RESEARCH ARTICLE

ANTIMICROBIAL ANALYSIS OF SCHIFF BASE LIGANDS PYRAZOLE AND DIKETONE METAL COMPLEX AGAINST PATHOGENIC ORGANISMS.

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Abstract

Schiff base are flexi-dentate ligands, which are important to expand the Schiff base complexes. These complexes form excellent catalytic reaction by forming stable complex with metal ions and producing the biological activity against antibacterial, antifungal, anticancer and antimalarial activities. In this study, we have explore the Minimum Inhibitory Concentration (both liquid & Disc method) of Schiff base ligand such as Pyrazole, diketone and their metal (Ni, Cu, Co, Mn, Vn, Ca, Zn) bound complexes against various microbial pathogens. Minimum Inhibitory Concentration (MIC both liquid & disc method) were analyzed for the various human microbial pathogens such as Pseudomonas aeruginosa, Escherichia coli, Klebsilla pneumonia, Staphylococcus aureus. The sensitivity towards the various commercial antibiotics (Rifampicin, Streptomycin, Ampicillin, Gentamicin, Neomycin, Bacitracin, Erythromycin & penicillin) for the selected microbial human pathogens were also evaluated and compared. The MIC values indicate the most of the complexes have higher antimicrobial activity than the free ligand. The variation in the antimicrobial activity of different metal complexes against different microorganism depends on their impermeability of the cell or the differences in ribosomes in microbial cells. The lipid membrane surrounding the cell favours the passage of any lipid soluble materials and it is known that lipo solubility is an important factor controlling antimicrobial activity. The presence of low activity of some metal complexes is may be due to their low lipophilicity, because of which penetration of the complex through lipid membrane was decrease and neither block nor inhibit the growth of the microorganism.

Introduction:

Heterocyclic compounds are cyclic compounds, which have two different types of functional elements in the ring. Syntheses of heterocyclic compounds consist of potential pharmaceutical relevant properties and medical importance; therefore, these compounds have huge industrial application (Bhava et al., 2013; Tharmaraj et al., 2009) for the biological products. Schiff-base ligands are derived from an amino and carbonyl group of compounds typically...
importance class of ligand in chemistry for the development of Schiff base complexes. Due to this, Schiff-base ligands are potentially capable of forming stable complexes with metal ions via C=N azomethine nitrogen group for the biological activities such as antibacterial, antifungal, anticancer and anti-malarial activities (Lippard and Berg, 1995; Naeimi et al., 2007). The terms of bidentate and tridentate ligands, they are simultaneously coordinated with metal in the centre of ligand for the synthesis of homo and hetero metallic complexes with different stereochemistry. Among the many different dinucleating ligands, the phenol-based compartmental ligands had drawn particularly wide attention of scientific community. Over the decade, several reported studies on the application exits in the homogenous and heterogeneous catalysis, hence this work mainly enlighten the catalytic activity of Schiff base complexes(Dickson and Robson, 1974; Lippard and Berg, 1995; Naeimi et al., 2007) Syntheses of such compounds are highly importance in the pharmaceutical and chemists are focused in this area for the industrial application to develop the pharmaceutical products to human health. Schiff-base compound has donors imine group in the structure, which has structure similarities with neutral biological systems that are utilized to elucidating the mechanisms of transformation in the biological system(Gibson et al., 1970). This ligand formation requires some conditions that are not very stringent, necessitating in the method of dry solvent or a removing the water produced in the reaction between an aldehyde/ketone and an amine. Precisely, the lone pair on the nitrogen atom of the imine moiety in the Schiff base compounds offers an appropriate donor atom for coordination with metal ion. Therefore, these compounds are highly active with adept at binding transition metal ions, in the diverse range of uses to catalysts biological mimics(Reaven et al., 2010). Schiff bases are utilized as initial source in the synthesis of industrial products and are regarded as privileged ligands. Depending on their binding mode with transition metal ions in the living system have been of quite interesting in this field. In addition, metal bound complex of schiff bases have been reported to be high enzyme inhibitors and pharmacological applications(Pervez et al., 2016). These compounds would pave the antitumor effect against some Gram-negative bacteria and are used to be distinct in the field of microbiological testing for the tumour chemotherapy. Moreover, Copper coordination complexes have oxidation state of the copper ion in +1 or +2 with most stable conformation. The Copper (II) metal ion complexes derived from S-benzylthiocarbazate and saccharinate consist of anticancer properties against the leukemic cell line. Nickel (II) complexes have different variety of geometries that may vary from octahedral to square planar including the tetrahedral coordination modes. Consequently, in this work, Schiff-base ligands Pyrazole, diketone and their metal bound complexes were analyzed against anti-microbial activity. The activities of the standard drugs such as Rifampicin, Streptomycin, Ampicillin, Gentamicin, Neomycin, Bacitracin, Erythromycin and Penicillin were taken for comparative studies with pyrazole, diketone and their metal bound complexes.

