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Antimicrobial activities of copper, cobalt and nickel cyanex complexes

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Abstract: The related researches are based on preparation of some complexes of the first transitional metal ions [Cu(II), Co (II) and Ni (II)] with the new solvent extracting reagent cyanex – 301 [i.e. bis (2, 4, 4-trimethyl pentyl) dithiophosphonic acid] as a ligand and finally on the studies of antimicrobial (antibacterial and antifungal) activities of the same. For their synthesis the prepared aqueous solutions of metal chlorides are allowed to react with an ethanolic solution of cyanex -301 (containing KOH) in different ratio. Disc diffusion methods were employed for antimicrobial assays against four human pathogenic bacteria and fungi. The bacterial properties of the metal complexes reveal that the copper cyanex complex is more effective against almost all antibacteria tested. Furthermore the copper and nickel complexes showed the highest antifungal activity against fungi *Penicilium sp.* and *Asphorgillus flavus*.
Key words: Antimicrobial activities, metal complexes

Introduction

The frequency of life threatening infectious diseases such as tuberculosis, cancer, AIDS, etc., caused by pathogenic microorganisms is increasing day by day and becoming an important cause of morbidity and mortality in immunocompromised patients. Synthetic chemical compounds constitute important sources of various bioactive compounds such as antibacterial, antifungal and anticancer compounds (Islam, Part, Zakaria). The synthesized chemical compounds, which are used for the treatment of infectious diseases are known as chemotherapeutic agents. Every year thousands of compounds are synthesized with an aim to find a potential chemotherapeutic agents to combat pathogenic microorganisms. But a very few compounds withstand as therapeutic agent by various methodological tests. Antimicrobial screening is one of these tests required to perform for primary selection of compounds as the therapeutic agents.

Metal chelation or complexation is involved in many important biological process where the co-ordination can occur between a variety of metal ions and a wide range of ligand (Shulman). Many types of ligand are known and the properties of their derived metal chelate have been investigated (Curtis). Prior to 1980, search for anticancer drugs was focused primarily on organic compounds (Sadler). However, with the discovery of cis-diammine dichloro platinum (II) which shows excellent antitumor activity, keen interest arose in exploring other inorganic compounds. Copper, Silver and gold complexes are among the most promising inorganic compounds known to possess anticancer activity. Copper is found in human cells and is primarily associated with copper –dependent enzymes that are required for normal metabolic process. The complexation of Co, Fe, Mg, Zn and Cu with nitrogen containing chain in the enzymes are very diverse (Tipton). The antimalarial activities of a series of 2-acetyl pyridine and their Cu, Ni, Fe, and Mn complexes have been tested for their antimalarial and antiteukemic properties. These compounds have been found to possess significant antimalarial activities (Klyman).

Brada and Alman found copper containing compounds to be effective in preventing liver tumours. Kaur and co-workers reported Ni(II), Co(II), Fe(II) and Cu(II) complexes with theazoline and their fungicidal activity has been evaluated.

Virtually it is clear that some compounds which have therapeutic actions have increased reactivity when these are complexed with metal ions. There are many metallic compounds which have pharmacological effect and are used as active ingredients (Saryan). Pharmaceutically so far the most important complexes are ferrous fumarate and ferrous gluconate (hematinies), gallium citrate (diagnostic agent) magnesium salicylate (antirheumatic arthritic agent), sodium cromoglycate (anti-asthma), etc. (Soine, Desliva, Sheries).

In order to detect the antimicrobial activity of compounds some transitional metal Cu(II), Co(II) and Ni(II) with cyanex-301 as bidentate ligand for development as potential screening is found to be useful. In general antimicrobial screening is under taken in a primary qualitative assay to detect the presence or absence of activity of a pure active compound.

The primary assay can be performed in vitro by a number of methods, one of which is disc diffusion technique (Gnanamanickam). By this method, we could classify the organism as susceptible as well as resistance.

