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In vitro antibacterial studies of transition metal complexes of 2-hydroxynicotinic acid (2-hydroxypyridine-3-carboxylic acid) & 2-hydroxynicotinamide

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Abstract
The investigations on antimicrobial activity of different transition metal complexes are not only useful for the development of new drugs but it is also essential to ascertain the toxic nature of the compound. In the present work the biological activity in terms of their growth inhibitory property on specific known bacterial cultures of the two synthesized bioactive ligands 2-Hydroxynicotinic acid i.e., 2-Hydroxypyridine-3-carboxylic acid (2HnicO), 2-Hydroxynicotinamide (2HNICAM) and their complexes with Mn (II), Ni (II), Co (II) and Cu (II) have evaluated by standard "disc diffusion" method. The bacterial subcultures of Escherichia coli, Bacillus subtilis and Staphylococcus aureus have been used as test organisms and all the samples are tested against these bacteria at different concentration.

Keywords: antimicrobial activity, transition metal complexes, disc diffusion etc

Introduction
In recent years, there has been growing interest in research on bioactive ligands and their transition metal complexes, mainly because these ligands and their complexes possesses a wide range of bioactivities, chemical and pharmacological applications. The real impact toward developing of coordination chemistry is their physicochemical properties and significant biological activities. These compounds have a wide variety of biological activity such as antitumor, antifungal, antibacterial or antiviral. Many of these compounds possessed wide spectrum of medicinal properties, including activity against influenza, protozoa, smallpox, certain kinds of tumour, leprosy, bacterial and viral infections, psoriasis, rheumatism, trypanosomiasis, coccidiosis, malaria and as a pesticides and fungicides. These activities due to their ability to chelate trace metals and in few cases, it has been proved that metal ions enhance the biological activity of bioactive ligands. (1-5)
Biologically active ligands such as DNA intercalators, doxorubicin, oestrogen analogues, amino acids, ferrocene, phosphonates, and sugars have been selected as molecular carriers to obtain a specific delivery/accumulation of the antitumor drug to the target cells (or organs) or to obtain synergistic pharmacological activities. Several metal complexes have been used as probes of DNA structure in solution, as agents for mediation of strand scission of duplex DNA and as chemotherapeutic agents. (5-8).

Experimental
The evaluation of antimicrobial activities involved following general steps:

1. Sterilization and treatment a of glass apparatus
All the glass apparatus, including petri dishes were cleaned with chromic acid followed by washing with distilled water. These were then sterilized by heating at 120°C in an oven fully wrapped in inert foil for 6-8 hours. (9-12).

2. Preparation and sterilization of media
Nutrient agar and Czapek Dox agar slants were used as culture media for bacterial cells and fungal spores respectively.
Composition of Nutrient agar medium is as;

Peptone	= 5g
Sodium chloride	= 5g
Beef extract	= 1.5g
Yeast extract	= 1.5g
Agar	= 15g
Distilled water	= 1000ml (pH=7.4±0.2)

Czapek Dox Agar medium was composed of;

Sodium nitrate	= 2g
Dipotassium hydrogen Phosphate	= 1g
Magnesium sulphate	= 0.5g
Potassium chloride	= 0.5g
Ferrous sulphate	= 0.0lg
Sucrose	= 30.0g
Agar	= 15g
Distilled Water	= 1000ml (pH=7.3±0.2)

For the preparation of media, all the ingredients except agar were dissolved in half of the water with gentle warming wherever required. In the other half of distilled water, agar was dissolved by heating with constant stirring. The two solutions were mixed and heated to make a homogenous solution. The one litre solution of each media was filtered through cotton and a clear solution was obtained. This was then sterilized properly plugged in a conical flask by autoclaving at 120°C for 30 min. (12-15).

3. Pouring of the media into sterilized Petri dishes and its solidification
The 15-20 ml of sterilized media was poured homogenously into sterilized petri dishes and used for the inoculation.

4. Inoculation of the media with the test organisms.
Bacterial cells (0.5 ml) or of fungal spore suspension (0.2-0.3 ml) was added on the petri dishes, prepared by the

method as described above and spread with the help of a sterile spreader. These petri dishes were kept in laminar for 10 minutes for inoculation.

5. Preparation of the solutions and control

The standard solutions of compounds were prepared as reported in chapter II. Solutions of concentration range in between 25 to 100 ppm have been prepared by diluting stock solution appropriately and used for study of antimicrobial activity.