Materials:-

Physical Measurement:-

All the reagents are AR grade chemicals used from commercial sources with well purification. Nutrient broth, Potato Dextrose Agar, Muller Hinton Agar, Tris base, sodium chloride, hydrogen peroxide and other chemicals were purchased from SRL, India.

Preparation of Metal complexes:-
The compounds Co (II), Cd (II), Ni(II), Zn(II), Mn(II), Vn (II) and Cu(II) were reacted and formed using an ethanolic solutions of metal halides/nitrates. The mixture was heated in the water bath for 2-3 hrs. The mixture of metal ions was mixed with pyrazole and diketone ligands solution for metal complexes.

Collection, Identification and Cultivation of Microbial Pathogens:-
The various microbial pathogens such as P. aeruginosa, E. coli, K. pneumoniae, S. aureus, were cultivated in nutrient agar medium. The grown pathogenic cultures were further identified based on morphological, microscopy and biochemical test.

Antibiotic sensitivity assay:-
The sensitivity towards the various antibiotics such as (Rifampicin, Streptomycin, Ampicillin, Gentamicin, Neomycin, Bacitracin, Erythromycin, Pencillin) against the pathogenic microorganism was analyzed. Sterile 50 ml nutrient broths were prepared and inoculated with P. aeruginosa, E. coli, K. pneumoniae, S. aureus,). The flasks were incubated in shaker for 24 hours at 37°C. Sterile Muller Hinton agar plates were prepared and were aseptically inoculated with the pathogenic cultures after 5 minutes the different antibiotic standard discs (Rifampicin, Streptomycin, Ampicillin, Gentamicin, Neomycin, Bacitracin, Erythromycin, Pencillin) were placed over the culture plate(Nomiya et al., 2000). After 5 minutes the different antibiotic standard disc (Nystatin) were placed over the culture plate. The plates were incubated at 37°C for 24 hours. The Minimal inhibitory concentration (MIC) in various
antibiotic susceptibility discs were tested (Fig. 1). The antimicrobial activity of metal ligand complex by disc method were analyzed as referred(Rafi et al., 2016). The sterile disc with metal and ligand was prepared with the minimum inhibitory concentration. All the selected complexes and the parent ligand were screened for their activity against the test organisms.

Minimum Inhibitor concentration:-
The MIC of the various metal ligand complex and ligand were analyzed as referred(Andrews and Andrews, 2001). 9ml of sterile Muller Hinton broth medium was prepared, and added with various metal ions such as Copper, Zinc, Cobalt, Manganese, Vanadium, and Nickel with the various concentration 50,100,150,200,250µl/ml by dissolving with 1ml of dimethylsulfoxide solution. After preparation of 10ml of Muller Hinton, broth in that 100µl was discarded and added with 100µl of culture. (P. aeruginosa, E. coli, K. pneumoniae, S. aureus). After incubation, the tubes were incubated in shaker incubator for 24 hours at 37ºC. The Minimum inhibitory concentration of growth was determined.

**Figures 1:** Zone Formation in Various antibiotic susceptibility discs.

**Figures 2:** Minimal inhibitory concentration (MIC) with metal – Pyrazole complex.

**Figures 3:** Minimal inhibitory concentration (MIC) with metal – Diketones complex.
Figures 4:- Microbial inhibition study by disc method using Nickel-Pyrazole complex.

