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Structural Aspects and Microbial Analysis of Copper Surfactants Substituted 2-Amino-6-Chloro Benzothiazole

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Abstract

Biologically active compounds are very important materials for the future generations to be synthesized and analyze. Increasing number of microbial infectious diseases and resistant pathogens create a demand and urgency to develop novel, potent, safe and improved variety of antimicrobial agents. The copper surfactants substituted 2-amino-6-chloro benzothiazole were synthesized. The synthesized complexes have been characterized by IR, NMR, ESR spectroscopic methods. The microbial activities against Alternaria alternate fungi have been evaluated. All complexes showed good antifungal activity due to presence of nitrogen and sulphur atoms in the synthesized molecule.

Keywords: IR, Copper surfactants, Antifungal, *Alternaria alternate,* 2-amino-6-chloro benzothiazole.

1. INTRODUCTION

Surfactants and their complexes with nitrogen and sulphur atoms have become one of the most important requirements in daily life, and have great importance in modern engineering and pharmacological fields. The activity of the surfactant having transition metal against pathogens is enhanced on complexing with ligands containing N and S atoms [1,2]. The microbial activity of these derivatives governed by the nature and structure of ligands and on the presence of particular transition metal atom [3, 4].Surface active compounds are very useful in biological systems and play an important role in many industrial processes [5,6]. Their pathogenic activity is enhanced on complexation with ligands having N and S atoms [7,8]. Information about micellar behavior and structure of copper (II) soaps may play an significant role in its selection in various fields such as, herbicides, pesticides, in paints and varnishes, as wood preservatives, lubricants etc. [9,10]. To build a pollution free environment, it targets to synthesis some new alternatives which are biodegradable and eco-friendly. Present research work is a step towards this initiative. The copper surfactants substituted 2-amino-6-chloro benzothiazole were synthesized. The antifungal activities of these have been evaluated by testing against *Alternaria alternate.* The fungi toxicity results indicate that the strain of fungal species are susceptible towards these and suggests that antimicrobial activity increases with the increase in concentration of copper surfactant and its complexes

2. EXPERIMENTAL

The three fatty acids (n–caprylic acid, n–capric acid and n–lauric acid) have been taken for study in pure form. Further they were purified by keeping over anhydrous sodium sulphate for a week and then distilled under reduced pressure. The purity of acids was checked by the determination of their melting points. n - caprylic acid (16.3^{0}C) n - capric acid (31.2^{0}C), n - lauric acid (44.0^{0}C). Ligand 2-

amino-6-chloro benzothiazole (Figure 1) was synthesized using thiocyanation method [11]. The purity of the benzothiazole has been checked by thin layer chromatography in various non-aqueous solvent systems [12]. Copper soaps were prepared by direct metathesis of the corresponding sodium soap with slight excess of the required amount of copper sulphate solution at $50-55^0$C. After washing with hot water and alcohol, the samples were dried at $100-105^0$C. Finally under reduced pressure, they were recrystallized twice from hot benzene (Figures 2-4). The metal was analyzed by standard procedure. C, H, N, Cl and S analysis were performed at RSIC, CDRI Lucknow. All the solid complexes (about 90% yields) with bluish green periphery were obtained (Table 1). The solubility has been checked and found that these are soluble in ethanol, methanol, benzene and other organic solvents and insoluble in water [13]. All the complexes are quite stable at room temperature up to 170^0C. On the basis of elemental analysis, the complexes have been assigned the composition $Cu_2(C_nH_{2n+1}COO^-)_4L_2$, n=7,9,11 and suggested 1:1 type stoichiometry [14].

