STRUCTURE-ACTIVITY RELATIONSHIP IN THE ANTIALGAL ACTIVITY OF SOME SCHIFF BASE COMPLEXES

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Abstract: Schiff bases and their coordination complexes has been an interesting area of inorganic research most recently because of the promising results reported from many parts of the world regarding their antimicrobial activities. In the present work, the Schiff base $\mathbf{H_2L^2}$ synthesized by condensing 2-hydroxybenzaldehyde with 3-aminophenol and its transition metal complexes were evaluated for their antialgal activities against two species of algae, *Oscillatoria brevis* and *Chlorella pyrenoidosa.* Structure-activity relationship was analyzed based on the nature of central transition metal ion and different anions. All the complexes showed enhanced activity compared to the ligands. Among the complexes, those derived from Cu(II) ion exhibited maximum screening effect. Comparing the complexes with different anions, perchlorate complexes exhibited maximum growth inhibition of both the algae.

Keywords: Schiff base, Ligand, Antialgal studies, SAR studies.

Introduction: Harmful algal blooms are one of the major environmental issues faced by many western countries and an anticipated problem in India due to increasing water pollution. Algae are a family of simple plant-like organisms that range from unicellular species to large seaweeds [1]. These are the most important photosynthesizing organisms on earth and form the foundation of most food webs, which support an abundance of living forms in water. However, overgrowths of algae in aquatic system can be menacing to both human and animals.

Some species produce toxins that are harmful to aquatic organisms and may cause human illness. Nontoxic algae, on the other hand, may clog the gills of fish and invertebrates or strangle corals. Some others desecrate water bodies and cause drinking water and fish to taste bad. The growth of harmful algal blooms is complemented by sunlight, slow-moving water and nutrients. Nutrient pollution from human activities makes the problem worse, leading to more severe blooms. With increased usage of algicides to control the growth of algae, there is a chance of developing some strands of algae that are resistant to chemicals [2]. The survival mechanisms exhibited by algae reveal scope for research works to develop novel algicides that can effectively be used against harmful algae.

As a continuation of the work on hydroxyl substituted Schiff base complexes, the present research work deals with complexes of 3-{[(2-hydroxyphenyl)methylidene]amino} phenol, $\mathbf{H_2L^2}$ with perchlorate, chloride and nitrate salts of transition metal ions Co(II), Ni(II) and Cu(II).

Experimental:

Synthesis and Physico-chemical Analysis of Schiff Base Complexes: All chemicals used in this work were of analytical reagent grade supplied by Merck. The ligand $\mathbf{H_2L^2}$ and transition metal complexes were prepared and characterized as described in our previous paper [3].

Schiff base and the complexes were analyzed for carbon, hydrogen and nitrogen content on a Heracus CHN rapid analyzer. The metal content in the complexes were determined gravimetrically as oxides. Molar conductance in DMF and acetonitrile in 10^{-3}M solutions were measured at room temperature using an Elico CM–180 conductivity meter with a dip type cell of platinum electrodes (Cell constant = 0.986 cm^{-1}). The IR spectra of the complexes and ligand were recorded in the range 400-4000 cm^{-1} on a Schimadzu IR-470 spectrophotometer in KBr disc. The electronic spectra in ethanol solution (10^{-3}M) were recorded in the range 200-900 nm on a Schimadzu UV-160A spectrometer. ^{1}H NMR spectra of the selected compounds were recorded on BRUKER AV III FT NMR Spectrometer in DMSO-d_6 with TMS as the internal reference at Indian Institute of Technology, Madras. Thermogravimetric studies of the compounds were carried out on a Perkin Elmer STA 6000 Thermal Analysis system, in the range 15-800^{o}C at Sophisticated Analytical Instruments Facility (SAIF) in STIC, CUSAT, Kochi and at the School of Chemical Sciences, M.G. University, Kottayam.

The proposed structure of the complexes, $[M(HL^2)(H_2O)_3]X$ where M=Co(II), Ni(II) and Cu(II) and X= $-ClO_4$, $-NO_3$ and –Cl, is given in Fig 1.

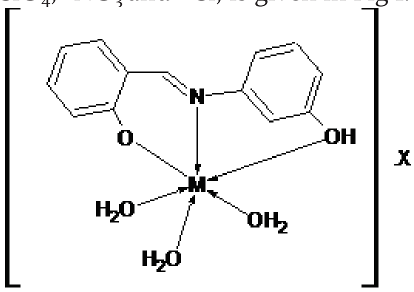

Fig 1: Structure of $[M(HL^2)(H_2O)_3]X$

Algal Defacement Test:

Test Specimen: Two algal species *Oscillatoria brevis* (filamentous blue green algae) and *Chlorella pyrenoidosa* (unicellular green algae) were used in the present study [4].