Figures 5:- Microbial inhibition study by disc method using Copper-Pyrazole complex.

Figures 6:- Microbial inhibition study by disc method using Cobalt-Pyrazole complex.

Figures 7:- Microbial inhibition study by disc method using Manganese-diketones complex.
Figures 8:- Microbial inhibition study by disc method using Zinc diketones complex.

Figures 9:- Microbial inhibition study by disc method using Cobalt diketones complex.

Figures 10:- Microbial inhibition study by disc method using Vanadium diketones complex.

Figures 11:- Microbial inhibition study by disc method using Cadmium diketones complex.
Results and Discussion:

Among the observations in the MIC of metal bound pyrazole complex and metal free pyrazole, Ni-Pyrazole complex showed MIC activity of 100 μg/ml against S. aureus; 150 μg/ml against K. pneumoniae; and 200 μg/ml against of E. coli. The Cu pyrazole complex has shown 100 μg/ml of MIC activity against K. pneumoniae; 150 μg/ml of MIC against E. coli; 200 μg/ml MIC against S. aureus and P. aeruginosa. In metal free pyrazole, it shows MIC of 150 μg/ml against P. aeruginosa, S. aureus; 300 μg/ml against E. coli; 350 μg/ml against K. pneumoniae (Bhattarai et al., 2012). The metal complex such as Nickel and copper showed very good MIC of 100 μg/ml against S. aureus and K. pneumonia respectively (Fig. 2).

In magnesium diketone complex, 100 μg/ml against K. pneumoniae and P. aeruginosa; 200 μg/ml against E. coli. The zinc diketone complex it shows, MIC of 100 μg/ml against P. aeruginosa, 200 μg/ml against E. coli, S. aureus, and K. pneumoniae. The cobalt diketone complex it shows MIC of 150 μg/ml against E. coli, S. aureus; 200 μg/ml against P. aeruginosa, K. pneumoniae. The vanadium diketone complex it shows MIC of 100 μg/ml against E. coli, P. aeruginosa; 150 μg/ml against K. pneumoniae; 200 μg/ml against S. aureus. The cadmium diketone complex shows MIC of 150 μg/ml against E. coli, S. aureus; 200 μg/ml against P. aeruginosa, K. pneumoniae. Nickel diketone complex it shows MIC of 50 μg/ml E.coli, 100 μg/ml against S. aureus; 150 μg/ml against P. aeruginosa, K. pneumoniae. In diketones shows MIC of 150 μg/ml against S. aureus, 200 μg/ml against Escherichia coli, P. aeruginosa, K. pneumoniae. (Yadav et al., 2013) Comparative results of MIC activity of diketone alone and diketone–metal bound complex, the metal complex shown very good MIC activity than the pyrazole complex alone (Fig. 2).

Antimicrobial activity of the Metal ligand complexes by Disc Method:

The disc method of microbial inhibition study was performed using nickel-pyrazole complex with the concentration ranges from (180, 190, 200, 210 and 220 μg) against the E. coli. The MIC activity showed 17 mm high zone of inhibition in the concentration of 200 μg against E. coli. In P. aeruginosa, the various concentration ranges (180, 190, 200, 210, 220 μg) were performed and the MIC activity shows high zone of inhibition 7 mm against the concentration 200 μg. In S. aureus, MIC activity of 20 mm zone of inhibition against the 100 μg. In K. pneumonia, the MIC of 6 mm zone of inhibition against the 150-μg concentration (Fig. 4).

In copper-pyrazole complex, the effect of the complex was analyzed with various concentration in the range of (130, 140, 150, 160, 170 μg) against E. coli. The MIC activity showed 12 mm high zone of inhibition in the concentration of 150 μg against E. coli. In P. aeruginosa, the various concentration ranges (180, 190, 200, 210, 220 μg) were performed and the MIC activity shows high zone of inhibition 15 mm against the concentration 200 μg. In S. aureus, MIC activity of 20 mm zone of inhibition against the 100 μg. In K. pneumonia, the MIC of 7 mm zone of inhibition against the 150-μg concentration (Fig. 5).