6. Preparation of test plates

Filter paper discs were soaked into above solution of test compound and these paper discs were placed on the Petri dishes and incubated at 37°C temperature for 24 hours.

7. Measurement of the zone of inhibition

Zone of inhibition was measured for each compound separately with respect to control and compared to a standard drug.

General Method used for the determination of antimicrobial activity

A saturated solution of Nutrient agar (75 g) was prepared in double distilled water and it was autoclaved for 15 min, then poured in Petri plates in the laminar. After its solidification loan of bacteria (i.e., Escherichia coli, Bacillus subtilis and Staphylococcus aureus) against which antimicrobial activity is to be investigated, was applied. Solutions were prepared of both ligands and their complexes with Mn (II), Ni (II), Co (II) and Cu (II). A separate paper disc was soaked in each solution for 10 minutes. Thus, prepared paper disc was placed into Petri plate and finally prepared Petri plates were kept in incubator at 37°C for 24 hours. After 24 hours, Petri plates were removed and checked for measuring zone of inhibition in mm (16-20).

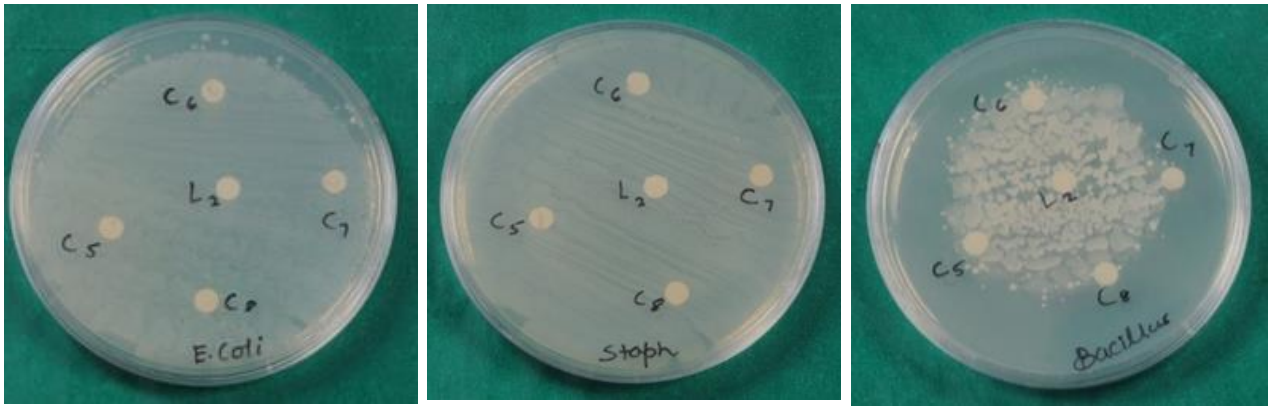

Fig 1: Biological Activity of 2HNICAM and its Complexes with Mn (II), Co (II), Ni (II) and Cu (II) (Medium-Nutrient Agar)

Fig 2: Biological Activity of H_2nicO and its Complexes with Mn (II), Co(II), Ni (II) and Cu (II) (Medium-Nutrient Agar)

Table 1: Biological Activity of the Bioactive Ligands (Medium - Nutrient Agar)

S. No.	Ligands	Sample Identity	Zone of Inhibition (in mm)		
			E. coli	*S. aureus*	*B. subtilis*
2	2-Hydroxynicotinamide(2HNICAM)	L2	4.5	5.5	4.5
4	2-Hydroxynicotinic acid(H_2nicO)	L4	6.5	6.5	4.5

Table 2: Biological Activity of Bioactive Ligand 2HNICAM and their Complexes (Medium-Nutrient Agar)

S. No.	Ligands/ Complexes	Sample Identity	Zone of Inhibition (in mm)		
			E. coli	*S. aureus*	*B. subtilis*
1.	**2HNICAM**	L2	4.5	5.5	4.5
	$[Mn (H_2O)_2(2HNICAM)_2] Cl_2$	C7	6.5	6.5	5.5
	$[Co (H_2O)_2(2HNICAM)_2] Cl_2$	C5	5.5	6.5	0.0
	$[Ni (H_2O)_2(2HNICAM)_2] Cl_2$	C8	0.0	6.5	6.0
	$[Cu (H_2O)_2(2HNICAM)_2] Cl_2$	C6	6.5	5.5	5.6

