SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND BIOLOGICAL EVALUATION OF P-METHOXY ISONITROSO ACETOPHENONE WITH CR(III), MN(II), CO(II) AND CU(II) AGAINST MULTIDRUG RESISTANT BACTERIAL AND FUNGAL PATHOGENS

Gajbhiye R. G., *Thakare S. R., **Raut R. D., ***Mahakale R. G.
Department of chemistry, Vidya Vikas Arts, Commerce & Science College, Samudrapur Dist-Wardha, 442305, India.
* Department of chemistry, Institute of Science, Nagpur, 440003, India.
**Department of chemistry, J. B. College Of Science, Wardha Dist- Wardha, 442001, India.
***Department of Chemistry, R. S. Bidkar College, Hinganghat Dist-Wardha, 442301, India
rahul.gajbhiye433@gmail.com

A B S T R A C T

A schiff base ligand p- methoxy isonitroso acetophenone (p-MINAP) was prepared from the interaction of anisole, acetic anhydride and n-amyl nitrite. The Cr(III), Mn(II), Co(II) and Cu(II) complexes of schiff base derived from p- methoxy isonitroso acetophenone (p-MINAP) have been synthesized and characterized through IR, ^{1}H NMR and elemental analysis. The ligand p-methoxy isonitroso acetophenone (p-MINAP) and its complexes have been screened for the antibacterial activities towards bacteria S. aureus, B. cereus (gram positive), and E. coli, K. pneumonae (gram negative) and antifungal activities towards fungi A. niger and C. albicans. It has been found that all the complexes show potent activity on the isolates.

Keywords: p-MINAP, IR,^{1}H NMR, antibacterial, antifungal, isolates.

1. Introduction

Coordination chemistry of schiff base metal complex is undergoing rapid growth due to their vital roles in chemical industries and biological systems described by Sarayna and Lakshami [1]. Recently, considerable attention has been paid to the chemistry of the metal complexes of schiff bases containing nitrogen as donor atom suggested by Saranya et. al. [2]. Transition metal complexes of schiff base has increased in the late 19th century and since then metal based drugs of schiff bases drew vital interest of researchers in the medical science for their immense biological activities shown by Mohmed et. al. and Adly [3,4]. Drug resistance against various pathogens is the major cause of morbidity and mortality throughout the world, so novel antimicrobial drugs are still in demand for biological monitoring of diseases reported by Rehman et. al., Mohamed et. al, Prakash and Adhikari [5-7]. In schiff bases the azomethine (C=N) linkage plays an important role in explaining the mechanism of transamination and resamination reactions in biological system suggested by Lau et. al. and Dholakiya et. al. [8, 9].

The main target of this paper is to synthesize new schiff base transition metal complexes derived from p-methoxy acetophenone with n-amyl nitrite and characterized through IR, ^{1}H NMR and elemental analysis. In addition, antibacterial and antifungal activities of the synthesized compounds were carried out.

2. Experimental Section

2.1 Chemicals:

The chemicals used were of analytical reagent grade. The ligand p- methoxy isonitroso acetophenone (p-MINAP) was prepared by the method described in the literature by Bhandakkar et. al. and Vogel [10, 11]. The metal complexes are prepared as follows:-

2.2 Preparation of Cr (p-MINAP)$_3$ Complex:

0.238 g of chromium sulphate is dissolved in a minimum quantity of alcohol and equal volume of water is added. Similarly,

0.358 g of p- methoxy isonitroso acetophenone is also dissolved in a minimum quantity of alcohol and equal volume of water is added. The aqueous solution of chromium is added to the reagent solution dropwise with continuous stirring in a conical flask. The pH is adjusted to 6.5 – 7 using buffer tablets. A green coloured complex is separated out. It is filtered, washed with water and then alcohol, dried at 120^{o}C for several hours and analyzed for chromium, carbon, hydrogen and nitrogen.

2.3 Preparation of Mn (p-MINAP)$_2$ Complex:

0.251 g of manganese chloride is dissolved in a minimum quantity of alcohol and equal volume of water is added. Similarly, 0.358 g of p-methoxy isonitroso acetophenone is also dissolved in a minimum quantity of alcohol and equal volume of water is added. The solution of manganese is added to the reagent solution dropwise with continuous stirring in a conical flask. The pH is adjusted to 6.3 – 6.9 using buffer tablets. This solution is refluxed on sand bath at 100^{o}C for 2 hrs & then kept in vacuum desiccators for overnight. A brown coloured complex is separated out. It is filtered, washed with water and then alcohol, dried at 124^{o}C for several hours and analyzed for manganese, carbon, hydrogen and nitrogen.