In cobalt-pyrazole complex, the effect shows the concentration ranges from 130, 140, 150, 160, 170 μg against E. coli. The MIC activity showed 17 mm high zone of inhibition in the concentration of 150 μg against E. coli. In P. aeruginosa, the various concentration ranges (180, 190, 200, 210, 220 μg) were performed and the MIC activity shows high zone of inhibition 15 mm against the concentration 150 μg. In S. aureus, MIC activity of 24 mm zone of inhibition against the 150 μg. In K. pneumonia, the MIC of 14 mm zone of inhibition against the 200-μg concentration (Fig. 6).

While analyzing all above, the maximum zone was absorbed in MIC of 150 μg with 24 mm in S. aureus. In ligand pyrazole, the effect shows the concentration from (280, 290, 300, 310, 320 μg) performed in E. coli the MIC of 300
μg shown the zone which was obtained as 14 mm. *P. aeruginosa* the concentration from (130, 140, 150, 160, 170 μg) was performed, the MIC of 150 μg shown the zone which was obtained as 7 mm. In *S. aureus* the concentration from (130, 140, 150, 160, 170 μg) was performed, the MIC of 150 μg shown the zone which was obtained as a mm. In *K. pneumoniae* the concentration from (330, 340, 350, 360, 370 μg) was performed the MIC of 350 μg shown the zone which was obtained as 9 mm. Resulting from the above, the maximum zone was obtained for nickel-pyrazole 200 μg with 17 mm zone of inhibition in *E. coli*, for copper pyrazole 200 μg with 15 mm in *P. aeruginosa*, for 14 mm in *E. coli*.

In Magnesium-diketone complex the effect shows the concentration from (180, 190, 200, 210, 220 μg) in *E. coli* was performed, the MIC of 200 μg shown the zone which was obtained as 5 mm. In *P. aeruginosa* the concentration from (130, 140, 150, 160, 170 μg) was performed, the MIC of 150 μg shown the zone which was obtained as 10 Mm. In *S. aureus* the concentration from (80, 90, 100, 110, 120 μg) was performed the MIC of 100 μg shown the zone which was obtained as 12 mm. In *K. pneumoniae* the concentration from (130, 140, 150, 160, 170 μg) was performed, the MIC of 150 μg shown the zone which was obtained as 8 millimeter (Fig. 7).

In Zinc-diketones complex shows the concentration (180, 190, 200, 210, 220 μg) in *Escherichia coli* was performed, the minimal inhibition concentration of 200 μg shown the zone which was obtained as 10 mm. In *P. aeruginosa* the concentration from (80, 90, 100, 110, 120 μg) was performed, the MIC of 100 μg shown the zone which obtained 9 mm. In *S. aureus* the concentration from (180, 190, 200, 210, 220 μg) was performed, the MIC of 200 μg shown the zone which was obtained as 6 mm. In *K. pneumoniae* the concentration from (180, 190, 200, 210, 220 μg) was performed, the MIC of 200 μg shown the zone which obtained as 20 mm (Fig. 8).

In cobalt-diketones complex the concentration from (130, 140, 150, 160, 170 μg) in *E. coli* was performed, the MIC of 150 μg shown the zone which was obtained as 7 mm. In *P. aeruginosa* the concentration from (180, 190, 200, 210, 220 μg) was performed, the MIC of 200 μg shown the zone which was obtained as 15 mm. In *S. aureus* the concentration from (130, 140, 150, 160, 170 μg) was performed, the MIC of 150 μg shown the zone which was obtained as 9 mm. In *K. pneumoniae* the concentration from (180, 190, 200, 210, 220 μg) was performed, the MIC of 200 μg shown the zone which was obtained as 7 mm (Fig. 9). In vanadium–diketone complex the concentration from (80, 90, 100, 110, 120 μg) was performed, the MIC of 100 μg shown the zone which obtained as 7 mm. In *P. aeruginosa* the concentration from from (80, 90, 100, 110, 120 μg) was performed, the MIC of 100 μg shown the zone which was obtained as 6 mm. In *S. aureus* the various concentration from (180, 190, 200, 210, 220 μg) was performed, the MIC of 200 μg shown the zone which was obtained as 9 mm. In *K. pneumoniae* the concentration from (130, 140, 150, 160, 170 μg) was performed, the MIC of 150 μg shown the zone which was obtained as 7 mm (Fig. 10). In cadmium-diketone complex the concentration from (130, 140, 150, 160, 170 μg) in *E. coli* was performed, the MIC of 150 μg shown the zone which was obtained as 7 mm. In *P. aeruginosa* the concentration from (180, 190, 200, 210, 220 μg) was performed, the MIC of 200 μg shown the zone which was obtained as 7 mm. In *S. aureus* the concentration from (130, 140, 150, 160, 170 μg) was performed, the MIC of 150 μg shown the zone which was obtained as 7 mm. In *K. pneumoniae* the concentration from (130, 140, 150, 160, 170 μg) was performed, the MIC of 150 μg shown the zone which was obtained as 8 mm (Fig. 12).

