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ANTIBACTERIAL ACTIVITY OF ORGANOMETALLIC COMPLEXES OF CHOLIC ACID

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Cholic acid and metal ions both have antibacterial activity; therefore, their organ metallic complexes were prepared to have synergistic effect. Cholic acid is one of the leading molecule for preparing organo-metallic complexes and their complexes were found to have larger antibacterial activity.

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1. Introduction

Cholic acid, a main bile acid, is a biosurfactant involved in the digestion of dietary lipids .It is commercially available at low cost. Furthermore, it has an unusual molecular structure with some special characteristics, such as the facial amphiphilicity. The carboxylic acid and three hydroxylic groups can act as synthesis handles. For these reasons cholic acid is a suitable building block for new functional molecules. Because of the differences in steric hinderance each hydroxyl group can be derivatized individually. Therefore, cholic acid can be used as a scaffold for combinatorial chemistry and asymmetric synthesis. Not only the co-directed hydroxyl groups are useful for these purposes, but also the side chain with a carboxylic group is important since it provides attachment point to the solid phase. The rigid steroid unit of cholic acid has a curvature, which facilitate the construction of cyclic compounds, so called cholaphanes. These compounds are widely used as receptors for small molecules. Also many other types of receptors were built from cholic acid in which usually two or more molecules are linked together to form a tweezer-type of receptor.

Cholic acid having nitrogen containing groups attached to the hydroxylic groups, permeabilizes the outer bacterial membrane .A short alkyl chain at the place of carboxylic group promotes transport through the membrane. Therefore, exhibit antimicrobial activity. Because of their interaction with bilayers, cholic acid derived facial amphiphiles can also be used as membrane fusogens. Other medical applications of cholic acid derivatives are as drug-delivery agents, as transfection agents & as X-ray contrast agents. In all these cases cholic acid facilitates transport of more polar molecules across the membrane bilayer by shielding them from the a polar interior. Cholic acid derivatives have been used for chiral separations, e.g. as stationary phase for HPLC & in inclusion chemistry.

Cholic acid, a natural biodetergent has been reported to exhibited antibacterial[11-14], antiviral[5], antifungal[4], antimalarial[10], antitubercular[10], anticancer[9], sperrmicidal[2,3], antiallergic [6-8] etc. Since cholic acid is a suitable building block for new molecules or in other words, it is a leading substance for the development of various compounds. Therefore, it is thought worthwhile to select it for the above research work. The antimicrobial activity of metal chelates was found to be in the order[1]: $Cd^{II} > Ni^{II} > Mn^{II} > Cu^{II} > Zn^{II} > Co^{II} > Fe^{II}$ .Cholic acid is one of the lead molecule for preparing organometallic complexes & their complexes were found to have more active antibacterial activity because of synergistic effect of cholic acid as well as metal ions.

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2. Experimental

Material Required

Micro-organism used: *Micrococcus luteus (106), Bacillus subtilis (121), Klebsiela pneumonia (109), Pseudomonas aeruginosa(424), Streptococcus pneumonia (267)* Collected from NBRI, Lucknow.Dimethyl sulfoxide (DMSO) Potato dextrose agar (PDA) purchased from Hi Media Laboratories Pvt. Ltd., Mumbai , India

Method:

Preparation of the tested organisms:

A) Preparation of standard bacterial suspensions:

The average number of viable, *Micrococcus luteus* (106), *Bacillus subtilis* (121), *Klebsiela pneumonia* (109), *Pseudomonas aeruginosa* (424),*Streptococcus pneumonia* (267) organisms per ml of the stock suspensions was determined by means of the surface viable counting technique. About (10^8 - 10^9) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

B) In vitro testing for antibacterial activity:

Antimicrobial activity was determined against five bacterial pathogens by the agar disc diffusion assay (NCCLS (National Committee for Clinical Laboratory Standards), 2005). The test compounds were dissolved in Dimethyl Sulfoxide (DMSO) and then antimicrobial effect of test compounds were tested. Petri dishes (measuring 90 mm each side) containing 20 mL of nutient agar. At the same time, 6 mm diameter sterile Whatman Antibiotic disc were placed on the surface of the inoculated agar plates, and then appropriate concentration of the test compounds in DMSO were applied onto the discs. The plates were incubated at 37^0C for 24 h. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs. Standard discs of the antibiotic Gentamycin (10 μg) and cholic acid (10 μg) served as the positive antibacterial controls. After that, the diameter of inhibition zone was measured in millimeters. All tests were repeated three times to minimize test error [18,19].