Generally, more susceptible the rest organism, the larger is the zone of inhibition. Anti microbial activities of the test samples are expressed by measuring the zone of inhibition observed around the area. The diameter of the inhibition zone is usually measured to understand the extent of inhibition in diffusion concentration.

Materials and Methods

The bacteria and fungi (test organisms) were collected from the Department of Botany, University of Rajshahi. All steps of the work were carried out at the Plant Pathology and Mycology laboratory, Department of Botany, University of Rajshahi.

Antibacterial activity: The complexes were screened for antibacterial activity against *Streptococcus-β-haemolitycus* (Gram positive), *Bacillus subtilis* (Gram positive), *Salmonella typhi* (Gram negative) and *Escherichia coli* (Gram negative). The activities were carried out with the help of disc diffusion technique. Each disc contained 100 μg of compound and it was placed on bacteria inoculated plates. The growth inhibition results were compared with standard antibiotic Kanamycin (K-30) and either carbon tetrachloride (CCl_4) or dimethylsulfoxide (DMSO), which were used as control. The instant nutrient agar (DIFCO) medium was weiged (28 grams), dispersed in one liter of distilled water, allowed soaking for 10 minutes starred to

mix and autoclaved at 15 lb./(inch)^2 pressure at 121^{0}C. It was then cooled at 47^{0}C and poured into 120 mm petridishes. 50 mL of borth medium was poured in a conical flask. The test microorganisms of pure culture were streaked on the nutrient broth media with the help of sterile loop in an aseptic condition and incubated at 37^{0}C for 24 hours. The broth culture thus obtained was considered fresh culture. Fresh culture of the type was always used throughout the sensitivity testing. The medium was poured into sterile petridishes in an aseptic condition on a level horizontal surface so as to give a uniform depth of approximately 4 mm. Then the medium had been allowed to cool to room temperature in order to solidify the medium. Sterile filter paper discs were taken and the test material of known concentration was applied on the discs with the help of a micropipette. The solvents from the discs were evaporated by hot air blower. In the similar way control discs (containing only the solvents) were also prepared. The solidified agar plates were seeded with 70 μL of fresh culture with the help of a micropipette and spread the microorganisms with the help of a sterile spreader in an aseptic condition. The prepared discs of samples were placed gently on the freshly seeded solidified agar plates with a sterile forceps. Standard discs and control discs were also placed on the test plates to compare their effect with tested samples. Then the plates were kept in a refrigerator at 4^{0}C for 24 hours in order that the materials had sufficient time to diffuse to a considerable area of the plates. After this, the plates were incubated at 37^{0}C for 16 hours. After incubation, the diameter of the zone of inhibition were observed and measured in mm by a transparent scale.

Antifungal activity: The Antifungal activity of the complexes was carried out against *Tricophyton* sp., *Penicillium* sp., *Aspergillus flavus* and *Bipolaris sorokiniana* with the help of disc diffusion technique. The compound were dissolved in either carbon tetrachloride (CCl_4) or dimethylsulfoxide (DMSO) and each disc contained 100 μg compound. Antifungal activity of the compound was compared with standard antifungal agent fluconazol (F-30) and either CCl_4 or DMSO, which were used as control. The technique of preparation of fresh culture, placement of the disc and incubation for antifungal activity was nearly same as those of antibacterial activity. The procedures for calculation of the zone of inhabitation were also same as those followed in the previous case.

Results and Discussion

Antibacterial and antifungal activities of these complexes were tested in the present studies and results are presented in Tables 1-6 and Figures 1-8. The antimicrobial activity of the compounds A, B and C were determined at the concentration of 100 μg/disc against a series of Gram positive and Gram negative pathogenic organisms. From the zone of inhibition, it has observed that the complex A was more active than the others. The compound B also has showed substantial antimicrobial activity. It may conclude that most of the complexes have antibacterial effect except complex no. C, which has less antibacterial effect.

From the zone of inhibition, it was also observed that the complexes A and B showed that the highest antifungal activity against the fungi *Penicillium* sp. and *Aspergillus flavus*. All the complexes did not show antifungal activities against the fungi *Trichophyton* sp. But the lowest antifungal activity against the fungi *Bipolaris sorokiniana* was (9 mm) only.