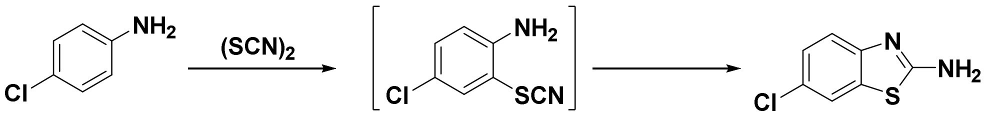

Figure 1: synthesis of ligand by thiocyanation method

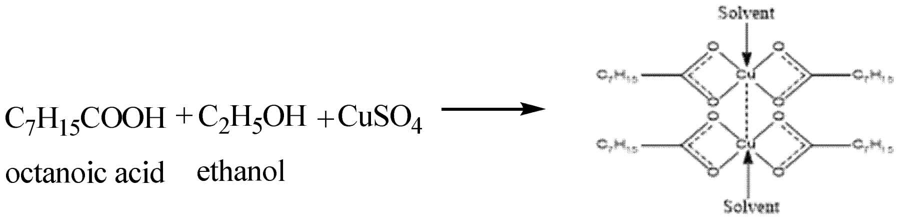

Figure 2: Synthesis of Copper-caprylate CC_{pl}

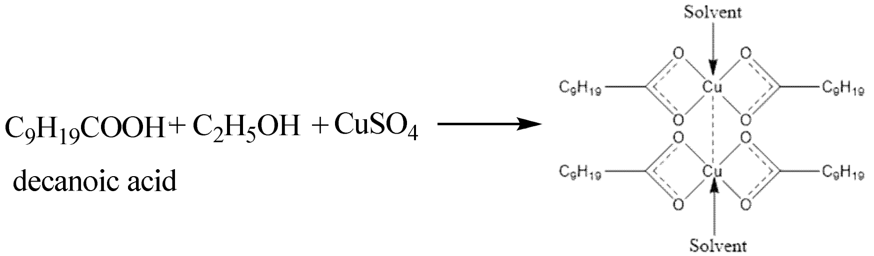

Figure 3: Synthesis of Copper-caprate CC_{pr}

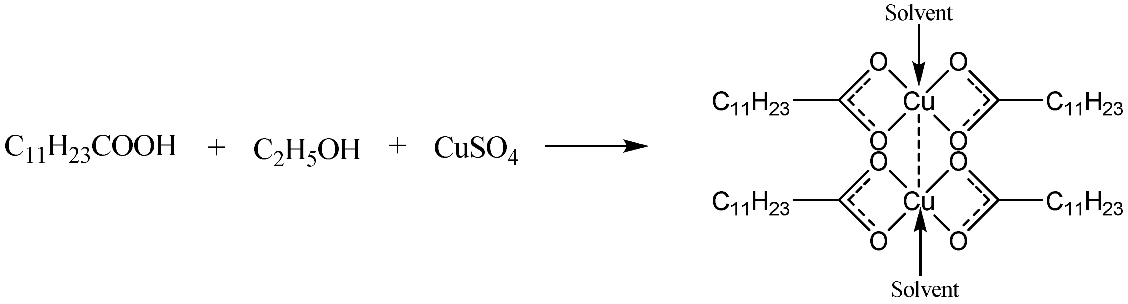

Figure 4: Structure of Copper-laurate CL_{rt}

Table 1: Analytical and physical data of the complexes

Molecular Formula of Complex	Colour	% Yield	M.P. °C	%Cu	%C	%H	%O	%N	%S	%Cl
$Cu_2(C_7H_{15}COO^-)_4.$ $(C_7H_5N_2SCl)_2$ CC$_{pl}$ACB	Dark Greenish Blue	90	175	11.67 [11.89]	51.07 [51.68]	6.23 [6.55]	11.72 [11.98]	5.02 [5.24]	5.89 [5.99]	6.56 [6.64]
$Cu_2(C_9H_{19}COO^-)_4.$ $(C_7H_5N_2SCl)_2$ CC$_{pr}$ACB	Greenish Blue	92	190	10.54 [10.76]	54.85 [54.91]	7.03 [7.28]	10.78 [10.84]	4.59 [4.74]	5.22 [5.42]	5.98 [6.01]
$Cu_2(C_{11}H_{23}COO^-)_4.$ $(C_7H_5N_2SCl)_2$ CL$_{rt}$ACB	Blue	94	205	9.80 [9.83]	48.06 [48.29]	7.77 [7.89]	9.81 [9.90]	4.25 [4.33]	4.86 [4.95]	5.38 [5.49]

The studied systems were abbreviated as:

1. Copper-caprylate with 2-amino-6-chloro benzothiazole: CC$_{pl}$ACB (Figure 5)
2. Copper-caprate with 2-amino-6- chloro benzothiazole: CC$_{pr}$ACB (Figure 6)
3. Copper-laurate with 2-amino-6- chloro benzothiazole: CL$_{rt}$ACB (Figure 7)

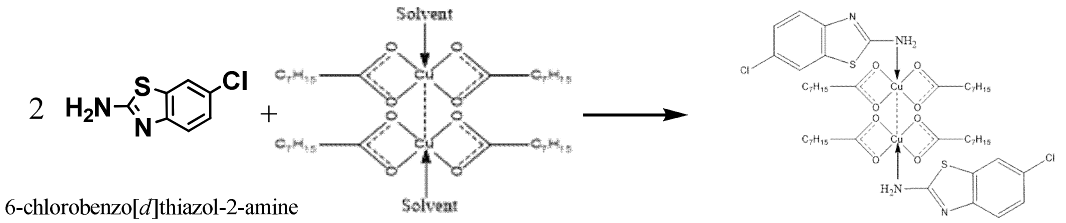

Figure 5: Synthesis of Copper-caprylate with 2-amino-6-chloro benzothiazole: CC$_{pl}$ACB

