International Research Journal of Natural and Applied Sciences
ISSN: (2349-4077)
Impact Factor- 5.46, Volume 5, Issue 3, March 2018
Website- www.aarf.asia, **Email** : editor@aarf.asia , editoraarf@gmail.com

SYNTHESIS, STRUCTURE ELUCIDATION AND ANTIMICROBIAL ACTIVITY OF NEW COMPLEXES OF CO (II) AND NI (II).

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ABSTRACT

The conjunction of Co and Ni ions with biologically important and less explored kynurenic acid ligand to form metal complex is an important area of current research. These complexes were characterized by elemental analysis, molar conductance measurements, magnetic susceptibility measurements, mass spectrometry, FTIR, electronic spectral studies, TGA techniques. The stoichiometry of the complex has been found to be 1: 3 (metal: ligand). The antimicrobial activities of the complexes have been studied by screening the compounds against the bacteria Bacillus subtilis, Salmonella typhii A, Escherichia coli and Staphylococcus aureus and also results have been compared with standard drugs streptomycin and ampicilin.

Key words: Kynurenic acid, metal complexes, Antibacterial activity, Physico chemical study.

INTRODUCTION

Study of the metal coordination chemistry of hetero cyclic compounds is interesting, not only from an analytical point of view, but also because of the biological implications of this type of compounds.

Kynurenic acid (KYNA) was discovered in 1853 by the German chemist Justus von Liebig in dog urine, which it was apparently named after. It is formed from L-kynurenine in a reaction catalyzed by the enzyme kynurenine—oxoglutarate transaminase. Kynurenic acid is one of the endogenous products of tryptophan metabolism, formed along the kynurenine pathway. This pathway is a link between the immune and the nervous system; it also plays an immunoregulatory role during infections, pregnancies, autoimmunological processes, neoplasm growth and also after organtransplantations. Moreover, KYNA, being a selective ligand of the GPR35 receptor, is involved in the modulation of the immune response because this receptor is expressed mainly on cells connected with the immune system [1].

EXPERIMENTAL:

Analytical grade chemicals were used throughout the course of experimental work. Spetroscopic grade solvents were employed for recording the spectra. The compound kynurenic acid (Sigma) was used as the ligand. All metal carbonates used were also A.R. grade. A calculated volume of 70% $HClO_4$ was diluted with water to obtain 0.2M perchloric acid solution. The exact strength was determined by pH metric titration against standard 0.2M NaOH solution. 75 ml 0.2 M perchloric acid was taken and solid metal carbonate was added in it till effervescences observed (slight excess addition was done). The solution was stirred for 30 minutes and filtered and thus the metal perchlorate in aqueous solution was obtained. The formation of complexes was carried out by mixing 50 mL (0.2M) metal perchlorate solution and 50 mL(0.2M) ligand in DMSO solution. The mole ratio of ligand and metal was (1:1). The reason for this ratio is lack of prior knowledge. The reaction mixture was refluxed for around 3.0 h at 95 0C temperature. After 3.0 h the reaction mixture was cooled. There was no immediate precipitation, then into this solution, ice water was added and immediately precipitates were obtained. The complexes thus obtained were washed well with double distilled water and alcohol for removal of unreacted metal and ligand. All the complexes were dried in an oven at 40° C to 50 ° C[2]. These Co(II) and Ni(II)

complexes were then characterized by chemical and instrumental methods to elucidate their structures.

RESULTS AND DISCUSSION

Table 1: RESULTS OF INFRARED SPECTRA

IR spectral band (cm^{-1}) of KYNA ligand and its complexes (values mentioned in cm^{-1}).

KYNA	O-H Phenolic (3434), Acidic –OH (3105), Ar-CH- stretching(2967), C=N(1593), C=O Aromatic stretching(1758), bending, vibrations (748-OH out of plane1245, 1264 CH, CH_2 ,OH in plane1380- wagging and twisting).		
	Changed	**New peaks**	**Eliminated**
Co-KYNA	O-H Phenolic(3200), Ar-CH- stretching (3085),C=N(1446), C=O Aromatic stretching (1654), bending vibrations(1446),	M-N, M-O (521,664)	Acidic –OH
Ni-KYNA	O-H Phenolic(3199), Ar-CH- stretching (3085),C=N(1437), C=O Aromatic stretching (1664), bending vibrations(1437)	M-N, M-O (518,636,664)	Acidic –OH

Table 2: RESULTS OF PHYSICO CHEMICAL MEASUREMENTS

TLC (solvent toluene: methanol 7:3) and M.P. was taken by melting point apparatus. Metal complex formations were confirmed by TLC single spot reading. The UV – visible spectra were measured on a UV-1800 Shimadzu (Double beam) spectrophotometer.