In ligand diketone the concentration from (180, 190, 200, 210, 220 μg) in *E. coli* was performed, the MIC of 200 μg shown the zone which was obtained as 12 mm. In *P. aeruginosa* the concentration from (180, 190, 200, 210, 220 μg) was performed, the MIC of 200 μg shown the zone which was obtained as 12 mm. In *S. aureus* the concentration from (130, 140, 150, 160, 170 μg) was performed, the MIC of 150 μg shown the zone which was obtained as 20 mm. In *K. pneumoniae* the concentration from (180, 190, 200, 210, 220 μg) was performed, the MIC of 200 μg shown the zone which was obtained as 12 mm.

Results shows the maximum zone was obtained 150 μg with 10 mm in *S. aureus*, 200 μg with 20 mm in *K. pneumonia*, 200 μg with 15 mm in *P. aeruginosa*, 200 μg with 9 mm in *S. aureus*, 50 μg with 25 mm in *E. coli*. Antibiotic susceptibility test of streptomycin, it consists of 17 mm (sensitive) in *E. coli* when compared to the metal ion concentration of 200 μg it was obtained as 17 mm (Sensitive), the zone formation is sensitive. In *S. aureus* it
consists of 20 mm (sensitive) when compared to metal ion concentration 100 μg is 20 mm sensitive. Antibiotic susceptibility test of Gentamicin, the highest zone in S. aureus and E. coli as 25 mm (Sensitive) when compared to metal ion it gives the 25 mm (Sensitive) in Nickel complex. Antibiotic susceptibility test of Neomycin in E. coli consists of zone is 25 mm sensitive as like the Nickel complex. Antibiotic susceptibility test of Erythromycin in S. aureus consists of zone is 25 mm sensitive as like the ligand diketones.

**Conclusion:**

The various metals Nickel, Copper, cobalt, Zinc, Magnesium vanadium, cadmium and ligand bound metal complexes (Schiff base) ligands such as pyrazole and diketones were prepared. The various microbial pathogens such as Pseudomonas aeruginosa, Escherichia coli, Klebsilla pneumoniae Staphylococcus aureus, were cultivated in nutrient agar medium. The sensitivity towards the various antibiotics such as Rifampicin, Streptomycin, Ampicillin, Gentamicin, Neomycin, Bacitracin, Erythromycin and penicillin was analyzed the activity against the pathogenic microorganism. Result enlightens that, the minimal inhibitory concentration (MIC) values found to have higher antimicrobial activity with ligand bound complexes than the free ligands compared with antibiotics. Moreover, in the pyrazole bound complex, the maximum zones were obtained in nickel-pyrazole complex in the concentration of 200 μg with 17 mm zone of inhibition against E. coli, copper-pyrazole complex in the concentration of 200 μg with 15 mm in P. aeruginosa. In the case of diketone complex, the maximum zone was obtained 150 μg with 10 mm in S. aureus, 200 μg with 20 mm in K. pneumonia, 200 μg with 15 mm in P. aeruginosa, 200 μg with 9 mm in S. aureus and 50 μg with 25 mm in E. coli. Overall from this study, the prepared shiff base compounds showed higher activity against the pathological microorganism and potential to inhibit the growth of organisms.

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