, N(1), N(3), N(7), and N(9) of adenine, N(9) is the most basic and hence bears a proton, rendering it the most preferred metal binding site. But in most of the studies, N (9) is alkylated to mimic the natural system.

Many reports on silver ion coordination with N-9 substituted adenine and 6-chlopurine. It is important to note that silver coordinates to all ring nitrogen atoms, as reported by Purohit etal[27, 29–31]. This coordination is used to design many coordination assemblies. (Verma et al. 2010)Also, Venkatesh etal synthesized luminescent silver purine helicate and study for its antibacterial activity[32,33].

In this manuscript, wereport the complexation of copper and silver ions with an unnatural derivative of adenine where the N-7 position is substituted with a methyl group. We also study their antibacterial and antifungal activity.

EXPERIMENTAL SECTION

Synthesis of N-7-Methyladenine



Scheme-1 (Synthesis of N-7-Methyladenine**)**

STEP-1: Synthesis of N-7- methyl-6-chloropurine

The N-7-Methyl-6-chloro purine was synthesized as per a literature method[34].6-Chloropurine (500mg, 3.3995mmol) was taken in dry THF in a flame-dried RB under N_2 atmosphere. Methyl magnesium bromide (3M in THF, 266mg, 3.55mmol) was added drop wise. After addition,methyl iodide (1.18ml, 9.7mmol) was added, and the color of the solution changed to brown. The reaction was stirred for 12 hrsat room temperature. After completion of the reaction by observation through TLC, the reaction mixture was diluted with chloroform, and the organic layer was dried in Na_2SO_4 and purified through column chromatography (Silica DCM: Methanol, 3% Rf =0.5) to give faint yellow color solid**. (Scheme-1)**. The ^{1}H NMR of the compound was taken and was confirmed bymatching with the literature spectral data details in Figure-1.

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.77 (s, 1 H), 8.74 (s, 1 H), 4.08 (s, 3 H)

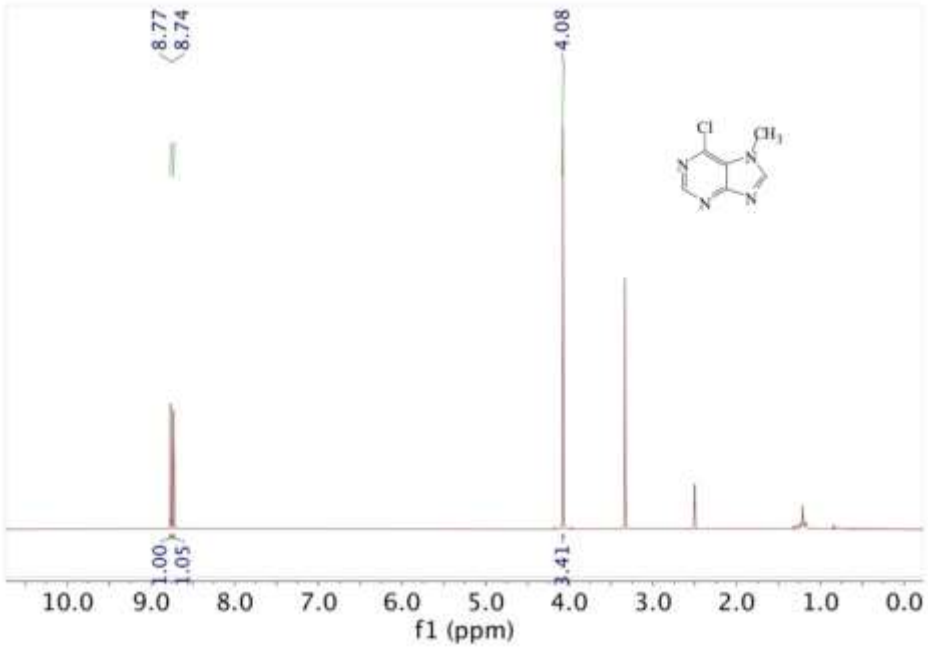


Figure 1: ^{1}H NMR ofN-7- methyl-6-chloropurine

STEP-2: SYNTHESIS OF N-7- METHYLADENINE

N-Methyl-6-chloropurine (300mg,) and excess Ammonium hydroxide were taken in an autoclave and heated at 130^{0}C for 16 hrs. It was then cooled down to room temperature.TLCindicated the complete consumption of the starting material. The solvent was reduced through a rotary evaporator and purified through column chromatography (Silica,DCM:methanol, 15% Rf =0.5) to give a white solid.**(Scheme 1).**

The compound was confirmed by matching with ^{1}H NMR spectral data with literature as given in Figure-2.

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.15 (s, 1 H), 6.90 (s, 1 H), 3.96 (s, 3 H)

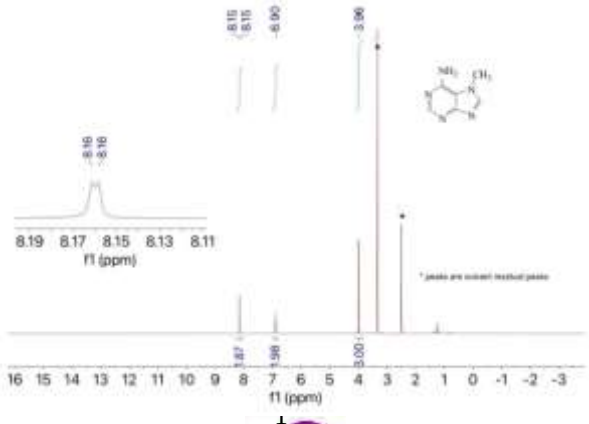


Figure 2:^{1}H NMR of N-7- methyl Adenine

Preparation of Metal complexes

1. **N-7-Methyl adenine:Cu complex (1:0.5)** Methyl adenine (5mg, 0.033mmol) was dissolved in 7.5ml of methanol and was placed in RB (100ml) with a magnetic stirring bar and was placed on a stirrer. Copper perchlorate hexahydrate $Cu(ClO_4)_2.6H_2O$ (6.11mg,0.0165 mmol,) in 5ml of methanol was slowly added with continuous stirring. Within 5 minutes, a precipitate was formed. The product thus formed was filtered and washed with methanol several times to remove theunreacted ligand and metal salt. The product was dried under a high vacuum, and 3.26 mg product wasobtained, the complex was extremely low solubility with organic solvent and water, therefore, NMR studies could not be carried out. But, 1mg of the complex was stirred with 5 mL of Methanol, and the supernatant was studied with Mass spectroscopy.The Mass studies revealed that probable Molecular Formula of complex would be $\mathbf{C_{26}H_{36}Cu_2Cl_2N_{20}O_{10}^{2+}}$ and Molecular weight984.09 (m/z = 493.04 expected, Experimental Value m/z = 493.35)was given in Figure- 3and theprobable structure with Figure-4