Chlorella pyrenoidosa: These are light green coloured unicellular algae with a eukaryotic cell containing chloroplast. These are capable of photosynthesis using primary pigment *chlorophyll*, the same green pigment used in plants. Many eukaryotic algae have whip like appendages called *flagella* attached to their cell walls.

Oscillatoria brevis: It belongs to the class of cyanobacteria and is also known as filamentous blue-green algae. These are unicellular organisms lacking a nucleus and other membrane bound organelles. These have many characteristics that resemble bacteria. At the same time, like plants, these have rigid cell walls composed of cellulose. Secondary pigments are responsible for the blue-green colour of *Oscillatoria brevis*.

Preparation of Algae Stock Solution: The algae were grown in Ward and Parrish medium [5] which is prepared according to the following composition.

1. Macronutrient Stock Solution (1L)	
$NaNO_3$	25.5g/L
$MgCl_2.6H_2O$	12.2g/L
$MgSO_4.7H_2O$	14.7g/L
$CaCl_2.2H_2O$	4.41g/L
$NaHCO_3$	15.0g/L
K_2HPO_4	1.044g/L
2. Micronutrient Stock Solution (1L)	
$CoCl_2$	0.78g/L
$CuCl_2$	0.9g/100mL

0.1855g of H_3BO_3, 0.2643g of $MnCl_2$, 0.327g of $ZnCl_2$, 0.0073g of $Na_2MoO_4.H_2O$, 0.096g of $FeCl_3$ and 0.3g of Na_2EDTA along with 1mL each of macronutrient solution and micronutrient solution were mixed in 1L of double distilled, deionised water. The stock cultures were maintained in borosil conical flasks (1L) plugged in with sterilized, nonabsorbant cotton. The conical flasks were illuminated with day light; the duration of light and dark period was 10:14 hours. The temperature was maintained between 28-32^{o}C.

Procedure: The test was carried out according to ASTM D 5589-97 [6]. A filter paper of 1.5mm diameter was dipped in solution (0.3mg/mL), dried and used as the substrate for algal growth. Allen's agar medium was prepared in a petri dish. The test specimen was placed over the solidified Allen's agar in plates which had been sterilized in advance. Algal inoculums were transferred from the flask using a sterilized sprayer and applied a thin coat of algae suspension to each specimen making sure that the surface was equally covered. The inoculated plates were kept in daylight at temperature 28-32^{o}C and at humidity of >85%. The duration of light and dark period was set to be 10:14 hours. The samples were examined weekly for visible growth up to four weeks.

Results and Discussion: The observations of antialgal studies after week 1 and week 4 are reproduced in Table 1. Traces of *Chlorella pyrenoidosa* and *Oscillatoria brevis* were found over the ligand after one-week observation, which turned to a *light* growth in the case of *Oscillatoria brevis* after four weeks. All the complexes could effectively resist the growth of the algae *Chlorella pyrenoidosa*.

The blue green algae *Oscillatoria brevis* was found to be extremely sensitive to copper complexes, BP12, BC13 and BN14 exhibiting *nil* growth over their plates after four weeks. BP9 could also bring about complete growth inhibition. *Light* growth was observed over BN11 while traces of algae were found over BP6, BC7, BN8 and BC10.

Table 1: Results of Algal Defacement Test of BP6-BN14

Sample	***Oscillatoria brevis***				***Chlorella pyrenoidosa***			
	Week 1		**Week 4**		**Week 1**		**Week 4**	
	#OG	***Rating**	**#OG**	***Rating**	**#OG**	***Rating**	**#OG**	***Rating**
DMSO	T	1	H	4	T	1	M	3
H_2L^2	N	0	L	2	N	0	T	1
$[Co(HL^2)(H_2O)_3]ClO_4$ **BP6**	N	0	T	1	N	0	N	0
$[Co(HL^2)(H_2O)_3]Cl$ **BC7**	T	1	T	1	N	0	N	0
$[Co(HL^2)(H_2O)_3]NO_3$ **BN8**	N	0	T	1	N	0	N	0
$[Ni(HL^2)(H_2O)_3]ClO_4$ **BP9**	N	0	N	0	N	0	N	0
$[Ni(HL^2)(H_2O)_3]Cl$ **BC10**	T	1	T	1	N	0	N	0

