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In-vitro study of antifungal activity of nickel (II) complex with phenylbiguanide and p-phenylenedibiguanide ligand against *Aspergillus versicolor* & *Aspergillus niger*

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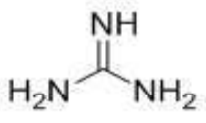
Abstract- In last two decades, it has been observed that many of the antimicrobial drugs became non-functional any more. In other words, one can say all of them are now inefficient to control microbial growth. In the 2nd wave of COVID – 19, many of the patients have been found infected with different fungi after defeating corona disease. This has resulted in taking out different organs in order to save the life of the patients. In this research, attention has been dragged towards biochemical activity of some complexes of biguanide derivatives to check its action towards antifungal activity of two common fungi of ascomycetes family. In the present research work, it was checked whether after complexation with transition metal nickel (II) do it has such properties or not. It was found that the complexes have a positive response against the growth of fungi *Aspergillus versicolor* and *Aspergillus niger*. Antifungal activity has been compared in PDA (Potato Dextrose Agar) and SDA (Sabourand and Dextrose Agar) media by disc dilution test method & mycelium growth inhibition was calculated. It was found that p-phenyledibiguanide ligand complex was more effective than phenylbiguanide complex in both media.

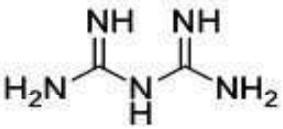
Key words: ***Aspergillus versicolor, Aspergillus niger*, PDA, SDA, ascomycetes, bidentate**

INTRODUCTION

Biguanide[1,2] is a class of drug which is used to reduce the sugar level in blood. This drug is given to diabetes type-2. Its oral intake reduces the sugar absorption by intestine and prevents liver to convert fats & protein into glucose. Also it stimulates the body to be more sensitive towards insulin release by pancreas. Biguanide as the name predict it to be diguanide i.e. formed by condensation of two guanidine molecules.



guanidine



biguanide

Biguanide[2] have been found to act as ligand and form numerous complexes with divalent and trivalent metal ions. Each biguanide moiety gets bonded to metal ion by one covalent bonding and one coordinate bonding. One of the imino hydrogen gets replaced by covalent bonding with metal ion and one amino N gets connected by

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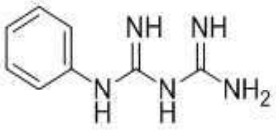
coordinate bond. Biguanide and its substituted derivatives have been synthesized by different researchers and scientists and complexed with transition metals. Its physical and chemical properties were studied by different scholars. Beside these, nowadays their spectral studies are also been done but still biochemical studies are still lacking in the field of above said ligand complexes. Hence, in this research work attention have been dragged towards antifungal activity of nickel (II) ion complexed with phenylbiguanide & p-phenylenedibiguanide ligands. After complexation both the complexes were treated against fungus- *Aspergillus versicolor* and *Aspergillus niger*. It was found that both the complexes showed positive result towards reduction in the fungal growth.

MATERIALS & METHOD

In order to do the research work, the mentioned ligands were prepared in the form of phenylbiguanide hydrochloride and p-phenylenedibiguanide sulphate. After the preparation of the ligands, complexation was done by reacting with the metal salt. The complexes so obtained were confirmed by different analysis such as elemental analysis, spectral analysis and magnetic properties.

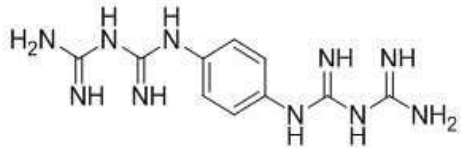
Phenylbiguanide hydrochloride (m.pt.=244-247°C)[3-6] - It was prepared by refluxing aniline hydrochloride with dicyandiamine by dissolving both with ethyl alcohol in round bottom flask on water bath for 2 hrs. On cooling shining crystals get deposited which was filtered and recrystallized with hot water.



Phenylbiguanide

p-phenylene dibiguanide[7] ($C_{10}N_{10}H_{16}$•$2H_2SO_4$)

It was prepared by refluxing a mixture of p-phenylenediamine, dicyandiamide with 32% hydrochloric acid and water for 2 hours. Refluxing was done in round bottom flask on water bath. After cooling the resulting refluxed solution was treated with alcohol. The solution so obtained was coloured and so boiled with activated charcoal to eradicate the colouration. The solution was then filtered in beaker and dil. sulphuric acid was added. The filtrate was then kept for obtaining crystals of the ligand. After 2-3 days crystals was deposited in the beaker. It was filtered and washed first with cold water and then alcohol & dried in air.



p-Phenylenedibiguanide

Nickel phenylbiguanidiumchloride or Bis (phenylbiguanidium) nickel (II) chloride[8] $[Ni(PhBigH)_2]Cl_2.\ 2.5H_2O$

It was prepared by action of phenylbiguanide hydroxide on a solution of nickel chloride. As a result, golden yellow crystals of the above said complex was obtained. The precipitate was filtered. The crystals were purified by recrystallisation with absolute alcohol and dried in air.

p-phenylenedibiguanidiumnickel (II)sulphate[9-10] $[Ni(C_{10}N_{10}H_{14})]SO_4.2H_2O$

It was prepared by mixing ammonical solution of p-phenylenedibiguanide sulphate with aqueous solution of nickel sulphate solution. As a result, a brick red flocculent precipitate of p-phenylenedibiguanidumnickel (II) sulphate was obtained. The red precipitate so obtained was filtered and washed with water and dried in air. The complex was sparingly soluble in hot water and insoluble in alcohol.