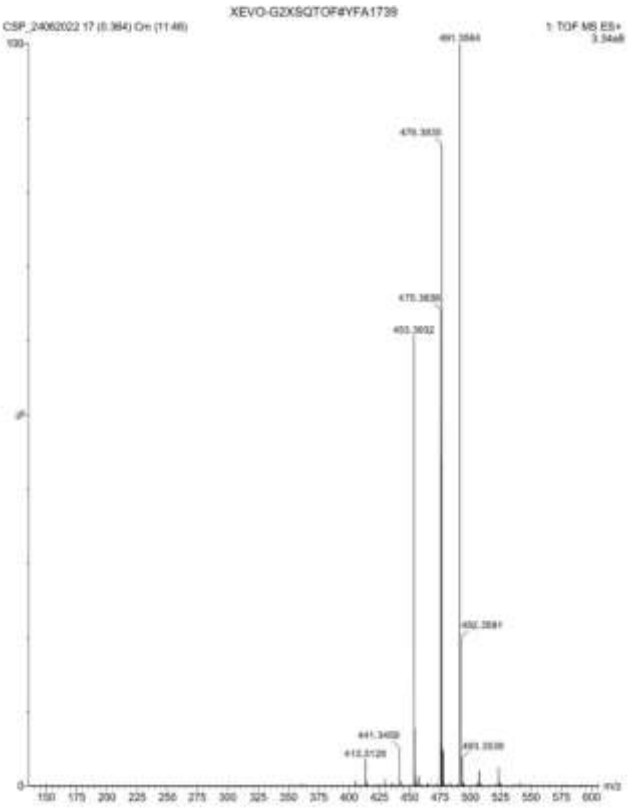


Figure 3: (Mass spectrum of N-7-Methyl adenine: Cu complex)

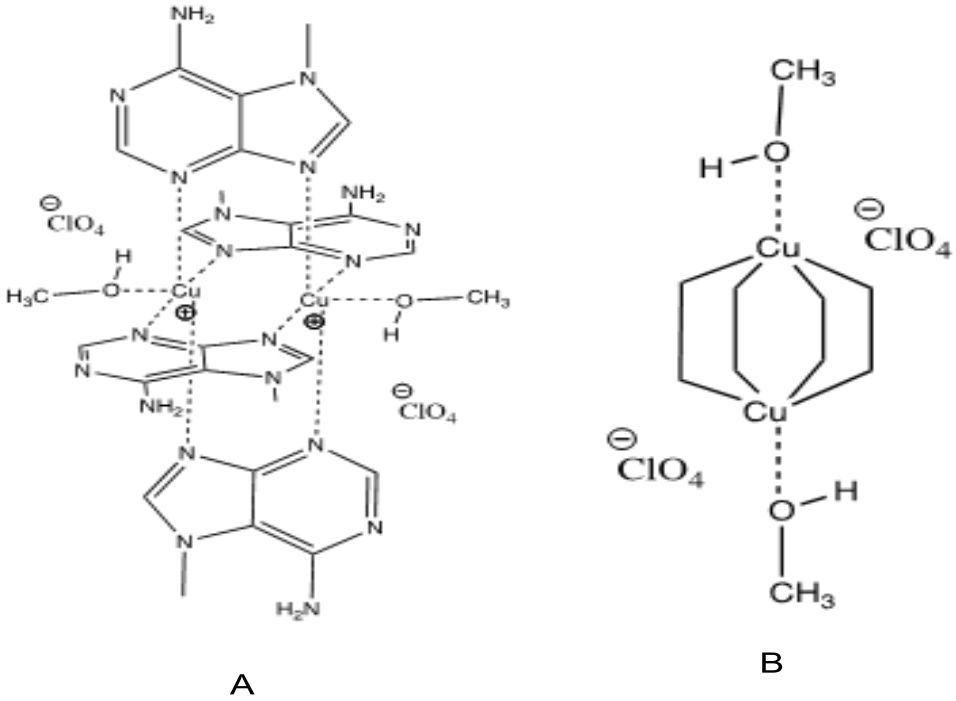
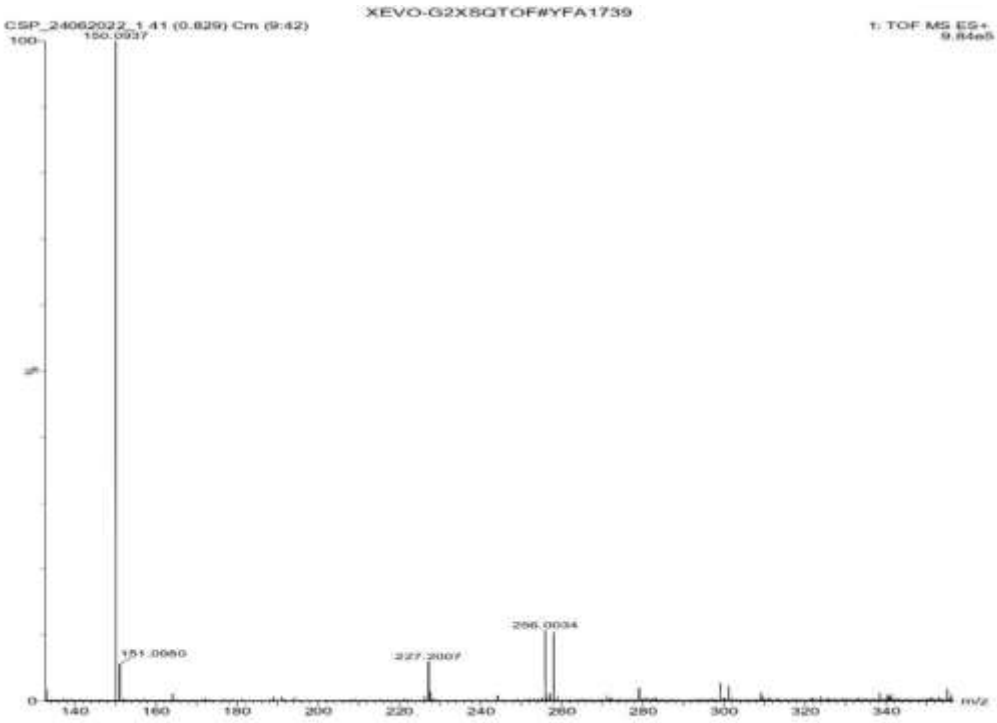


Figure4: A) Probable structure of copper complex. B) Cartoon representation of the complex, lines connecting copper centres are the adenine derivative.