$[Ni(HL^2)(H_2O)_3]NO_3$ **BN11**	T	1	L	2	N	0	N	0
$[Cu(HL^2)(H_2O)_3]ClO_4$ **BP12**	N	0	N	0	N	0	N	0
$[Cu(HL^2)(H_2O)_3]Cl$ **BC13**	N	0	N	0	N	0	N	0
$[Cu(HL^2)(H_2O)_3]NO_3$ **BN14**	N	0	N	0	N	0	N	0

#OG=Observed Growth on Samples: N = *Nil*, T = *Traces*, L = *Light*, M = *Moderate*, H = *Heavy*.
*Standard Ratings are given as <1=0, 1-10% = 1, 10-30% = 2, 30-60% = 3 and 60-100% = 4.

Structure-Activity Relationship in the Antialgal Activity of Complexes: Transition metal complexes containing three different anions were compared and contrasted for their antialgal activity and the results are tabulated in Table 2.

Here *light* to *moderate* growth of the algae was observed over nitrate complexes. Only the nitrate complex BN14 could bring complete growth inhibition of both the algae. The nitrate complex BN8 allowed *trace* growth of the species over the substrate while BN11 permitted *light* growth of *Oscillatoria brevis*.

Nitrate containing complexes were found to promote the growth of algae instead of the expected inhibition [7]. Out of the chloride complexes, BC13 could inhibit the growth of *Oscillatoria brevis*. And among the perchlorates, all complexes, except BP6, completely inhibited the growth of *Oscillatoria brevis*.

No growth of *Chlorella pyrenoidosa* was observed over any of the chloride or perchlorate complexes. The observed antifungal spectrum follows the pattern perchlorates>chlorides>nitrates.

Schiff base complexes containing three different transition metal ions were compared and contrasted for antialgal activity and the results are given in Table 3. Considering the growth rates over different metal complexes, *Chlorella pyrenoidosa* was effectively inhibited by all the complexes irrespective of the difference in central metal ion.

Oscillatoria brevis was more sensitive to Cu(II) complexes and was tolerant to Co(II) and Ni(II) complexes. The activity of Cu(II) complexes is related to their ability of some redox coupling interactions inside an a living cell, which may lead to damages to cell organelles [8].

The algicidal activity of the complexes was the highest against the raphidoflagellate, *Chlorella Pyrenoidosa,* irrespective of the structural variations. This may be because cell envelopes of raphidoflagellate are mainly composed of glycocalyx and plasma membranes. And morphologies of raphido flagellate are easily changed by chemical and physical treatments. Therefore it was not surprising to observe that the efflux of intracellular components from algal cells of Chlorella pyrenoidosa was quickly induced by the complexes compared to the other algal species [9].

Table 2: Comparison of Antialgal Activity of Complexes based on Nature of Anion

Ligand	Algal Species	Anion								
		Perchlorate			Chloride			Nitrate		
		BP6	BP9	BP12	BC7	BC10	BC13	BN8	BN11	BN14
H_2L^2	*O. brevis*	1	0	0	1	1	0	1	2	0
	C. pyrenoidosa	0	0	0	0	0	0	0	0	0

Table 3: Comparison of Antialgal Activity of Complexes based on Nature of Metal ion

Ligand	Algal Species	Central Transition Metal Ion								
		Co			Ni			Cu		
		BP6	BC7	BN8	BP9	BC10	BN11	BP12	BC13	BN14
H_2L^2	*O. brevis*	1	1	1	0	1	2	0	0	0
	C. pyrenoidosa	0	0	0	0	0	0	0	0	0

Conclusion: The activity of a chemical compound to an organism depends on several physical, chemical and biological factors. A comparative study of the antialgal properties of the Schiff base and complexes have been carried out based on structural aspects like nature of central metal ion and nature of anion. A cumulative effect of different favourable factors such as the presence of hydroxyl group, increase in polarity due to chelation, disinfecting effects of the selected metal ions, presence of specific anions and increased penetration through cell membrane owing to ionic nature may be reason for the observed activity of the compounds. Promising results have been obtained regarding the structural dependence of algicidal activity of Schiff base complexes, which may be effectively utilized in the synthesis of algicides after a thorough study of their toxic impacts on the marine ecosystem.

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