2.4 Preparation of Co (p-MINAP)$_2$ Complex:

0.364 g of cobalt nitrate is dissolved in a minimum quantity of absolute alcohol and equal volume of water is added; similarly 0.716 g of p-MINAP was dissolved in absolute alcohol and equal quantity of water. The cobalt nitrate solution is added to the ligand (p-MINAP) solution dropwise with constant stirring in a conical flask. The pH of mixed solution maintain to 6 – 6.5 using buffer tablets. On refluxing on sand bath at 110^{o}C for 2 hours and then kept in vacuum desiccators for overnight. A yellow coloured complex has been formed. It is filtered and recrystallised from chloroform and analyzed for cobalt, carbon, hydrogen and nitrogen.

2.5 Preparation of Cu (p-MINAP)$_2$ Complex:

Copper acetate and p-MINAP in the molar proportions of 1:2 are dissolved separately in absolute alcohol and then equal volume of redistilled water and then mixed their aqueous solution with each other by constant stirring in a conical flask. The pH of the resultant solution is adjusted to 6-7 using buffer tablets. The mixed solution refluxed on sand bath at 90^{o}C for 3 hours and a coloured complex is formed. It has been filtered, dried in air, recrystallised from chloroform and analyzed for copper, carbon, hydrogen and nitrogen.

2.6 Antibacterial and Antifungal Activity Tests

The p-methoxy isonitroso acetophenone (p-MINAP) and its divalent and trivalent metal complexes were dissolved separately in DMSO. They were placed on the surface of the culture and incubated at 37^{o}C for 24 hrs suggested by Ramon et. al. and Yeamin et. al. [12, 13]. The *in vitro* antibacterial activity was carried out by disc diffusion method. The diameter of zone of inhibition produced by the schiff base and its metal complexes were compared with Gentamycine and Micanozole for bacterial and fungal standard respectively.

3. Result and Discussion

The schiff base p-methoxy isonitroso acetophenone prepared is powdery brown solid, with percentage yield 80.2 % and melting point of 96^{o}C. The colour of Cr(III), Mn(II), Co(II) and Cu(II) schiff base complexes is green, brown, yellow and light green respectively. The elemental analysis data and molar conductance are shown in Table 1.

Table 1: Elemental analysis and molar conductance of complexes of p-MINAP

Ligand/Complexes	Colour	% yield	% of C	% of H	% of N	% of M	Molar conductance (Sm^2mole^{-1})
p-MINAP	Brown	80.2	60.30 (60.33)	5.12 (5.06)	7.75 (7.82)	---	--
Cr(p-MINAP)$_3$	Green	63.58	54.96 (55.10)	4.58 (4.62)	7.12 (7.83)	8.82 (9.07)	17.2
Mn(p-MINAP)$_2$	Brown	77.45	52.29 (52.31)	4.37 (4.35)	6.75 (6.77)	13.25 (13.29)	16.4

$Co(p\text{-}MINAP)_2$	Yellow	71.19	51.14 (51.18)	4.38 (4.34)	6.68 (6.71)	14.16 (14.12)	11
$Cu(p\text{-}MINAP)_2$	Light Green	74.62	51.30 (51.25)	4.25 (4.30)	6.60 (6.64)	15.03 (15.06)	7.35

The elemental analysis of the schiff base and its divalent chelates for H, C and N determined showed that the observed and the calculated percentages of the elements are in good agreement and the result suggested 1:2 metal-schiff base ratio in all the complexes.

The molar conductance values of the metal (II) schiff base complexes determined in DMSO solution suggesting that they are non-electrolytes by Geary [14].

3.1 Infrared Spectroscopy

The infrared spectra of the p-MINAP showed a band at 3317 cm^{-1} which is assigned to *ν*(O-H) stretching vibrations of =N-OH which was in agreement with Das et. al., Deshmukh and Thakkar [15, 16]. This band was absent in all the complexes, revealing deprotonation on coordination of the p-MINAP to the respective metal ions suggested by Ahmed and Akhtar [17]. The peak at 1610 cm^{-1} in the free p-MINAP was attributed to azomethine *ν*(C=N) vibration frequencies. However, this band was observed to shift to the lower frequencies in all the metal schiff base complexes, suggesting the participation of azomethine nitrogen of the schiff base p-MINAP on coordination to the respective metal ions. The absorption bands in the ranges 610 – 660 cm^{-1} and 1230 – 1238 cm^{-1} observed in all the complexes were assigned to *ν*(M—N) and *ν*(N→O) vibrational frequencies respectively, confirming coordination of the schiff base to the respective metal ions (Table 3).