2. **N-7-Methyl adenine:Cu complex (1:1)** Methyl adenine (5 mg, 0.033mmol) was dissolved in 7.5 ml of methanol and was placed in RB (100ml) with a stirring bath magnetic stirrer, it was placed on a stirrer and copper perchlorate hexahydrate Cu $(ClO_4)_2.6H_2O$ (12.22mg, 0.033 mmol) in 5ml of methanol was slowly added with continuous stirring, within 5minutes a precipitate was formed. The product thus formed was filtered and washed with methanol several times to remove the unreacted ligand and metal salt. The product was dried under a high vacuum and 3.68 mg product was obtained. Similarly, thiscomplex also had extremely low solubility with organic solvent and water, NMR studies could not be carried out. But, 1mg of the complex was solubilized with 3ml of Methanol, and Mass studies carried out. As the Mass spectrum of this compound was the same as the N-7-Methyl adenine: Cu complex (1:0.5), So, the probable Molecular Formula of the complex would be $\mathbf{C_{26}H_{36}Cu_2Cl_2N_{20}O_{10}^{2+}}$ and Molecular weight984.which was the same probable structure as Figure.4and are the same compound. 3358

3. **N-7Methyl adenine: Ag complex (1:1)** Methyl adenine (5mg, 0.033mmol) was dissolved in 7.5 ml of methanol and was placed in RB (100ml) with a stirring bath magnetic stirrer, it was placed on a stirrer and Silver perchloratemonohydrate (6.84 mg, 0.033 mmol) in 5ml of methanol was slowly added with continuous stirring. Within 5 minutes a precipitate was formed. The product thus formed was filtered and washed with methanol several times to remove the unreacted ligand and metal salt, this product was dried under high vacuum and a 3.8 mg product was obtained. As the complex was poorly soluble with organic solvent and with water NMR studies of the compound were not possible. Thus, 1mg of compound mixed with 3ml of Methanol and Mass studies was carried out. The Mass studies revealed that probable Molecular Formula of complex would be $\mathbf{C_{12}H_{14}Ag_2N_{10}^{2+}}$and Molecular weight514.05 (m/z = 255.97 expected, Experimental Value m/z= 256.0034) was given in Figure- 5 and theprobable structure with as Figure-6

Figu



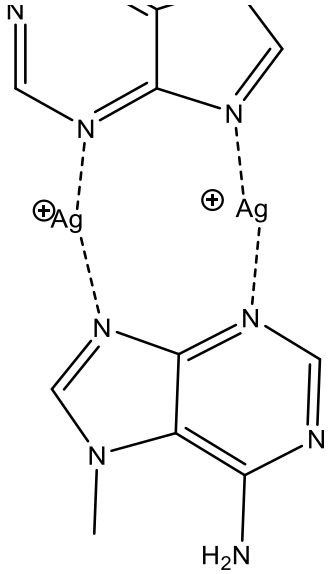


Figure6: Probable structure of N-7-Methyl adenine: Ag complex

INVITRO ANTIBACTERIAL STUDY

This study was carried out to investigate the antibacterial and antifungal potentials of synthesized compound N-7 methyl adenine, N-7 methyl adenine: Ag Complex (1:1), N-7 methyl adenine: Cu Complex (1:1) and N-7 methyl adenine: Cu Complex (1:0.5).

The study aims to assess the antimicrobial activity and to determine the zone of inhibition of synthesized uncomplexed and complexed compound on some bacterial and fungal strains. The antibacterial activity of the synthesized compound (200, 400, 600 & 800µg/ml) were tested against (*E. coli, S. typhimurium, P. aeruginosa,* and *S. aureus*) and the antifungal activity of synthesized compound (100, 200, 300 & 400 µg/ml) were tested against fungi strain (*S. pombe*) usingagardisc diffusion method.

The Zone of inhibition of synthesized compounds were compared with that of different standards like Ciprofloxacin for antibacterial and Fluconazole for antifungal activity.

The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms

Antimicrobial activity.

The Gram (-ve) bacterial strain of *Escherichia coli, Salmonellae typhimurium, Pseudomonas aeruginosa,* and Gram (+ve) *Staphylococcus aureus*were obtained from MTCC (Microbial Type Culture Collection and Gene Bank), Chandigarh. The microorganisms were maintained in slant tubes having 5 ml respective media. The organisms were sub-cultured in tubes having the broth media, which contain the same composition without agar. The tubes were incubated at $37^{o}C \pm 1^{o}C$ for 24 hours.

The synthesized compound N-7 methyl adenine was suspended in 10% DMSO. The solution was sterilized by passing through a Millipore filter (0.22 mm). The stock solutions were diluted to 10µg/ml, 25µg/ml, 50µg/ml, 100µg/ml, 125µg/ml, 150µg/ml, 175 µg/ml, and 200 µg/ml for determination of Minimum Inhibitory Concentration (MIC) and the MIC was found 200 µg/ml.

200 µg/ml, 400 µg/ml, 600, µg/ml, and 800 µg/ml of N-7 methyl adenine were taken for experimental work. Ciprofloxacin was suspended in 10% DMSO and diluted to 25 µg/ml, 50 µg/ml, 75 µg/ml, and 100 µg/ml.

The culture media was prepared by taking peptone (0.6%), yeast extract (0.3%), tryptone (0.4%), dextrose (0.1%), beef extract (0.15%), agar (1.75%), distilled water quantity sufficient to 100 ml. All components were assorted properly in distilled water by applying gentle heat, and a certain quantity of agar was dissolved by heating in a water bath. NaClwasused to adjust the pH of the medium to 7.4, and the volume of the medium was adjusted with distilled water. For sterilization, the broth was sterilized by autoclave for 15 minutes at 121 ^{0}C and 15 pounds of pressure per square inch.