Complex	Colour	M.W. (gm/mol)	M.P. °C	R.F. value *	Molar Conductance (ʊ) mho cm^{-1}	% yield
Ligand (KYNA)	Light cream	189.17	269	0.8503	2.55×10^{-3}	---------
Co-KYNA	cream	626.51	271	0.8545	2.43×10^{-3}	29.96
Ni-KYNA	cream	644.2	267.9	0.8145	3.23×10^{-3}	30.93

* Solvent system : (Toluene: methanol 7:3),).

Table 3: MAGNETIC AND ELECTRONIC SPECTRA.

Metal Complexes	Uv-vis spectral λmax (nm)	Magn. Sus. (BM)	Number of unpaired electrons	Oxidation No.	Coordination No.	Probable shape
KYNA	346.50 291.50 258.00	---------	____	_____	_____	_____
Co-KYNA	360.00 346.00 291.50 242.50	6.16	3 (hs)	(II)	6	octahedral
Ni-KYNA	360.00 346.50 291.50 231.00	6.34	2	(II)	6	octahedral

Uv-vis = ultra violet- visible, Magn. Sus. = magnetic susceptibility, hs = high spin.

Table 4: CHN AND METAL ANALYSIS

Elemental analyses were performed with a Vario-MICRO CUBE C, H, N analyzer.

Metal Complexes	C (%)		H (%)		N (%)		Metal (%) by TGA	
	Found	calculated	found	calculated	found	calculated	found	calculated
Co-KYNA	62.21	57.46	3.85	3.35	7.18	6.70	8.57	9.41
Ni-KYNA	60.56	55.88	4.021	3.25	6.90	6.51	8.89	9.11

The three columns of IR spectra results, single RF values in TLC, electronic spectra, magnetic susceptibility, elemental analyses, molar conductance, all indicated formation of complex compounds in case of both Co(II) and Ni(II). Furthermore, the over all results suggested six coordination with octahedral shape.