Table: 3 Biological Activity of Bioactive Ligand H_2nicO and their Complexes (Medium-Nutrient Agar**)**

S. n.	Ligands/ complexes	Sample identity	Zone of inhibition (inmm)		
			E.coli	*S. aureus*	*B. subtilis*
2.	H_2nicO	L4	6.5	6.5	4.5
	$Na_2[Mn(HnicO)_2Cl_2]$	C15	5.5	6.5	5.5
	$Na_2[Co(HnicO)_2Cl_2]$	C13	6.0	6.5	6.0
	$Na_2[Ni(HnicO)_2Cl_2]$	C16	5.5	6.0	6.0
	$Na_2[Cu(HnicO)_2Cl_2]$	C14	5.5	6.0	5.5

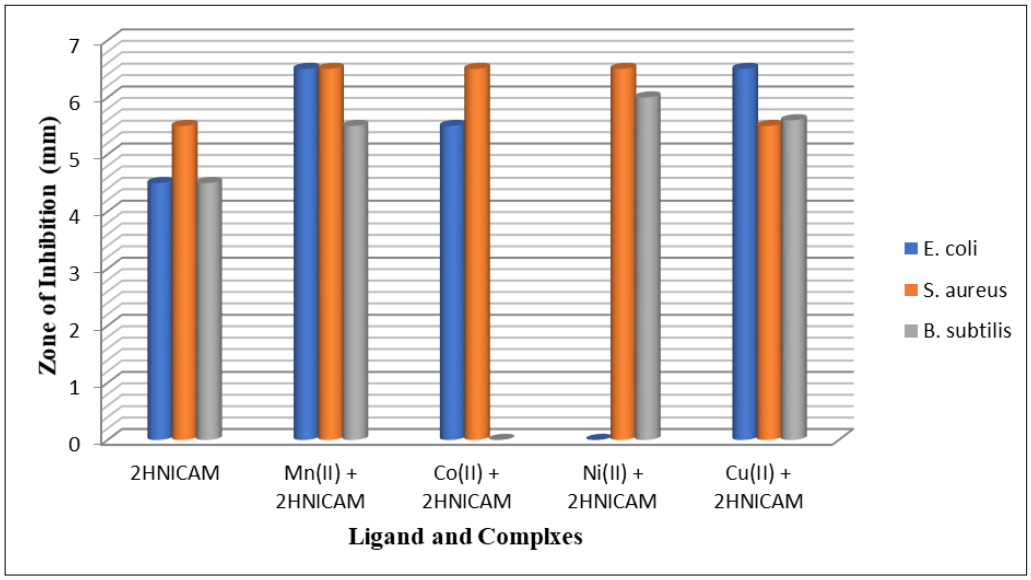

Fig:3 Biological Activity of the Ligand 2HNICAM and its Metal Complexes

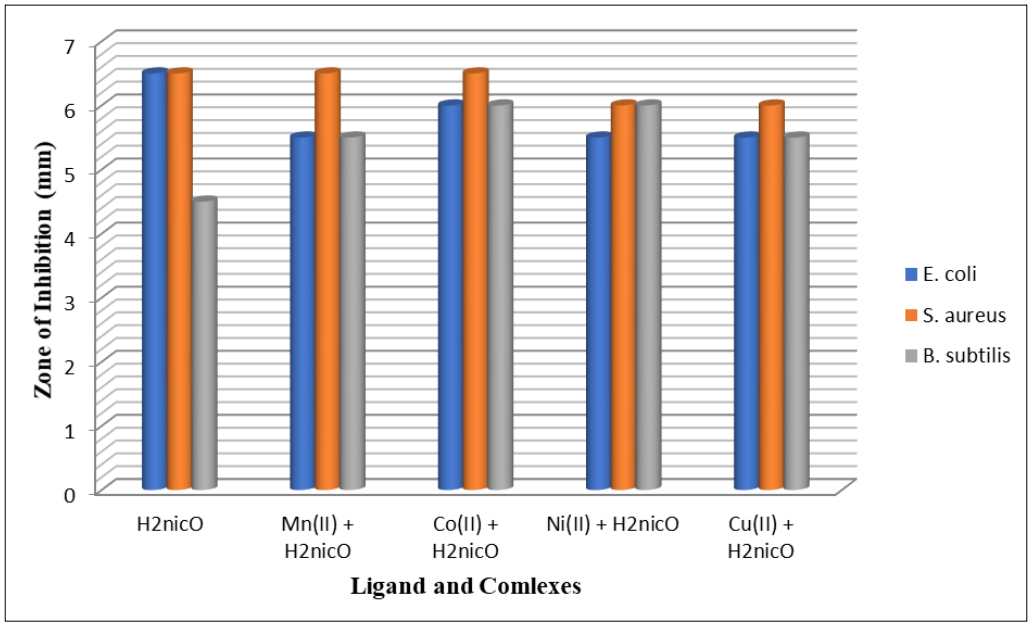

Fig: 4 Biological Activity of the Ligand H_2nicO and its Metal Complexes

Results and Discussion

Antibacterial activity of both bioactive ligands and their complexes with Mn (II), Co (II), Ni (II) and Cu (II) have been carried out on three microbes i.e., *E. coli*, *B. subtilis* and *S. aureus*.

The results of antibacterial activities of ligands have been given in table 1 and the results of antibacterial activity of complexes have been given in table 2 & 3 and have also been graphically represented in Fig.3 to Fig 4. The photographs of biological activities of ligand and complexes are given in Fig. 1 to Fig. 2.

1. Both bioactive ligands and their complexes reported here possess at least one type of biological activity up to substantial level.
2. Antibacterial activity of complexes in many cases greater than the bioactive ligands.
3. On the basis of results of antimicrobial activity, a trend of structure activity relationship may be developed after further investigations, at primary level a trend has been observed and reported for used bioactive ligand systems.

a. Against *E. coliI*
H_2nicO > 2HNICAM

b. Against *S. aureus*
H_2nicO > 2HNICAM

c. Against *Bacillus subtilis*