Antibacterial activity of these compounds was carried out by disc diffusion method using ampicillin as standard. In this technique, the filter paper(Whatmann No.1) sterile disc of 5 mm diameter, impregnated with the test compounds(10μg/ml of Dimethyl sulfoxide) along with standard was placed on the nutrient agar plate at 37°C for 24 hours in BOD incubator. The inhibition zones around the dried impregnated disc were measured after 24 hours Fig.[1-3].

Antibacterial activity

Accurate determination of bacterial susceptibility to antibiotics is essential to the successful management of bacterial infections and to the comparative analysis of antimicrobial agents. This can be done by a number of techniques, which include the disc diffusion method, the broth dilution assay and the E tests. The effectiveness of antibiotics can be assessed by their ability to suppress bacterial growth, described by the MIC, or by their ability to kill bacteria, characterized by the minimal lethal concentration (MLC). MIC is usually derived by means of tests in solid media, whereas both MIC and MLC can be determined in broth dilution assays [15-17]. Kirby-Bauer disc diffusion (DD) - Agar diffusion refers to the movement of molecules through the matrix that is formed by the gelling of agar. When performed under controlled conditions, the degree of the molecule's movement can be related to the concentration of the molecules. This phenomenon forms the basis of the agar diffusion assay that is used to determine the susceptibility or resistance of a bacterial strain to an antibacterial agent, (e.g., including antibiotics) .When the seaweed extract known as agar is allowed to harden, the resulting material is not impermeable. Rather, there are spaces present between the myriad of strands of agar that comprise the hardened polymer. Small molecules such as antibiotics are able to diffuse through the

agar. Typically, an antibiotic is applied to a well that is cut into the agar. Thus, the antibiotic will tend to move from this region of high concentration to the surrounding regions of lower antibiotic concentration. If more material is present in the well, then the zone of diffusion can be larger. This diffusion was the basis of the agar diffusion assay devised in 1944. A bacterial suspension is spread onto the surface of the agar. Then, antibiotic is applied to a number of wells in the plate. There can be different concentrations of a single antibiotic or a number of different antibiotics present. Following a time to allow for growth of the bacteria then agar is examined. If bacterial growth is right up to the antibiotic containing well, then the bacterial strain is deemed to be resistant to the antibiotic. If there is a clearing around the antibiotic well, then the bacteria have been adversely affected by the antibiotic. The size of the inhibition zone can be measured and related to standards, in order to determine whether the bacterial strain is sensitive to the antibiotic. This technique can also be done by placing disks of an absorbent material that have been soaked with the antibiotic of interest directly onto the agar surface. The antibiotic will subsequently diffuse out of the disk into the agar. This version of agar diffusion is known as the Kirby-Bauer disk-diffusion assay. The agar diffusion assay allows bacteria to be screened in a routine, economical and easy way for the detection of resistance.

Table 1: Antibacterial activity of organometallic complexes of Cholic Acid (Zone of Inhibition (mm) dia. ± S.E)

Compound Code	**Micrococcus luteus(106)**	**Bacillus subtilis(121)**	**Streptococcus pneumoniae(267)**	**Klebsiela pneumoniae(109)**	**Pseudomonas Ae aeruginosa(424)**
BSN-I	15.00±0.57	12.00±1.15	13.00±0.50	11.00±0.57	10.52±0.76
CN-II	16.00±0.57	15.00±0.57	16.00±0.57	07.83±0.44	13.00±0.50
ZA-III	18.66±0.66	07.83±0.44	15.00±0.57	08.50±0.29	15.66±0.33
MCL-IV	13.00±0.50	15.66±0.33	17.33±0.33	17.00±0.57	15.00±0.57
NA-V	11.33±0.66	11.00±0.57	12.00±1.15	18.66±0.66	16.00±0.57
MA-VI	08.00±0.28	19.00±0.57	08.50±0.29	15.66±0.33	17.33±0.6
CCL-VII	08.50±0.29	18.66±0.66	16.00±0.57	10.50±0.76	13.00±0.50
AN-VIII	17.33±0.66	19.00±0.57	13.00±0.50	12.00±1.15	18.66±0.66
LA-IX	13.00±0.50	17.00±0.57	18.66±0.66	17.33±0.60	10.50±0.76
CON-X	11.00±0.57	08.10±0.16	15.66±0.33	17.33±0.60	07.83±0.44
BN-XI	17.33±0.60	10.50±0.76	17.33±0.60	16.00±0.57	15.00±0.76
CDN-XII	15.66±0.33	16.00±0.57	10.50±0.76	13.00±0.50	12.00±1.15
CUA-XIII	17.00±0.57	13.00±0.50	07.83±0.44	19.00±0.57	08.50±0.29
CUS-XIV	12.00±1.15	17.33±0.60	17.00±0.57	15.00±0.57	08.50±0.29
CA	10.50±0.76	08.50±0.29	19.00±0.57	19.00±0.57	17.00±0.57
Gentamycin	18.00±0.21	18.26±0.42	18.11±0.32	18.08±±0.28	18.72±0.68