TGA- DSC ANALYSIS

Fig 1: Co-KYNA

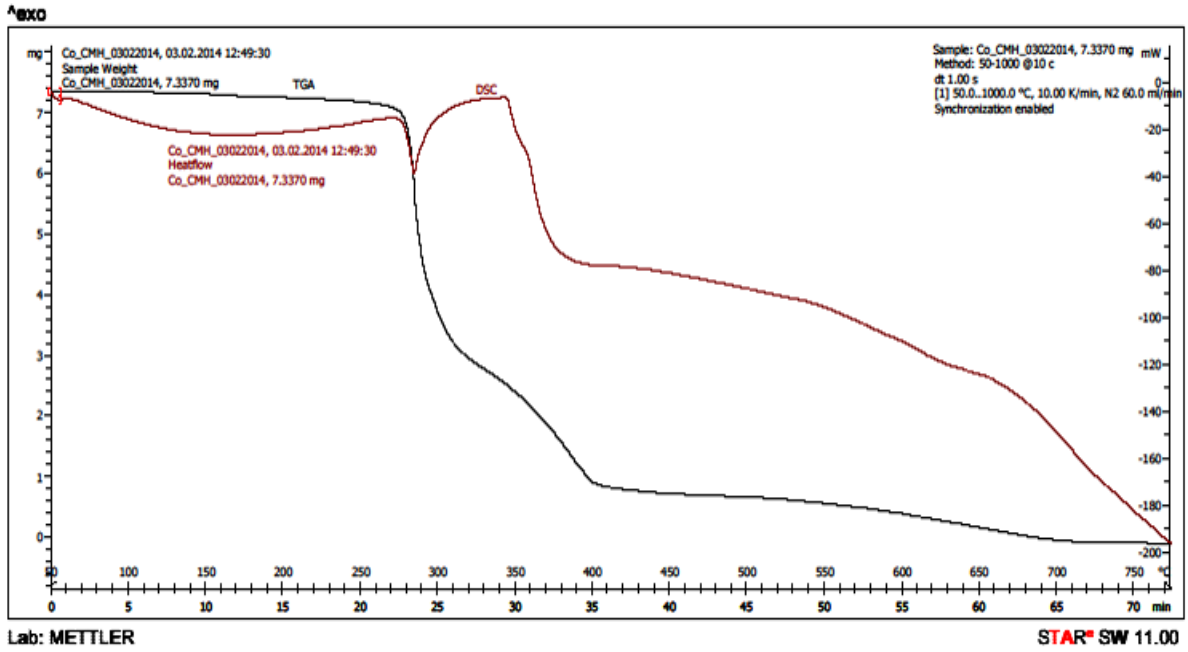
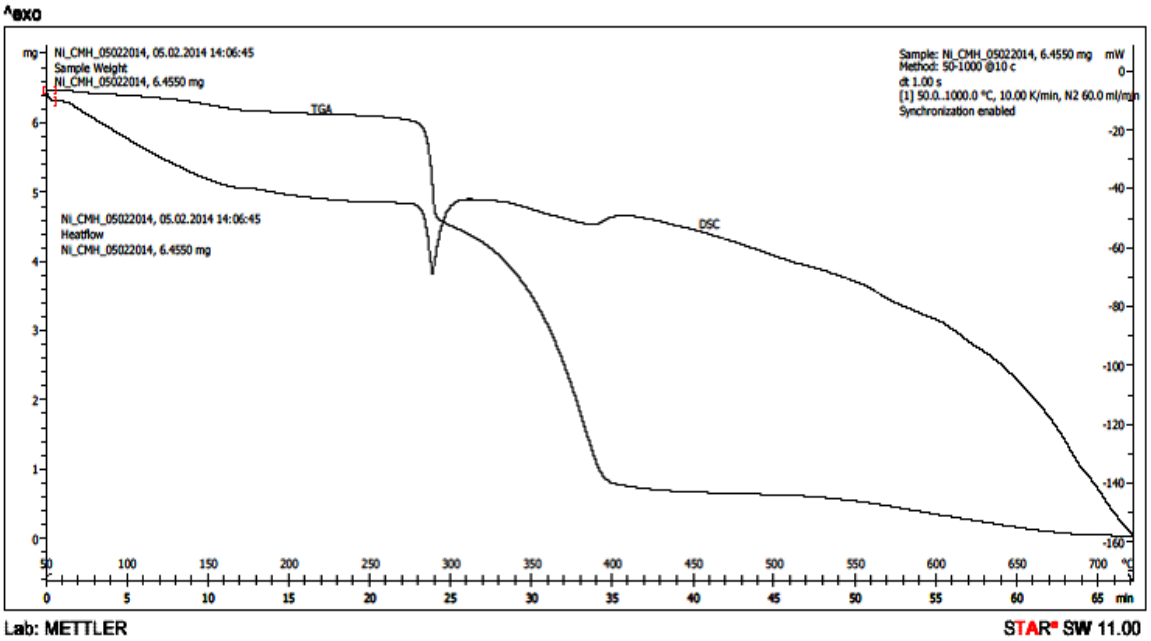


Fig 2: Ni –KYNA



It is observed that at 150^0C temperature in Co-KYNA complex weight loss per mole is 2.41 gm, which indicates that no H_2O molecule of crystallization with Co-KYNA is present and 7.91 gm weight loss occurred for one mole Co-KYNA complex at 250^0C temperature, which indicates that no water molecules coordinated with Co^{2+}complex.

Thermo gravimetric analysis for one mole of Ni-KYNA at 150^0C temperature, 13.84 gm weight loss occurred, which indicated that there is possibly one water molecule of crystallization present. At 250^0C temperature weight loss occurred by one mole Ni-KYNA complex is 12.46 gm. This loss is not accompanied by any appreciable DSC change. Therefore, we may infer absence of water of coordination.