Experimental protocol for antibacterial activity

For antibacterial activity studies, the synthesized compound and metal complexes were tested using the following experimental design.

Group-I
Negative Control: DMSO + *E. coli*

Positive Control: Ciprofloxacin (at 25, 50, 75, and 100 µg/ml.) + *E. coli*
Test Drug-1: N-7 methyl adenine (at 200, 400, 600, and 800 µg/ml) + *E. coli*
Test Drug-2: N-7 methyl adenine: Ag Complex (1:1) (at 200, 400, 600, and 800 µg/ml) + *E. coli*
Test Drug-3: N-7 methyl adenine: Cu Complex (1:1) (at 200, 400, 600, and 800 µg/ml) + *E. coli*
Test Drug-4: N-7 methyl adenine: Cu Complex (1:0.5) (at 200, 400, 600, and 800 µg/ml) + *E. coli*

Group-II
Negative Control: DMSO + *S. typhi*
Positive Control: Ciprofloxacin (at 25, 50, 75, and 100 µg/ml.) + *S. typhi*
Test Drug-1: N-7 methyl adenine (at 200, 400, 600, and 800 µg/ml) + *S. typhi*
Test Drug-2: N-7 methyl adenine: Ag Complex (1:1) (at 200, 400, 600, and 800 µg/ml) + *S. typhi*
Test Drug-3: N-7 methyl adenine: Cu Complex (1:1) (at 200, 400, 600, and 800 µg/ml) + *S. typhi*
Test Drug-4: N-7 methyl adenine: Cu Complex (1:0.5) (at 200, 400, 600, and 800 µg/ml) + *S. typhi*

Group-III
Negative Control: DMSO + *P.aeruginosa*
Positive Control: Ciprofloxacin (at 25, 50, 75, and 100 µg/ml.) + *P.aeruginosa*
Test Drug-1: N-7 methyl adenine (at 200, 400, 600, and 800 µg/ml) + *P.aeruginosa*
Test Drug-2: N-7 methyl adenine: Ag Complex (1:1) (at 200, 400, 600, and 800 µg/ml) *P.aeruginosa*
Test Drug-3: N-7 methyl adenine: Cu Complex (1:1) (at 200, 400, 600, and 800 µg/ml) + *P.aeruginosa*
Test Drug-4: N-7 methyl adenine: Cu Complex (1:0.5) (at 200, 400, 600, and 800 µg/ml) + *P.aeruginosa*

Group-IV
Negative Control: DMSO + *S. aureus*
Positive Control: Ciprofloxacin (at 25, 50, 75, and 100 µg/ml.) + *S. aureus*
Test Drug-1: N-7 methyl adenine (at 200, 400, 600, and 800 µg/ml) + *S. aureus*
Test Drug-2: N-7 methyl adenine: Ag Complex (1:1) (at 200, 400, 600, and 800 µg/ml) *S. aureus*
Test Drug-3: N-7 methyl adenine: Cu Complex (1:1) (at 200, 400, 600, and 800 µg/ml) + *S. aureus*
Test Drug-4: N-7 methyl adenine: Cu Complex (1:0.5) (at 200, 400, 600, and 800 µg/ml) + *S. aureus*

Antibacterial activity Assay by Disc Diffusion Method

The nutrient was transferred into a petri dish (sterilized) and permitted to solidify. The bacterial lawn was made by swab on nutrient agar plates from 10^3(CFU) colony-forming units (CFU) / ml of respective bacterial cultures. Sterilized paper discs (4 nos.) were soaked in a series concentration of standard and test drugs and placed on the bacterial lawn (inoculated plates), then incubated

for 24 hours at 37^0C. The antibacterial activity was assessed by measuring the diameter (in millimeters) of the zone of inhibition. All the tests were performed in triplicate. [35-37].

Invitro antifungal activity

For antifungal activity studies, the synthesized compound and metal complexes were tested using the following experimental design.

Group-V

Negative Control:	DMSO + *S. pombe*
Positive Control:	Fluconazole (at 25, 50, 75, and 100 µg/ml.) + *S. pombe*
Test Drug-1:	N-7 methyl adenine (at 100, 200, 300, and 400 µg/ml) + *S. pombe*
Test Drug-2:	N-7 methyl adenine: Ag Complex (1:1) (at 100, 200, 300, and 400 µg/ml) + *S. pombe*
Test Drug-3:	N-7 methyl adenine: Cu Complex (1:1) (at 100, 200, 300, and 400 µg/ml) + *S. pombe*
Test Drug-4:	N-7 methyl adenine: Cu Complex (1:0.5) (at 100, 200, 300, and 400 µg/ml) + *S. pombe*

Antifungal activity by Disc Diffusion Method

The synthesized compound N-7- methyl Adenine (Ligand) and its metal complexes in the ratio of Ligand metal complexes (L:M) as Cu (1:0.5), (1:1) and Silver (1:1) were screened for in vitro antifungal activity against *S.pombe*by paper disc diffusion method.
The selected fungus was seeded with 10^5 (CFU) ml^{-1} and suspension was inoculated with the agar plate, with the help of a spreader loop it was spread over the agar surface. Further paper disc of size 5 mm dipped in various concentrations (i.e.100, 200, 300, and 400µg/ml) of all the compounds and standarddrug Fluconazole. The entire discs were placed in the agar plate. Then, the plates were incubated at 27^0C for 72 hrs as per the reference procedure. After 72 hrs zone of inhibition was measured (in millimeters.) All the tests were performed in triplicate.[35, 38, 39].

RESULTS AND DISCUSSION

The compounds N-7 methyl adenine, N-7 methyl adenine: Ag Complex (1:1), N-7 methyl adenine: Cu Complex (1:1), and N-7 methyl adenine: Cu Complex (1:0.5) were synthesized and N-7 methyl adenine: Cu Complex (1:1) and N-7 methyl adenine: Cu Complex (1:0.5) probable structure was found to same. All the compounds were assessed for their antibacterial activity against two gram-negative bacterial strains (*E. coli, S. typhimurium)* two gram-positive bacteria *(P. aeruginosa,* and *S. aureus*) as well as one fungi strain (*S. pombe*) using paper disc diffusion method as per the reference methods. Zone of inhibition are recorded in Table 1 (bacteria) &Table 2 (fungi) and illustrated in Figure7-10(antibacterial activity) & Figure-11 (antifungal activity). The graphical antibacterial activity of synthesized compounds and complexes are shown in Figure 12-13.