Table 1. Antibacterial activity of the complex **A** [Cu(Cyanex-301)$_2$]

Name of bacteria	Diameter of inhibition zone of bacteria in mm	Kanamycin 30μg/disc
Streptococcus -β-haemolyticus (+ve)	17	18
Bacillus subtillus (+ve)	15	16
Salmonella typhi (-ve)	16	15
Escherichia coli (-ve)	18	19

Table 2. Antibacterial activity of the complex **B** [Cu(Cyanex-301)$_2$]

Name of bacteria	Diameter of inhibition zone of bacteria in mm	Kanamycin 30μg/disc
Streptococcus -β-haemolyticus (+ve)	9	18
Bacillus subtillus (+ve)	10	16
Salmonella typhi (-ve)	12	15
Escherichia coli (-ve)	8	19

Table 3. Antibacterial activity of the complex **C** [Ni(Cyanex-301)$_2$]

Name of bacteria	Diameter of inhibition zone of bacteria in mm	Kanamycin 30μg/disc
Streptococcus -β-haemolyticus (+ve)	8	18
Bacillus subtillus (+ve)	7	16
Salmonella typhi (-ve)	5	15
Escherichia coli (-ve)	7	19

Table 4. Antifungal activity of the complex **A** [Cu(Cyanex-301)$_2$]

Name of fungi	Diameter of inhibition zone of fungi in mm	Fluconazol 30µg/disc
Penicillium sp.	16	18
Bipolaris sorokiniana	0	15
Aspergillus flavus	13	19
Trychophyton sp.	0	17

Table 5. Antifungal activity of the complex B [Co(Cyanex-301)$_2$]

Name of fungi	Diameter of inhibition zone of fungi in mm	Fluconazol 30µg/disc
Penicillium sp.	14	18
Bipolaris sorokiniana	0	15
Aspergillus flavus	17	19
Trychophyton sp.	0	17

Table 6. Antifungal activity of the complex C [Ni(Cyanex-301)$_2$]

Name of fungi	Diameter of inhibition zone of fungi in mm	Fluconazol 30µg/disc
Penicillium sp.	10	18
Bipolaris sorokiniana	9	15
Aspergillus flavus	12	19
Trychophyton sp.	0	17

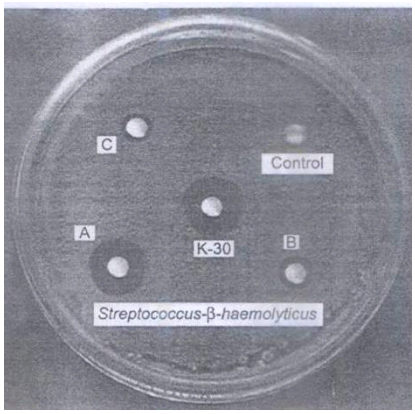

Fig. 1. Photographic representation of zone of inhibition of complexes A-C, control and Kanamycin (K-30) respectively against the bacteria *Streptococcus-β-haemolyticus*.

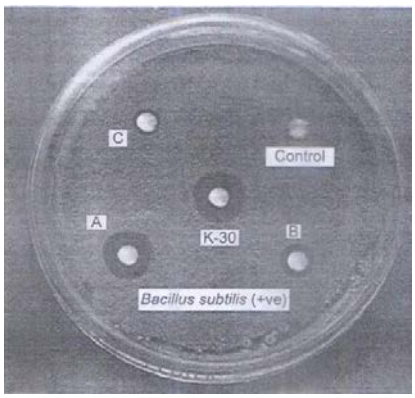

Fig. 2. Photographic representation of zone of inhibition of complexes A-C, control and Kanamycin (K-30) respectively against the bacteria *Bacillus subtilis* (+ve).