Table 5: Results of TGA

Compound	**RT-150 ^{0}C (Water of crystallization)**			**150 ^{0}C – 250 ^{0}C (water of coordination)**		
	% Loss	**Loss of weight(gm) for 1 mole complex**	**water molecules**	**% Loss**	**Loss of weight(gm) for 1 mole complex**	**water molecules**
KYNA	_____	_____	____	_____	_____	_____
Co-KYNA	0.54	2.41	0	1.77	7.91	0
Ni-KYNA	3.87	13.84	1	2.01	12.46	0

RT= Room Temperature

RESULTS OF MASS SPECTROMETRY

Fig 3: [KYNA mass spectra]

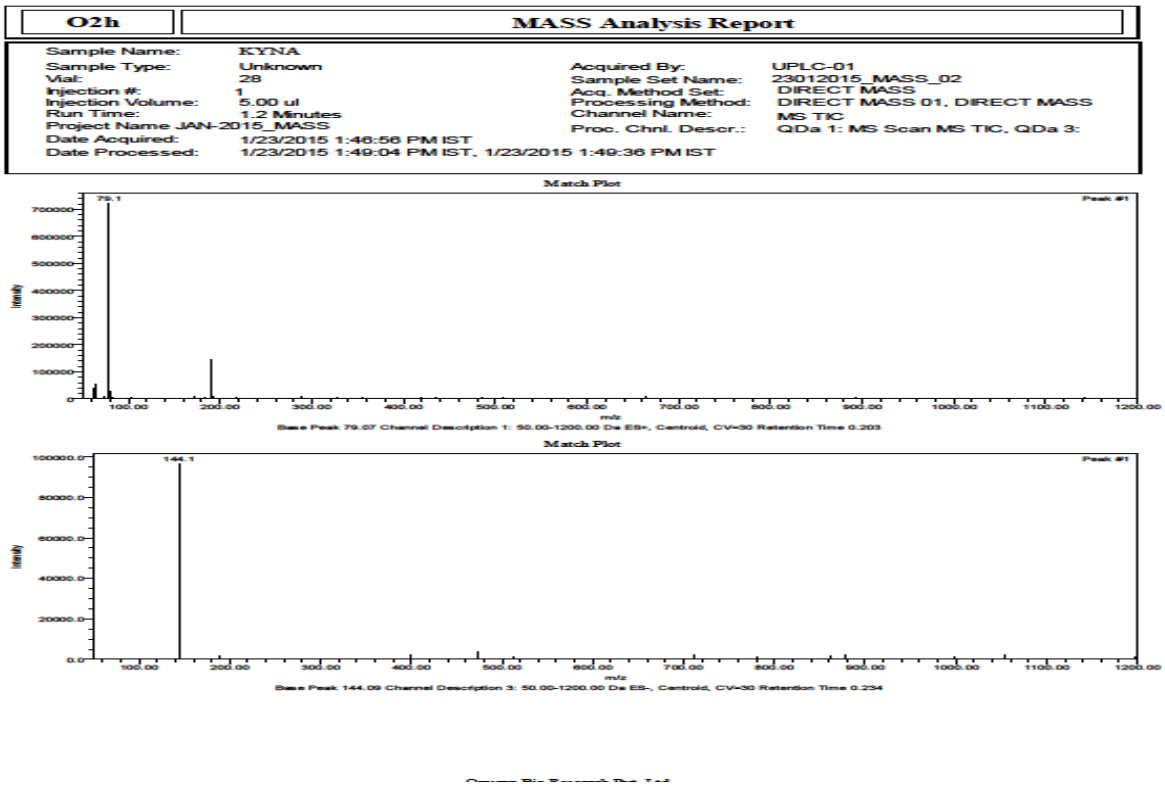


Fig 4: [Co-KYNA mass spectra]