The result revealed that the metal complex N-7 methyl adenine: Ag Complex (1:1) at a concentration of 800 µg/ml was potentially effective in suppressing microbial growth of all bacteria and fungi as compared to N-7 methyl adenine, N-7 methyl adenine: Cu Complex (1:1) and N-7 methyl adenine: Cu Complex (1:0.5) with reference to standards. The drugs N-7 methyl adenine showed variable antimicrobial activity against all four bacteria strains. N-7 methyl adenine: Cu Complex (1:1) was effective against three bacteria strains *E. coli, S. typhimurium,* and

S. aureus, whereas the compound N-7 methyl adenine: Cu Complex (1:0.5) exhibited an inhibitory effect against *E. coli* and*S. aureus* at only highest concentration both.

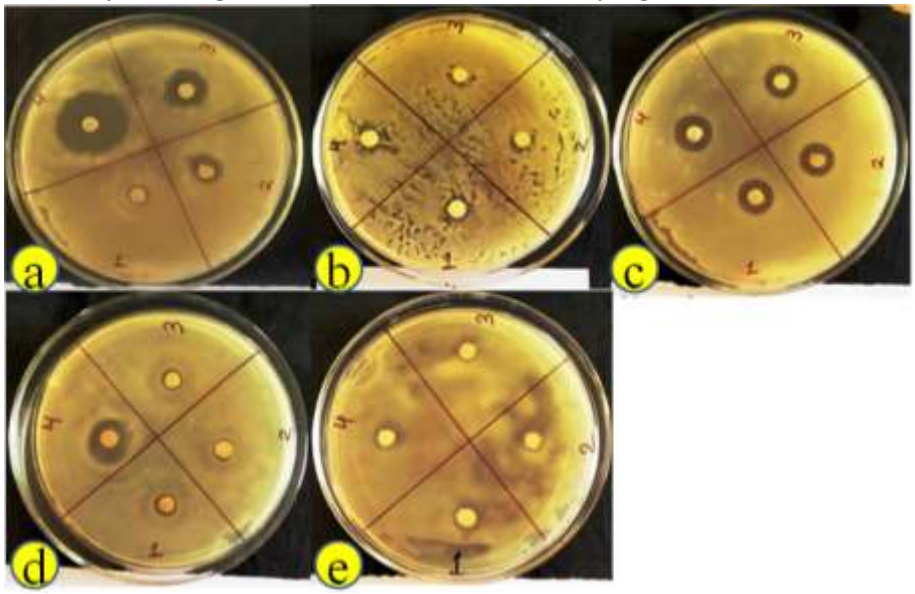


Figure 7: Antibacterial activity(*E.coli*) of drugs, a: Ciprofloxacin, b: N-7 methyl adenine, c. N-7 methyl adenine: Ag Complex (1:1), d. N-7 methyl adenine: Cu Complex (1:1), e. N-7 methyl adenine: Cu Complex (1:0.5)

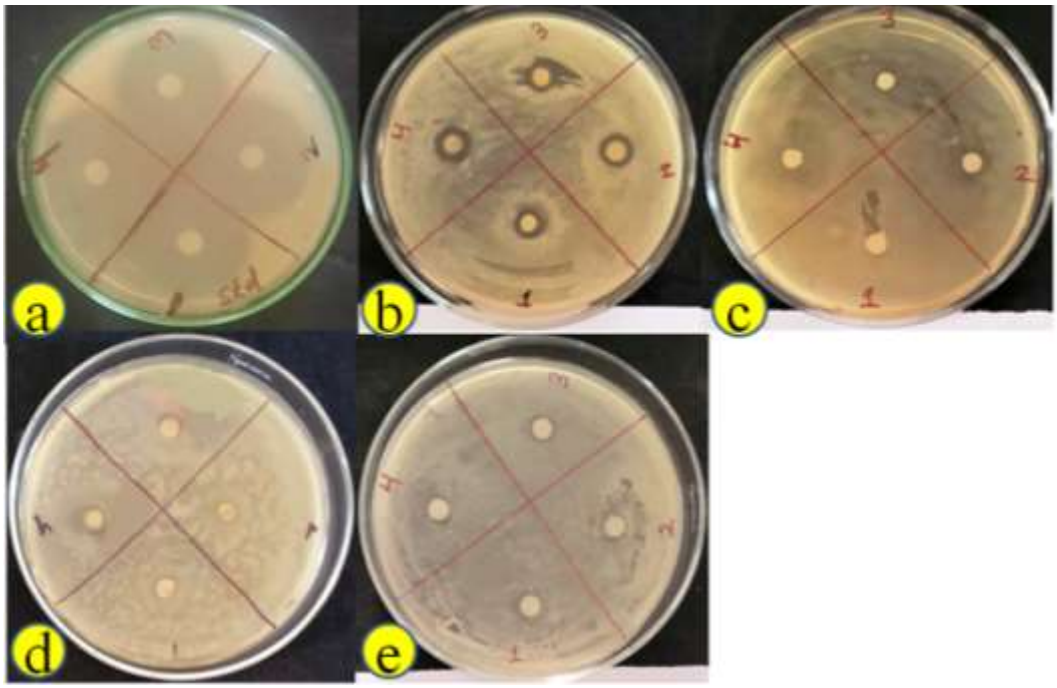


Figure 8:Antibacterial activity(*S. typhi*) of drugs, a: Ciprofloxacin, b: N-7 methyl adenine, c. N-7 methyl adenine: Ag Complex (1:1), d. N-7 methyl adenine: Cu Complex (1:1), e. N-7 methyl adenine: Cu Complex (1:0.5)