H_2nicO = 2HNICAM

4. Results of structure activity relationship of Mn (II), Co (II), Ni (II) and Cu (II) complexes of the bioactive ligands against *E. coli, S. aureus* and *B. subtilis* are as:

a.* Against *E. coli
1. [Mn(H2O)2(2HNICAM)2]Cl2 = [Cu(H2O)2(2HNICAM)2]Cl2>[Co(H2O)2(2HNICAM)2]Cl2 > [Ni (H2O)2(2HNICAM)2]Cl2
2. $Na_2[Co\ (HnicO)_2Cl_2] > Na_2[Mn\ (HnicO)_2Cl_2] = Na_2[Ni(HnicO)_2Cl_2] = Na_2[Cu\ (HnicO)_2Cl_2]$

b. Against *S. aureus*
1. $[Mn(H_2O)_2(2HNICAM)_2]Cl_2=[Co(H_2O)_2(2HNICAM)_2]Cl_2 =[Ni(H_2O)_2(2HNICAM)_2]Cl_2>[Cu(H_2O)_2(2HNICAM)_2]Cl_2$
2. $Na_2[Co\ (HnicO)_2Cl_2] = Na_2[\ Mn\ (HnicO)_2Cl_2] > Na_2[Ni\ (HnicO)_2Cl_2] = Na_2[Cu\ (HnicO)_2Cl_2]$

c. Against *Bacillus subtilis*
1. $[Ni(H_2O)_2(2HNICAM)_2]Cl_2>[Cu(H_2O)_2(2HNICAM)_2]Cl_2>[Mn(H_2O)_2(2HNICAM)_2]Cl_2>[Co(H_2O)_2(2HNICAM)_2]Cl_2$
2. $Na_2[Co\ (HnicO)_2Cl_2] = Na_2[Ni\ (HnicO)_2Cl_2] > Na_2[Mn\ (HnicO)_2Cl_2] = Na_2[Cu(HnicO)_2Cl_2]$

5. Antibacterial activity of metal complexes in general, greater against *Bacillus subtilis* than *S. aureus* and *E. coli* with few exceptions.

Conclusion
Biological activity of two bioactive ligands and their complexes with Mn (II), Ni (II), Co (II) and Cu (II) has been investigated in nutrient agar medium. The standard disc diffusion method has been employed for study. E. coli, S. aureus and B. subtilis as bacterial subcultures have been used for study of antibacterial activity of the compounds. The study indicates that two bioactive ligands and their complexes reported here, possess at least one type of biological activity up to substantial level and antibacterial activity of complexes in many cases greater than the bioactive ligands.

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