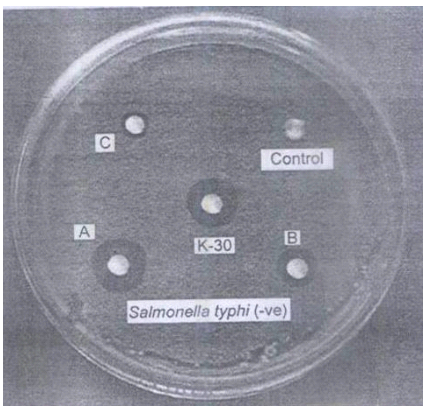

Fig. 3. Photographic representation of zone of inhibition of complexes A-C, control and Kanamycin (K-30) respectively against the bacteria *Salmonella typhi* (-ve).

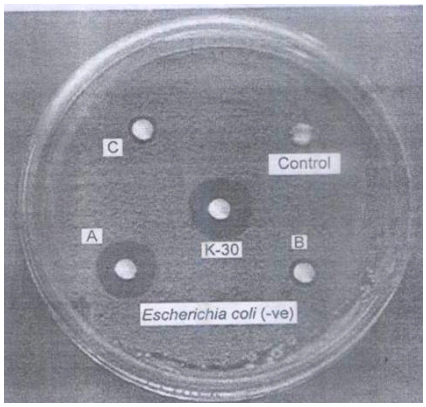

Fig. 4. Photographic representation of zone of inhibition of complexes A-C, control and Kanamycin (K-30) respectively against the bacteria *Escherichia coli* (-ve.).

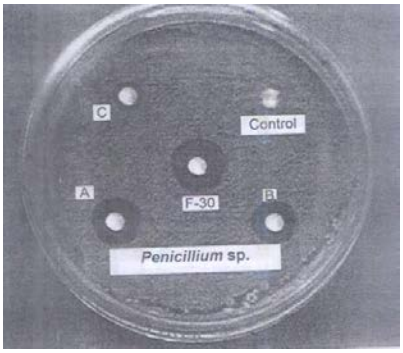

Fig. 5. Photographic representation of zone of inhibition of complexes A-C, control and Fluconazol (F-30) respectively against the fungi *Penicillium* sp.

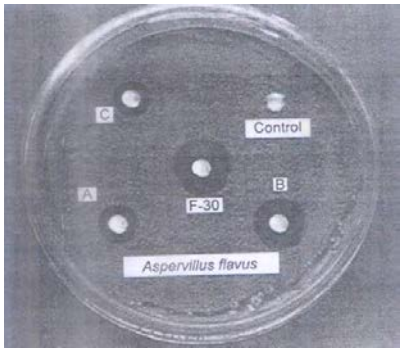

Fig. 6. Photographic representation of zone of inhibition of complexes A-C, control and Fluconazol (F-30) respectively against the fungi *Bipolaris sorokiniana*.

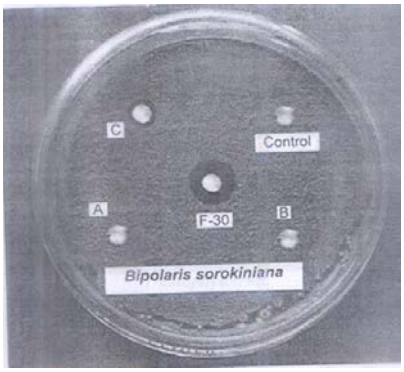

Fig. 7. Photographic representation of zone of inhibition of complexes A-C, control and Fluconazol (F-30) respectively against the fungi *Aspervillus flavus*.

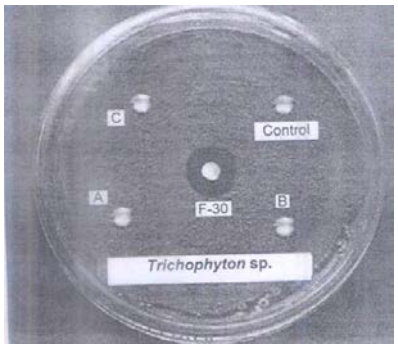

Fig. 8. Photographic representation of zone of inhibition of complexes A-C, control and Fluconazol (F-30) respectively against the fungi *Trichophyton* sp.

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