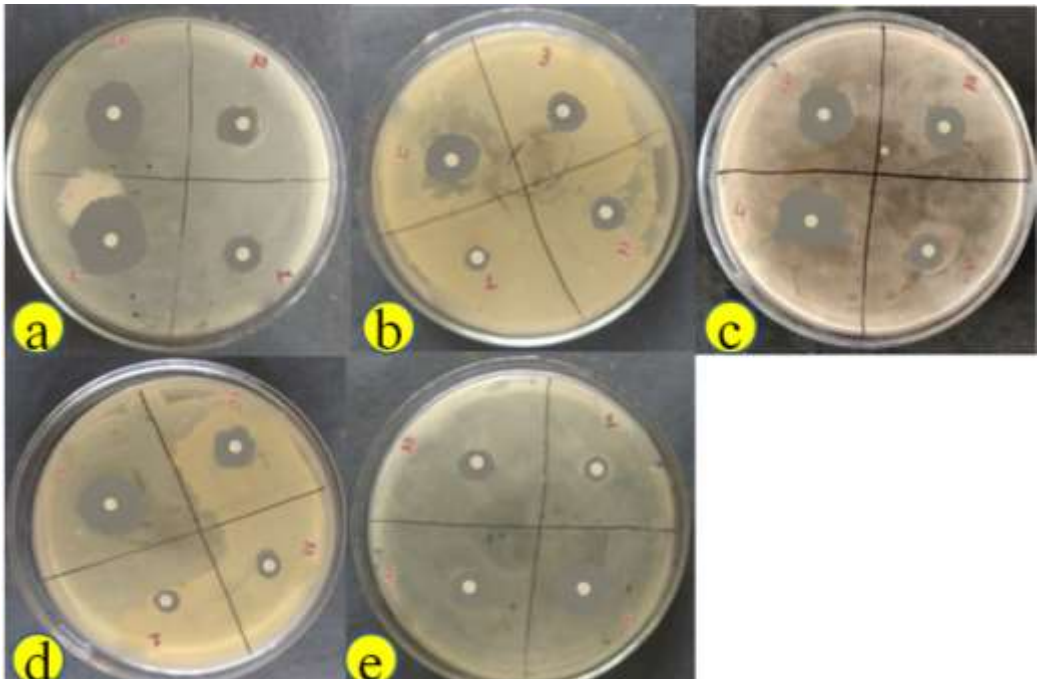


Figure 10:Antibacterial activity(*S. aureus*) of drugs, a: Ciprofloxacin, b: N-7 methyl adenine, c. N-7 methyl adenine: Ag Complex (1:1), d. N-7 methyl adenine: Cu Complex (1:1), e. N-7 methyl adenine: Cu Complex (1:0.5)

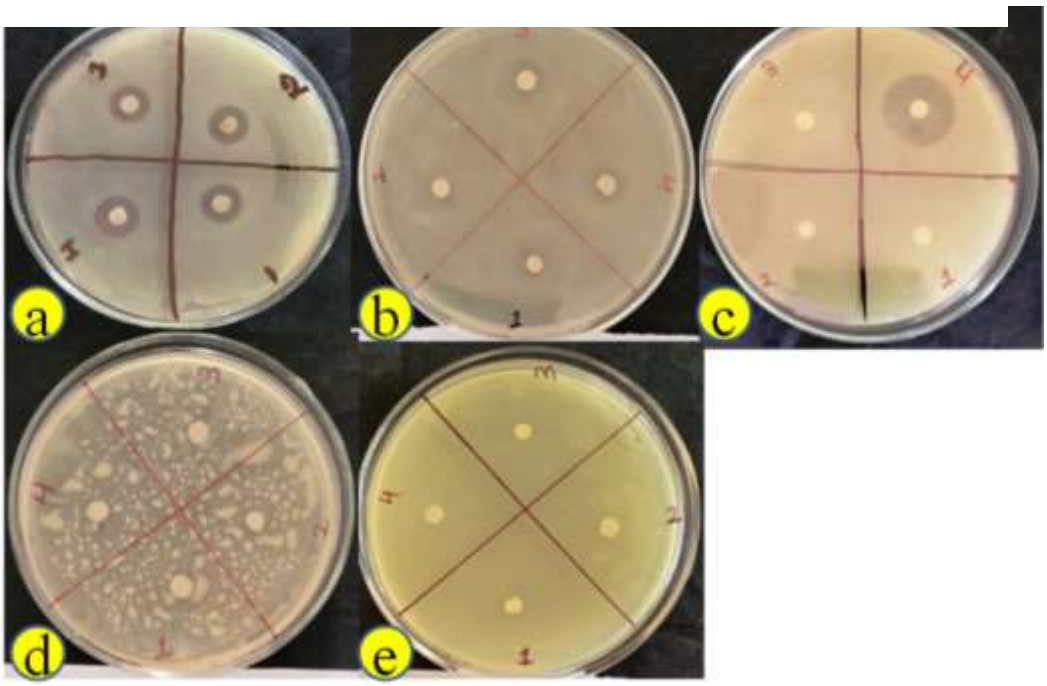


Figure 9:Antibacterial activity(*P. aeruginosa*) of drugs, a: Ciprofloxacin, b: N-7 methyl adenine, c. N-7 methyl adenine: Ag Complex (1:1), d. N-7 methyl adenine: Cu Complex (1:1), e. N-7 methyl adenine: Cu Complex (1:0.5)

- **Table 1:** Antibacterial activity of N-7 methyl adenine, N-7 methyl adenine: Ag Complex (1:1), N-7 methyl adenine: Cu Complex(1:1), and N-7 methyl adenine: Cu Complex (1:0.5)

• **Treatment**	• **Dose (µg/ml)**	• **Zone of inhibition (mm)**			
		• *E. coli* • (Gram -ve)	• *S. typhi* • (Gram -ve)	• *P. aeruginosa* (Gram -ve)	• *S. aureus* • (Gram +ve)
• Negative Control (DMSO + microorganism)	• 200 µl	• 0	• 0	• 0	• 0
	• 400 µl	• 0	• 0	• 0	• 0
	• 600 µl	• 0	• 0	• 0	• 0
	• 800 µl	• 0	• 0	• 0	• 0
• Positive	• 2	• 07	• 20	• 07 ±	• 08

Control (Ciprofloxacin + microorganism)	5 µg	± 0.89	± 0.13	0.29	± 0.19
	• 50 µg	• 08 ± 0.14	• 20 ± 0.36	• 07 ± 0.29	• 09 ± 0.14
	• 75 µg	• 11 ± 0.29	• 21 ± 0.32	• 08± 0.51	• 13 ± 0.22
	• 100 µg	• 16 ± 0.11	• 22 ± 0.18	• 09 ± 0.23	• 15 ± 0.16
• Test Drug-1 • N-7 methyl adenine	• 200 µg	• 0	• 0	• 0	• 0
	• 400 µg	• 0	• 07 ± 0.11	• 0	• 07 ± 0.26
	• 600 µg	• 07 ± 0.11	• 07 ± 0.24	• 07 ± 0.89	• 09 ± 0.19
	• 800	• 09 ± 0.39	• 08 ± 0.42	• 08 ± 0.36	• 11 ± 0.1