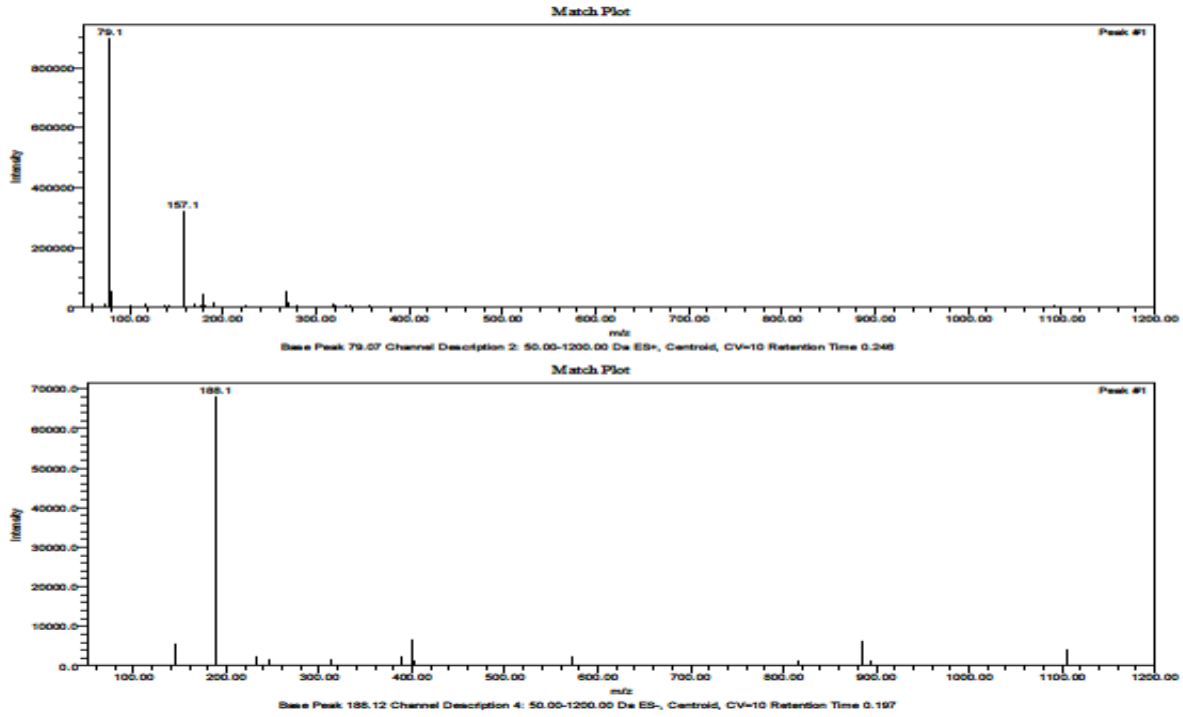
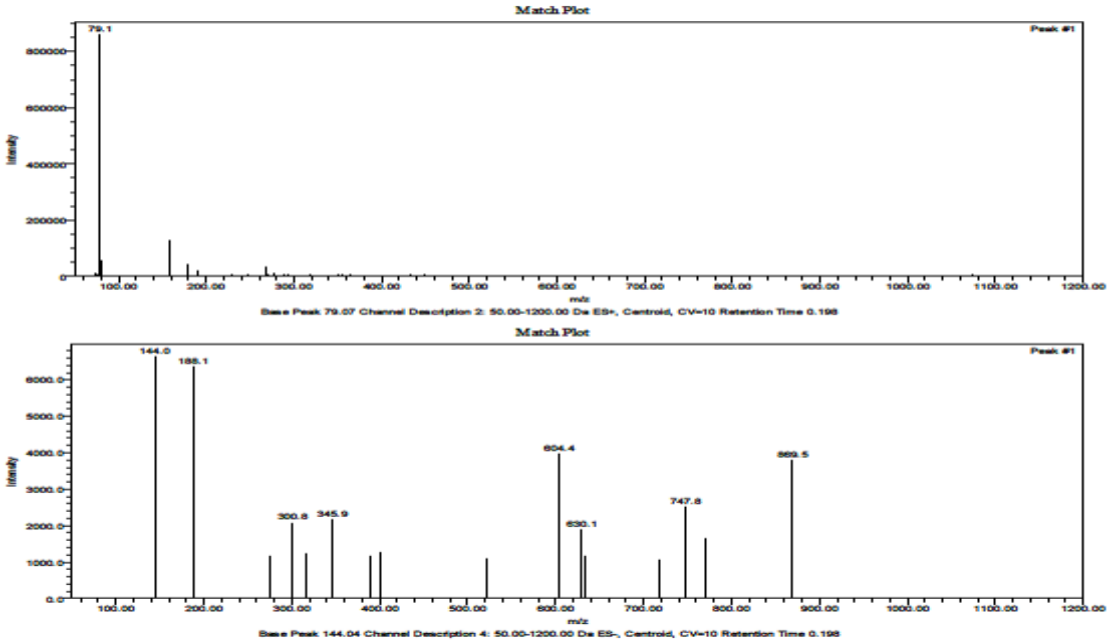


Fig 5: [Ni-KYNA mass spectra]



Mass spectra:

Mass spectrum of KYNA ligand

ES+ : 189 amu is the peak for $C_{10}H_8NO_3$ and 79amu is the base peak due to $(C_6H_6)H^+$ and it is present because of removal of C_4HNO_3 from the hetero cyclic part.