Table 2: - Infrared Spectra of p-MINAP and their metal complexes

Assignment	p-MINAP	Cr $(p\text{-}MINAP)_3$	Mn $(p\text{-}MINAP)_2$	Co$(p\text{-}MINAP)_2$	Cu$(p\text{-}MINAP)_2$
OH of =N-OH	3317.39	----	----	----	----
Aromatic C-H	3018.70	3051.07	3024.72	3011.07	3038.72
$-OCH_3$	2840.23	2858.42	2860.32	2819.42	2840.32
-C=O	1710.51	----	----	----	----
-C=N	1610.11	1598.84	1595.68	1600.04	1617.68
=C-H	1440.12	1445.46	1447.68	1445.46	1440.68
=N→ O	----	1234.67	1230.57	1230.67	1238.57
Para Substituent	766	791.34	790.30	795.47	790.32
M-N	----	659.25	660.68	610.25	627.30

3.2 ^{1}H NMR Spectroscopy

^{1}H NMR Spectra of the schiff base ligand p-MINAP, $Cr(p\text{-}MINAP)_3$, $Mn(p\text{-}MINAP)_2$, $Co(p\text{-}MINAP)_2$ and $Cu(p\text{-}MINAP)_2$ in DMSO as a solvent shows peaks due to –CH group, $-OCH_3$ group & Ca and C_b of aromatic ring proton. In metal complexes with p-MINAP does not show any proton signal due to =N-OH group. This recommend that their complexes are formed by the replacement of the proton of the -OH group in p-MINAP by the metal atoms. It's very interesting to note that the peaks due to –CH proton in transition metal complexes with ligand p-MINAP seem at similar value compared to that of –CH proton within the ligand p- methoxy isonitroso acetophenone. Further signals of aromatic ring protons in these complexes occur at higher field

side with regard to that of aromatic ring signal in the ligand p-MINAP. The donor atom is nearest to the metal particle that concerned within the formation of metal ligand bond.

Table 3: Assignments of ^{1}H NMR Spectral Signals in p-MINAP & its Metal Complexes

All Values in δ scale

Compounds	=N-OH group	Aromatic Ring $-C_aH$ group	Aromatic Ring $-C_bH$ group	-CH Group	$-OCH_3$ Group
p-MINAP	8.48	7.92	7.60	2.42	3.90
Cr(p-MINAP)$_3$	--	7.96	7.68	2.42	3.90
Mn(p-MINAP)$_2$	--	7.90	7.66	2.44	3.90
Co(p-MINAP)$_2$	--	7.90	7.68	2.42	3.84
Cu(p-MINAP)$_2$	--	7.94	7.68	2.44	3.84

3.3 Antimicrobial Activity

The antibacterial activity tested for the schiff base ligand p- methoxy isonitroso acetophenone and its transition metal complexes have been determined. The diameter of inhibition zone (mm) was measured for each treatment. *E. coli* is active on the schiff base ligand. The result for *S. aureus* showed minimal activity against all the metal complexes with p-MINAP. The Co(p-MINAP)$_2$ complex showed strong activity on the isolates, while Mn(p-MINAP)$_2$, Cr(p-MINAP)$_3$ and Cu(p-MINAP)$_2$ showed some activity on all the isolates. The results are shown on Table 5. Sensitivity of fungal isolates (*A. niger* And *C. albicans*) showed that the schiff base ligand p-MINAP is active on both the isolates. The result for *A. niger a*nd *C. albicans* showed maximal activity against all the complexes except that of Copper (II), which showed some activity (Table 6). In general, the results indicate that the complexes show potent activity than the schiff base ligand under similar experimental conditions. Fehmi et. al. would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedys chelation theory [18]. Chelation considerably reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with the donor groups and possible electron delocalization over the whole chelate ring. Such chelation could also enhance the lipophilic character of the central metal atom, which subsequently favours its permeation through the lipid layer of the cell membrane suggested by Tuner et. al. [19].

Table 4: Zone of Inhibition Antibacterial Activity Data of p-MINAP & their Complexes

Compounds	Isolates			
	***S. aureus* (a)**	***B. Cereus* (b)**	***E. coli* (c)**	***K. pneumonae* (d)**
p-MINAP	14	15	17	16
Cr(p-MINAP)$_3$	16	21	16	18
Mn(p-MINAP)$_2$	13	14	12	15
Co(p-MINAP)$_2$	15	23	19	21
Cu(p-MINAP)$_2$	14	14	18	15
Gentamycin	22	20	20	19

Table 5: Zone of Inhibition Antifungal Activity Data of p-MINAP & their Complexes