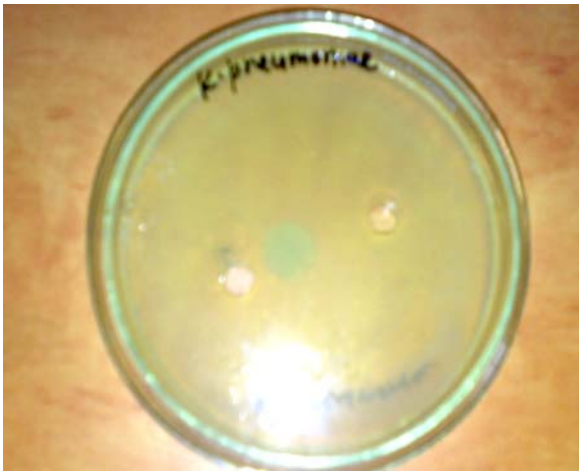

Fig.1. Zone of inhibition of Klebsiela pneumoniae (109) by CUS- XIV

Fig.2. Zone of inhibition against Micrococcus luteus (106) by CN-II

Fig.3. Zone of inhibition of CUA XIII & CA against S.aureus

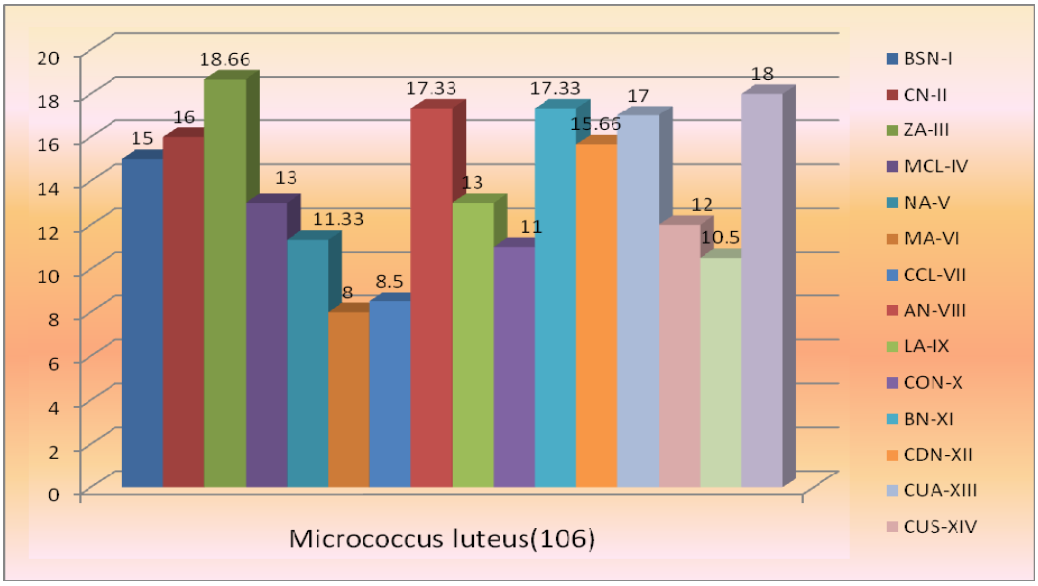

Fig. 5. Antibacterial activity against Micrococcus luteus (106)