ES- : 144 amu is the base peak because of removal of –COOH group from the KYNA molecule. Feeble 189 peak is observed.

Mass spectrum of Co-KYNA

ES^+ : 157 amu is a peak due to removal of 0_2 (deoxygenation) from KYNA

79 amu is the base peak due to $(C_6H_6)H^+$

58 amu is a peak due to metal (Co).

ES^- : 248 amu is a peak because of Co – KYNA.H^+

188 amu is the base peak due to (KYNA - H^+)

145 amu peak because of removal of (KYNA – COO H)

Mass spectrum of Ni-KYNA

ES^+ : 190 amu is a peak due to (KYNA.H^+)

158 amu is a peak due to removal of 0_2 (deoxygenation) from (KYNA.H^+ - O_2)

79 amu is the base peak due to $(C_6H_6)H^+$

ES- : 188 amu is the peak for (KYNA - H^+)

144 amu is the base peak because of (KYNA – COO H)

FLUORESCENCE SPECTRA

Fig 6: Combined Fluorescence spectra

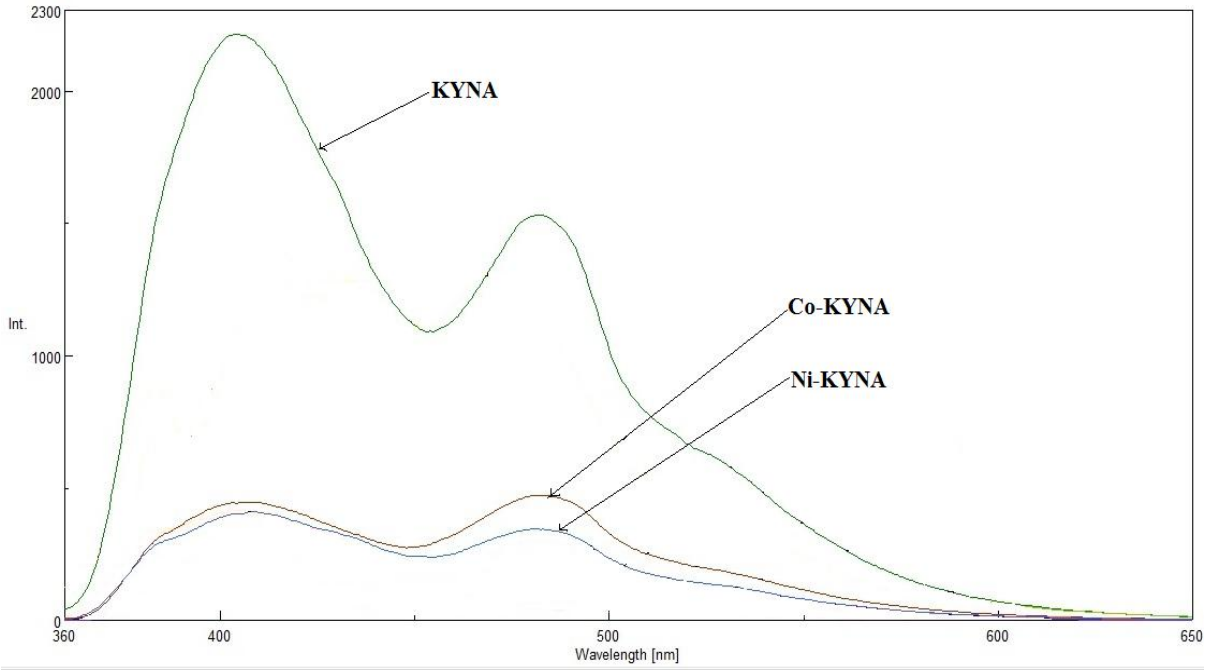


Fig.6. shows the fluorescence spectra of the ligand and the complexes. The spectra are of emission type and taken in the range of 360 nm to 650 nm. The metal ions generally do not exhibit fluorescence activity under normal conditions. However the ligand KYNA indeed exhibits fluorescence activity, therefore it was considered to study the fluorescence behavior of the complexes. The ligand KYNA exhibits UV-visible absorption with λ_{max} below 400 nm but the fluorescence peaks around 405 nm and 480 nm. On co-ordination with metal ions, the fluorescence diminishes to a great extent. The order of reduction in fluorescence intensity is $Ni^{+2} > Co^{+2}$. The probable reason seems to be due to change in the π(pi) bonding and lone pair electron sharing for the metal coordination.