	µg				5
• Test Drug-2 • N-7 methyl adenine: Ag Complex (1:1)	• 200 µg	• 0	• 0	• 0	• 08 ± 0.36
	• 400 µg	• 07 ± 0.11	• 07 ± 0.17	• 0	• 09 ± 0.21
	• 600 µg	• 07 ± 0.21	• 07 ± 0.29	• 0	• 11 ± 0.09
	• 800 µg	• 09 ± 0.45	• 13 ± 0.24	• 14 ± 0.26	• 13 ± 0.89
• Test Drug-3 • N-7 methyl adenine: Cu Complex(1:1)	• 200 µg	• 0	• 0	• 0	• 07 ± 0.63
	• 400 µg	• 0	• 0	• 0	• 07 ± 0.14
	• 600	• 08 ± 0.63	• 0	• 0	• 09 ± 0.8

	µg				8
	• 800 µg	• 11 ± 0.11	• 07 ± 0.67	• 0	• 12 ± 0.35
• Test Drug-4 • N-7 methyl adenine: Cu Complex (1:0.5)	• 200 µg	• 0	• 0	• 0	• 07 ± 0.21
	• 400 µg	• 0	• 0	• 0	• 08 ± 0.23
	• 600 µg	• 0	• 0	• 0	• 08 ± 0.63
	• 800 µg	• 07 ± 0.71	• 0	• 0	• 09 ± 0.19

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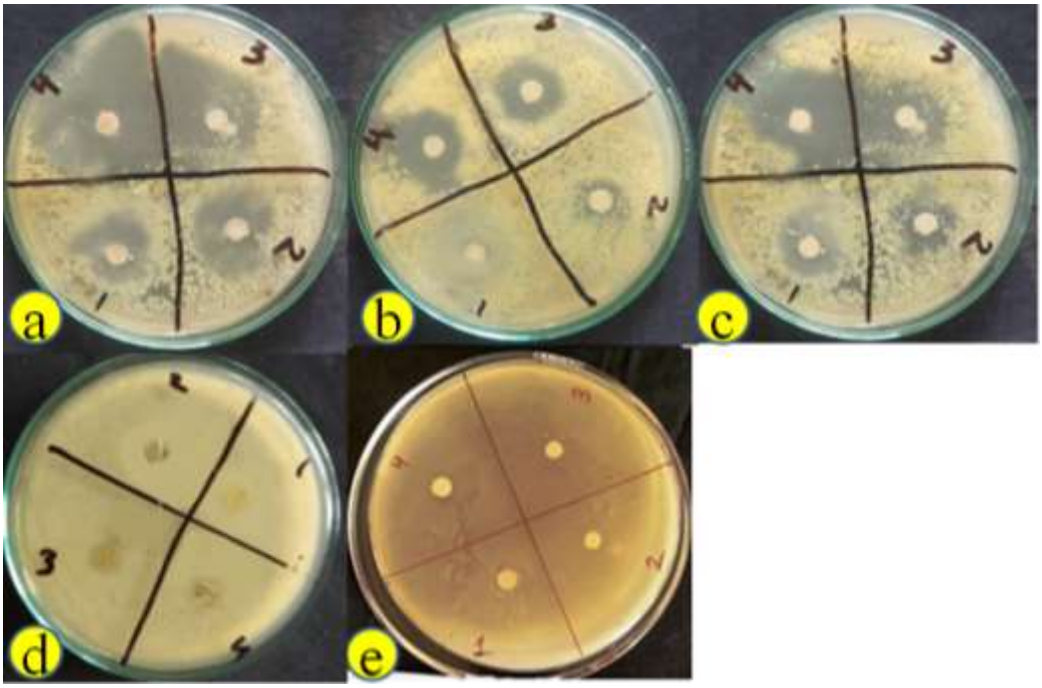

Figure 11:Antifungal activity(*S.pombe*) of drugs, a: Ciprofloxacin, b: N-7 methyl adenine, c. N-7 methyl adenine: Ag Complex (1:1), d. N-7 methyl adenine: Cu Complex (1:1), e. N-7 methyl adenine: Cu Complex (1:0.5)

Table 2: Antifungal activity of N-7 methyl adenine, N-7 methyl adenine: Ag Complex (1:1), N-7 methyl adenine: Cu Complex(1:1), and N-7 methyl adenine: Cu Complex (1:0.5)

Treatment	**Dose (**µg/ml**)**	**Zone of inhibition (mm)**
		S.pombe
Negative Control (DMSO + microorganism)	200 µl	0
	400 µl	0
	600 µl	0
	800 µl	0
Positive Control (Ciprofloxacin + microorganism)	25 µg	13 ± 0.14
	50 µg	15 ± 0.26
	75 µg	18 ± 0.24
	100 µg	23 ± 0.13
Test-1 N-7 methyl adenine	100 µg	07 ± 0.11
	200 µg	08 ± 0.28
	300 µg	10 ± 0.33
	400 µg	12 ± 0.11
Test-2 N-7 methyl adenine: Ag Complex (1:1)	100 µg	11 ± 0.22
	200 µg	11 ± 0.13
	300 µg	13 ± 0.19
	400 µg	19 ± 0.15
Test -3 N-7 methyl adenine: Cu Complex(1:1)	100 µg	0
	200 µg	0
	300 µg	7
	400 µg	7
Test -4 N-7 methyl adenine: Cu Complex (1:0.5)	100 µg	0
	200 µg	0
	300 µg	0
	400 µg	0