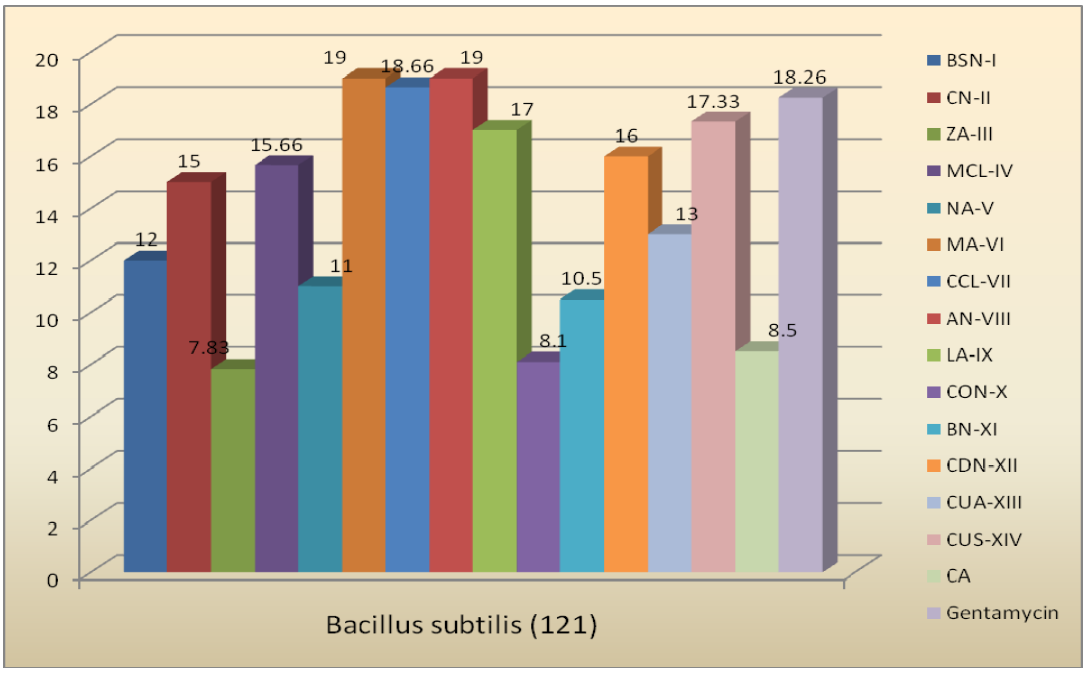

Fig.6. Antibacterial activity against Bacillus subtilis (121)

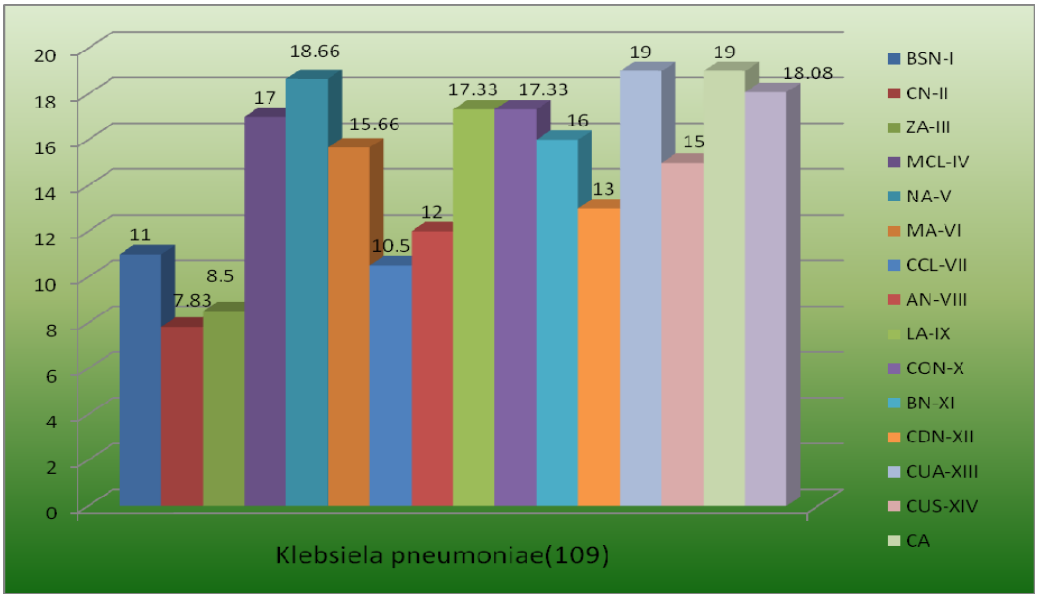

Fig.7. Antibacterial Activity against Klebsiela pneumonia (109)