STRUCTURES

Based upon the physico chemical analyses, the structures of the two complexes can be shown as below.

Fig 7: Co-KYNA structure

Fig 8: Ni-KYNA structure

ANTIMICROBIAL ACTIVITY

In vitro biological screening effects of the compounds under investigation were carried out and that was tested against the bacteria: Salmonella typhii, Staphylococcus aureus, Escherichia coli and Bacillus subtilis [3]. The well-diffusion method, using agar nutrient as the medium was used to check antimicrobial activity. In this method, generally compounds are loaded into well made in agar plates and their activities were tested against different organisms streaked on the surface of agar medium [4].

The stock solution of compounds (10^{-2} M) was prepared by dissolving the compounds in DMSO.The given cultures were streaked on agar plates and wells were made with the help of cork borers. The well was filled with the test solution using a micropipette and the plate was incubated 24 h for bacteria at 35°C. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration was noted[5].

ANTIBACTERIAL ACTIVITIES OF ANTIBIOTICS

Zone size	Antimicrobial disc used in practical
+++ 2.6 to 3.0 cm	Streptomycin (25μg/disc) for E-coli, S.typhy and S. aureus
++ 2.0 to 2.5 cm	Ampicilin (25μg/disc) for Bacillus sp.
- No zone 0.8 cm	

All antibiotics in standard condition gave +++ results.

Table 6: RESULTS OF ANTIBACTERIAL STUDIES

Culture	Well no.	Compound Co-KYNA	Ni-KYNA	KYNA
	1. 25 mcg/ml	+	+	+
Bacillus subtilis	2. 50 mcg/ml	+	+	+
(Gram-possitive)	3. 75 mcg/ml	+	+	++
	4. 100 mcg/ml	+	+	+++
Staphylococcus aureus	1. 25 mcg/ml	_	_	_
(Gram-possitive)	2. 50 mcg/ml	_	_	_
	3. 75 mcg/ml	_	_	+
	4. 100 mcg/ml	++	++	++
Escherichiacoli	1. 25 mcg/ml	_	_	_
(Gram negative)	2. 50 mcg/ml	_	_	+
	3. 75 mcg/ml	_	_	_
	4. 100 mcg/ml	_	++	_
	1. 25 mcg/ml	_	_	_
Salmonella typhii A	2. 50 mcg/ml	_	_	_
(Gram negative)	3. 75 mcg/ml	_	_	_
	4. 100 mcg/ml	_	_	_

The KYNA has inhibitory effect on gram +ve bacteria namely Bacillus subtilis, Staphylococcus but no inhibitory effect on gram –ve cultures. The ligand and complexes did not exhibit any inhibitory effect on gram negative bacteria in general. Another observation was that on coordination of KYNA, the overall antibacterial activity decreased. The antibacterial activity of ligand and complexes was much less compared to the standard antibiotics selected. The antibacterial activity of Co-KYNA and Ni-KYNA are nearly same for all the four microorganisms selected. The ligand showed higher antibacterial activity which increased with increased concentration compared to the complexes.

CONCLUSION

Biological activities of Ni^{+2}, Co^{+2} as well as of the ligand, kynurenic acid, led us to prepare their complexes which showed 6 coordination number . The complexes are found to be fairly stable and these were characterized by important instrumental and routine chemical methods. Fluorescence analysis showed that on complexation, the fluorescence behaviour of the ligand was reduced but not totally eliminated. Although the antimicrobial activities of both the complexes are found to be quite low but we earnestly are hopeful of getting encouraging results in other applications of these complexes.

AC KNOWLEDGEMENT

One of the authors, Jwalant vora is thankful to GUJCOST for the financial support in the form of a minor research project (GUJCOST/MRP/14-15/1460). Authors are also thankful to the principal and faculty members of M.G. Science institute, Ahmedabad. Our sincere thanks to Dr. Devang Pandya for his valuable discussions.

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