Compounds	Isolates	
	C. albicans (a)	*A. niger (b)*
p-MINAP	12	15
$Cr(p\text{-}MINAP)_3$	18	15
$Mn(p\text{-}MINAP)_2$	17	16
$Co(p\text{-}MINAP)_2$	19	18
$Cu(p\text{-}MINAP)_2$	16	14
Miconazole	22	21

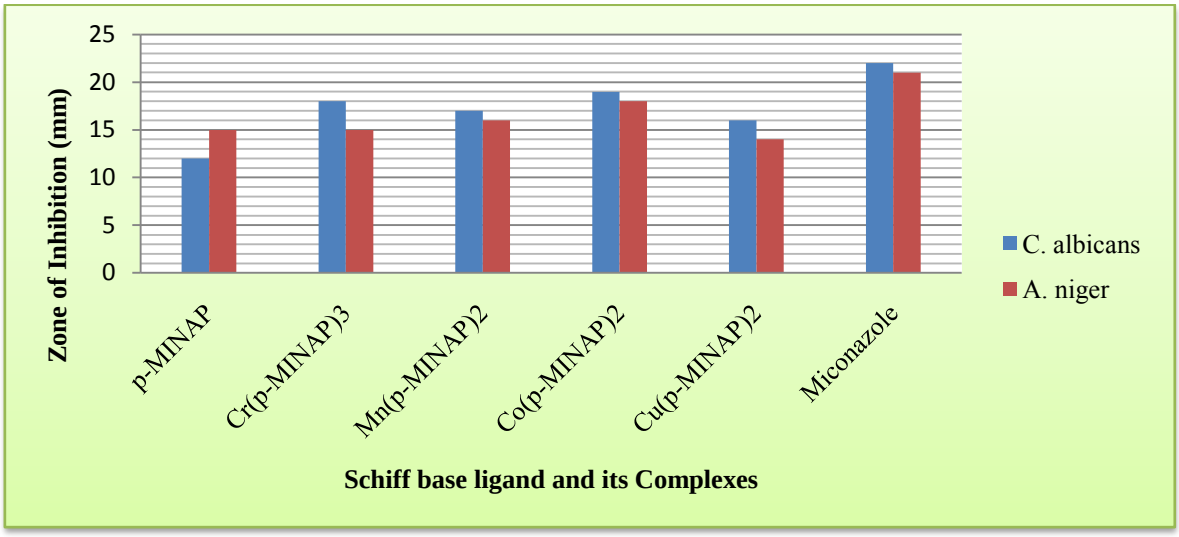

Figure 1: Antifungal activity of p-MINAP and its complexes with isolates

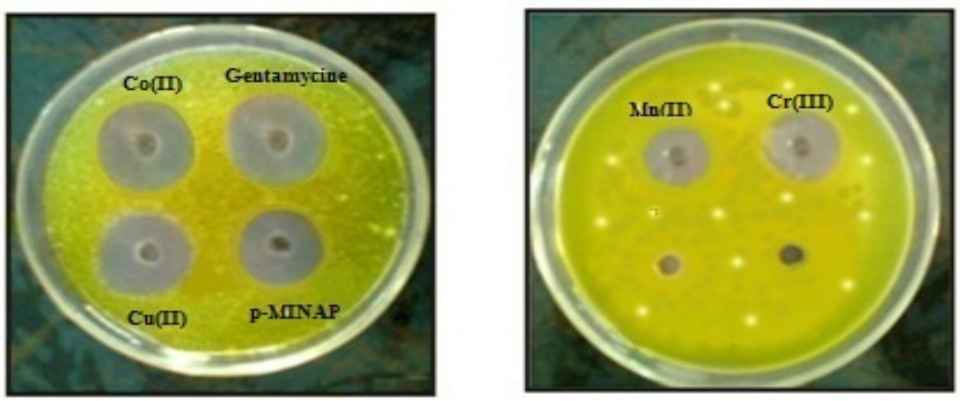

Figure 2: Inhibition zone by p-MINAP, its complexes and Standard Gentamycin against *E. coli* isolate

4. Conclusion

In the present work, the bioinorganic material p- methoxy isonitroso acetophenone containing azomethine linkage (C=N) has been studied extensively and is an entity which has interesting biological features. The ligand p-MINAP coordinates to metal ions using the azomethine nitrogen atom. The free ligand p-MINAP coordinates to metal ions in the ratio of 2:1. The synthesized complexes were

characterized by elemental analysis, Infrared and ^{1}H NMR. All the synthesized compounds that are free ligand and metal complexes were evaluated *in vitro* against four bacterial and two fungal pathogens and showed potent antibacterial activity.

Acknowledgment

The authors want to thanks each and every teaching and non teaching staff member of the Department of Chemistry, Govt. Institute of Science, Nagpur for all the support rendered throughout this study.

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