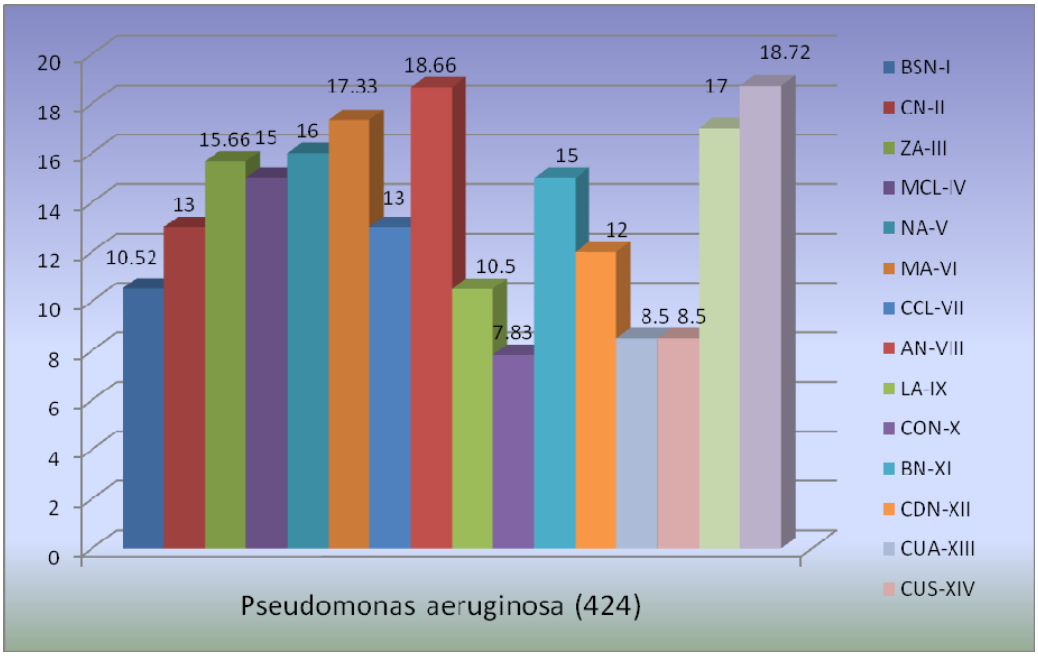

Fig.8. Antibacterial Activity against Pseudomonas aeruginosa(424)

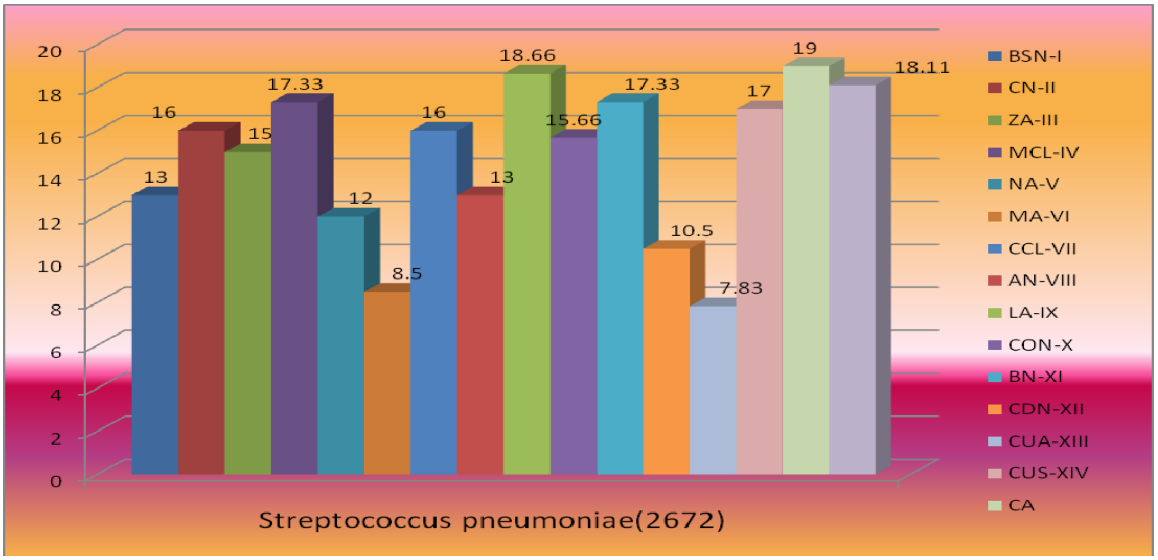

Fig.9. Antibacterial Activity against Streptococcus pneumonia (267)