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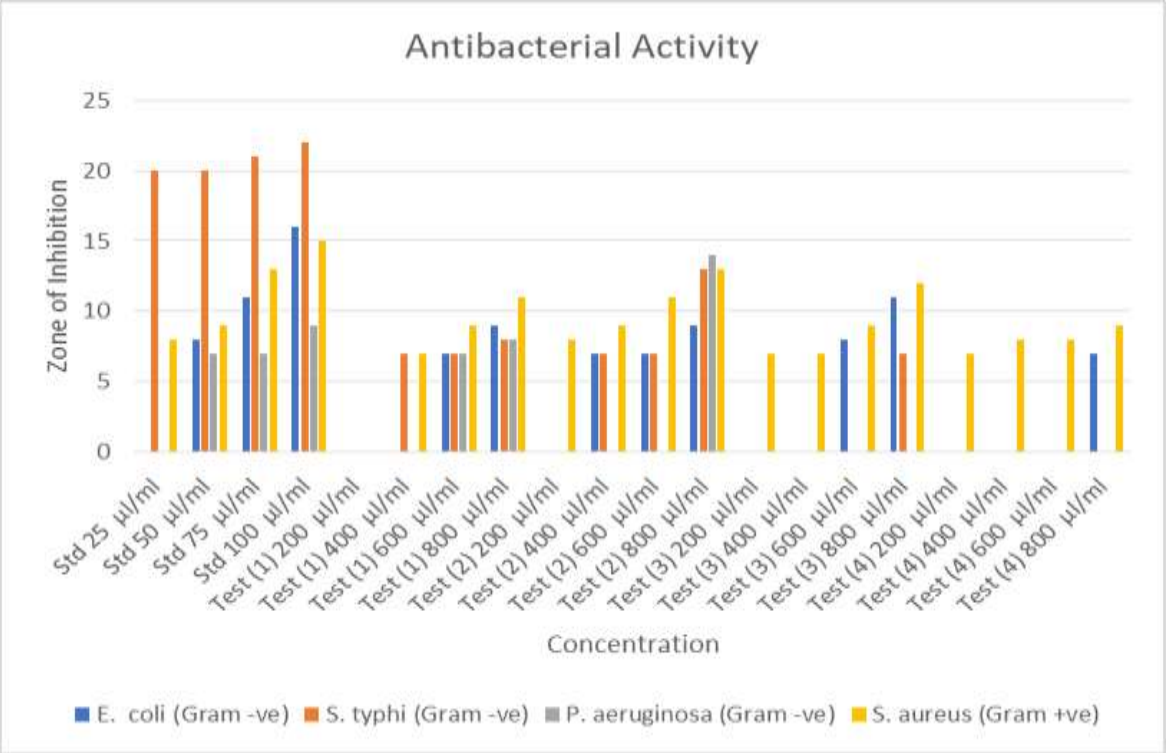

Figure 12: The graphical figure for Antibacterial activity

(Std. Ciprofloxacin drug, **Test-1.**N-7 methyl adenine,**Test-2**N-7 methyl adenine: Ag Complex (1:1),**Test -3. N-**7 methyl adenine: Cu Complex (1:1), **Test -4.**N-7 methyl adenine: Cu Complex (1:0.5)**)**

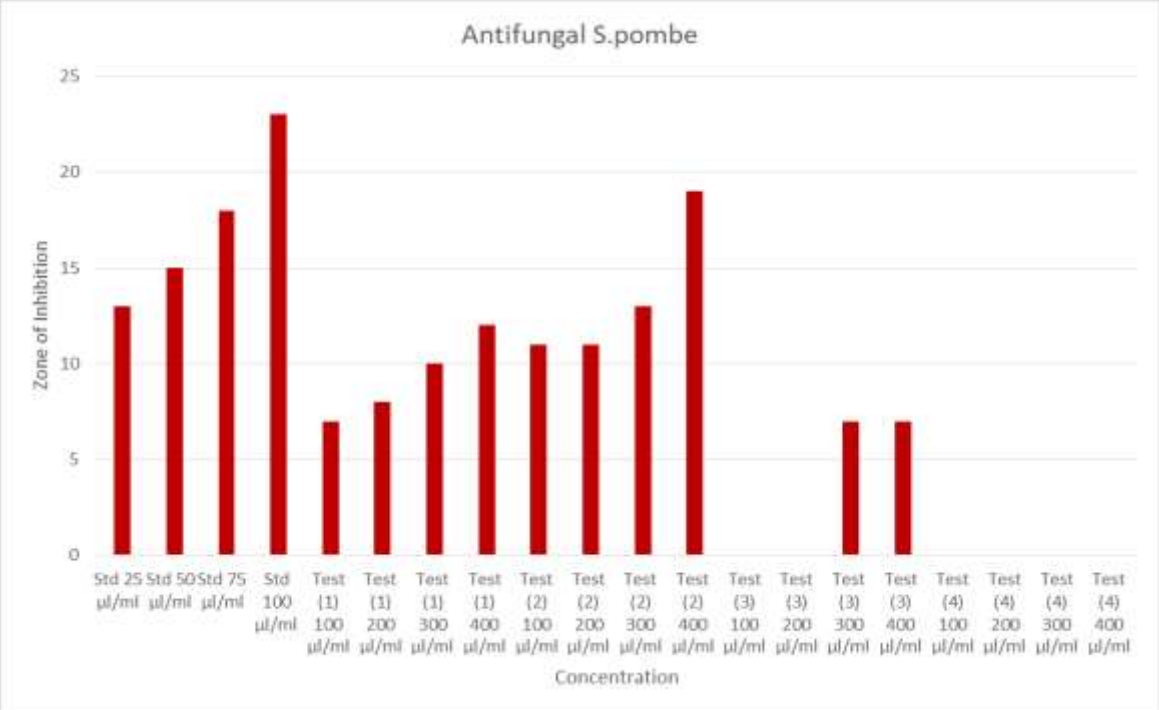

Figure 13: The graphical figure for Antifungal activity

(Std. Fluconazole drug, Test-**1.**N-7 methyl adenine, **Test-2.**N-7 methyl adenine: Ag Complex (1:1),**Test-3. N-**7 methyl adenine: Cu Complex (1:1), **Test -4.**N-7 methyl adenine: Cu Complex (1:0.5)**)**

CONCLUSION

Adenine analogous is widely used as different therapeutic agents as an anticancer, antibacterial, antiviral, antifungal, antiparasitic, and antitubercular agent, But, Current urgently requires newer antimicrobial agents for different infectious diseases due to resistance of various Pathogens. We prepare unnatural N-7 adenine derivative and their Cu (II) complexes and Ag (I) complex for the study of antibacterial activity.

TheCu complex (1:1) and (1:0.5) are found same probable structure, but higher antimicrobial activity is shown by Cucomplex (1:1) than Cu complex (1:0.5), it may be due to higher Cu^{2+} concentration, the N-7 methyl adenine: Ag Complex (1:1) shown better activity than Cu complexes and uncomplexedcompound. Further synthesizing and exploring other biological activityof N^7 -substituted adenine analogous and other metal complexesmay be developed as novel clinical therapeutic agents. So, it can be used to discover synthesized compounds that may serve as leads in the development of new pharmaceutical research activities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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