Antifungal activity[11-13]

Antifungal activity was done by inoculating the above mentioned fungus to PDA (Potato Dextrose Agar) and SDA (Sabourand and Dextrose Agar) medium. The medium was autoclaved before use and the ratio used for complex solution and medium was1:10. Further inhibition of mycelium was calculated by disc dilution test method. PDA and SDA were prepared as follows:

PDA medium preparation[14-15]- 200gms of potato tubers were peeled off and chopped into small pieces. The pieces were boiled with 100ml water in a beaker for 20 minutes and filtered with muslin cloth. The filterate so obtained is called potato extract. 20gm of dextrose, 15gm agar and 2gm peptone was then added to the extract and heated gently. The solution was then made to 1litre. pH of the solution is maintained to 5.6 by 1N HCl or 1N NaOH solution and stored in Erlenmeyer flask.

SDA medium preparation[14-15] - In this case, premix was dissolved in distilled water and boiled. The filterate so obtained was filtered and diluted to 1litre and heated gently for half an hour to make uniform solution. pH was maintained to 5.6 and stored in Erlenmeyer flask by putting glass wool on its mouth.

After preparation of the media, pure culture of the fungal spores are prepared and isolated by Warcup method.[15] On successive inoculation in new petridish with the medium, pure culture of a particular fungal spore had been obtained. After getting separated, the fungus was identified under microscope and testing was done in UV chamber of the Chemistry Department of Magadh Mahila College, Patna University.

RESULTS & DISCUSSION

Table 1- Percentage composition of elements and ions

Complexes	Formulae	Percent composition of elements and ions				
			Ni	N	Cl^-	H_2O
Bis-(phenylbiguanidium) nickel(II) chloride	$[Ni(PhBigH)_2]Cl_2.2.5H_2O$	By calculation	11.02	26.50	13.45	8.52
		Found	11.08	26.30	13.20	8.43
p-phenylenedibiguanidium nickel(II)sulphate	$[Ni(C_{10}N_{10}H_{14})]SO_4.2H_2O$		Ni	N	SO_4^{-2}	H_2O
		By calculation	12.57	29.98	20.50	7.70
		Found	12.30	29.84	20.50	5.68

The nickel absorption spectra of Ni (II) complex, Bis(phenyldibiguanidium) nickel (II) chloride (A), display one broad distinct band at 240nm & 220nm attributed to the charge transfer and $^1A_{1g} \longrightarrow {}^1E_g$ transition. The diamagnetism and UV electronic absorption pattern suggested square planer geometry of nickel (II) complexes.

The electronic absorption of Ni (II) complexes display one band (distinct) at 470nm for [Ni(p-phenylenedibiguanide)]sulphate. This medium band due to $A_{1g} \longrightarrow B_{1g}$ transition in square planer field. The strong absorption band located at 270nm attributed to the charge transfer and $^1A_{1g} \longrightarrow {}^1E_g$ transition. The diamagnetic and uv electronic absorption pattern suggested square planar geometry of Ni (II) complexes.

Table 2. Percentage inhibition of growth of fungus *Aspergillus versicolor* at indicated dose

Complex	Medium	Concentration in µg/ml	Percentage inhibition of fungus
Bis-(phenylbi guanidium) nickel(II)chloride Complex A	PDA	400	92.31
	PDA	200	78.73
	PDA	100	50.72
	SDA	400	88.50
	SDA	200	70.58
	SDA	100	47.41
p-phenylenedibi guanidium nickel(II)sulphate Complex B	PDA	400	98.51
	PDA	200	81.20
	PDA	100	59.51
	SDA	400	95.20
	SDA	200	79.25
	SDA	100	56.31

Table 3. Percentage inhibition of growth of fungus *Aspergillus niger* at indicated dose

Complex	Medium	Concentration in µg/ml	Percentage inhibition of fungus
Bis-(phenylbi guanidium) nickel(II)chloride Complex A	PDA	400	84.01
	PDA	200	65.72
	PDA	100	44.36
	SDA	400	82.09
	SDA	200	62.37
	SDA	100	42.27
p-phenylenedibi guanidium nickel(II)sulphate Complex B	PDA	400	95.80
	PDA	200	70.25
	PDA	100	50.73
	SDA	400	84.11
	SDA	200	69.59
	SDA	100	48.45

When the data mentioned in table ii and iii were compared, it was concluded that when concentration of the complex taken was 400µg/ml, minimum inhibitory concentration (MIC) in PDA and SDA media were as follows:

Table 4. Percentage inhibition of growth of fungal growth

Complex	Concentration (µg/ml)	Fungus	Percentage inhibition of fungal growth	
			PDA medium	SDA medium
A	400	*A.versicolor*	92.31	88.50
B	400	*A.versicolor*	98.51	95.20
A	400	*A. niger*	84.01	82.09
B	400	*A. niger*	95.80	84.11

From the data, it was found that in PDA (potato dextrose agar) medium, the complex B was more effective to control the fungal growth in *A.versicolor* than *A. niger.* At 400 µg/ml concentration in the case of SDA (Sabourand

Dextrose Agar) medium also, complex B was more effective to control fungal growth of *A. versicolor* than *A.niger*.

For both the fungi, *A. versicolor* and *A. niger*, when MIC was compared, it was found that under 400µg/ml concentration in PDA & SDA media, complex B was more effective in controlling the fungal growth with 98.51% & 95.20% and 95.80 % & 84.11% inhibition respectively.

CONCLUSION

From the result and discussion as described above, it is concluded that for complex B i.e. nickel complexed with p-phenylenedibiguanide ligand controls the mycelium growth more efficiently than complex A i.e. nickel (II) complexed with phenylbiguanide ligand for both the fungi- *A.versicolor* and *A.niger*.

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