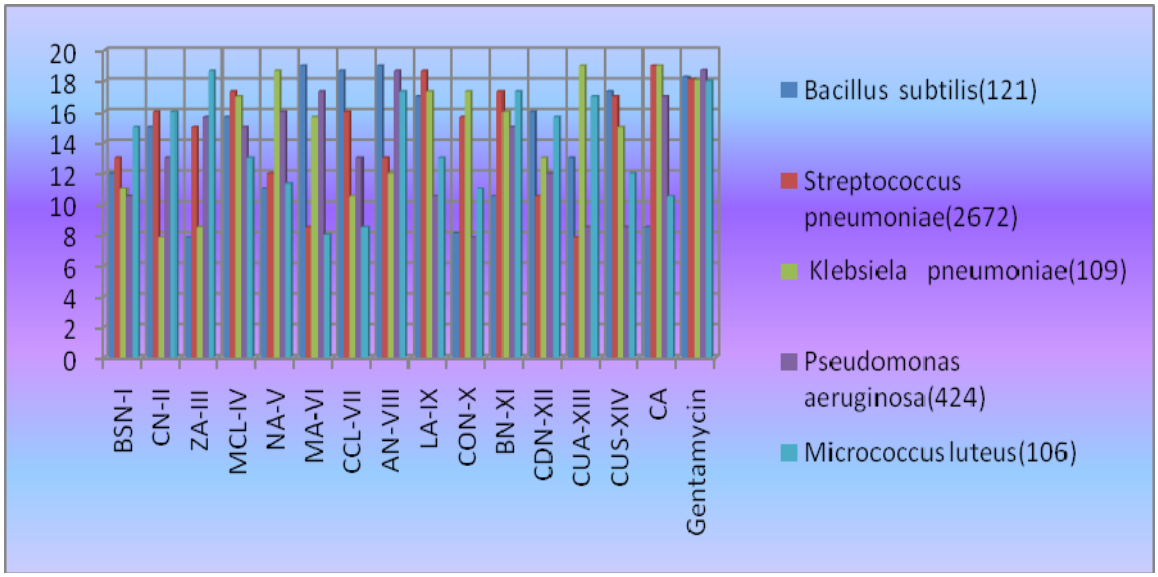

Fig.10.Antibacterial Activity against Streptococcus pneumonia (267), Pseudomonas aeruginosa(424), Klebsiela pneumonia (109), Bacillus subtilis (121) & Micrococcus luteus (101)

3. Results and discussion

From Table 1, it is observed that ZA III was more active than standard i.e Gentamycin against *Micrococcus luteus (106)*, AN VIII & BN-XI were equally potent to standard.MA-VI & CCL-VII were least active. Except MA-VI, all compounds were more active than CA i. e cholic acid.MA-VI ,CCL-VII and AN-VIII were found more active than standard i.e Gentamycin against *Bacillus subtilis (121)*, LA-IX & CUS-XIV were equally potent to standard.ZA-III & CON-X were least active. Except ZA-III & CON-X, all compounds were more active than CA i. e cholic acid.LA-IX & CA were found more active than standard i.e Gentamycin against *Streptococcus pneumonia (2672)*, MCL-IV,BN-IX & CUS-XIV were almost equally potent to standard.MA-VI & CUA-XIII were least active. None of the compounds were more active than CA i. e cholic acid.NA-V, CUA-XIII & CA were found more active than standard i. e Gentamycin against *Klebsiela pneumonia(109)* ,MCL-IV,LA-IX,CON-X were almost equally potent to standard.CN-II & ZA-III were least active. Except CUA-XIII, all other compounds were less active than CA i. e

cholic acid. AN-VIII was equally potent to standard i. e Gentamycin against *Pseudomonas aeruginosa (424)* MA-VI & CA were slightly less potent than standard i. e Gentamycin & CON-X,CUA-XIII & CUS-XIV were least active.MA-VI & AN-VIII were more active than CA i. e cholic acid.ZA-III,MCL-IV,NA-V & BN-XII were almost equally potent to CA i. e cholic acid Fig.[5-10].

4. Conclusions

Some of the organometallic complexes of cholic acid like ZA-III were found to be more active against *Micrococcus luteus (106),* MA-VI ,CCL-VII and AN-VIII were found more active against *Bacillus subtilis (121),* LA-IX & CA were found more active against *Streptococcus pneumonia (2672),* NA-V, CUA-XIII & CA were found more active against *Klebsiela pneumonia(109)*&AN-VIII was equally potent against *Pseudomonas aeruginosa (424)* against standard i. e Gentamycin.

We conclude that organometallic complexes of cholic acid have more active antibacterial activity than cholic acid alone.

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