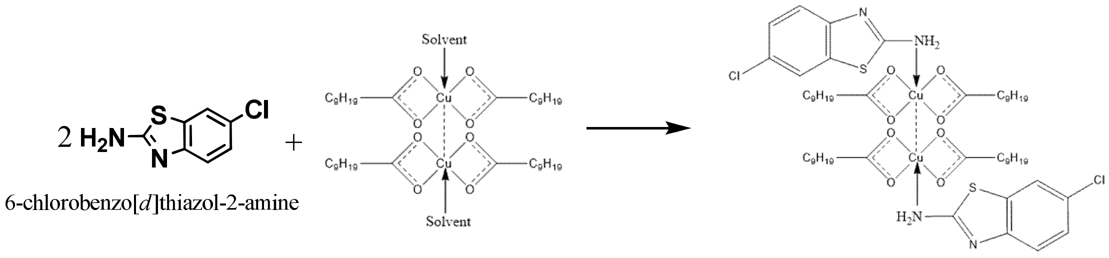

Figure 6: Synthesis of Copper-caprate with 2-amino-6- chloro benzothiazole: CC$_{pr}$ACB

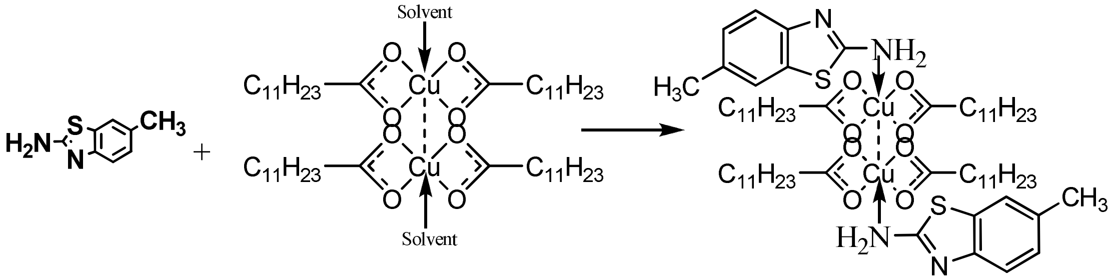

Figure 7: Synthesis of Copper-laurate with 2-amino-6- chloro benzothiazole: $CL_{rt}ACB$

2.1 Fungicidal activities:

The solutions of pure soaps and complexes were prepared with different concentrations .1 ml of each solution was aseptically transferred into sterile petriplates. Into these plates, 20 ml of P.D.A. [15] was poured and was mixed with soap solution by rotating the petriplates in clockwise and anticlockwise direction 3-4 times and was allowed to solidify. After the solidification of the above medium, single hypha / spore of test organism was aseptically transferred in the centre of the petriplates. The plates were incubated at 30 ^{0}C for 3 days. After the period of incubation, the plates were observed for the growth of fungus in different concentration of the complex solutions used in the present study. The data were statistically analyzed according to the following formula [16].

% Inhibition = (C-T)*100/C

Where C = diameter of fungal colony in control plates after 3 days
T = diameter of fungal colony in test plate after 3 days

2.2 Instrumentation:

The structures of complexes have been studied using various spectroscopic techniques. The infrared absorption spectra of compounds were obtained on a Perkin–Elmer 5100.4367 Spectrophotometer [4000-200 cm^{-1}] and ESR analysis was made on Marker-TCNE analyzer at RSIC, IIT Powai, Mumbai. ^{1}H NMR spectra (C_6D_6) was recorded on a NMR spectrometer at RSIC, IIT Powai, Mumbai. The ESR spectra of the complex recorded on X-band at frequency 9.07 GHz under the magnetic field strength of 2000 ± 4000 and 3000 ± 2000 gauss at room temperature. ESR Spectrum is characterized by the position intensity and shape as to component lines. The position of ESR is referred as g values and is directly determined by the energy levels. The variation in g value is interpreted in terms of first and the second order spin orbit interaction. The g values for ESR signal are calculated by the formulae [17].

$g = h\nu/\beta H$

Where,
ν = Freq. of band in kH$_z$.
β= Bohr magneton
H = magnetic field
h = Planck's constant

3. CHARACTERIZATION

3.1 IR Studies

The detailed infrared spectral investigation confirms the synthesis of complexes as there are marked differences have been observed between the spectra of pure copper soaps and pure ligand than those of corresponding complexes (Table 2) The absorption bands observed in the region 2960-2950 cm^{-1} and 2900-2950 cm^{-1} respectively are assigned to the antisymmetric stretching for methyl group of the soap segment present in the complex. Symmetric stretching bands for methyl group has been assigned in the region 2820-2850 cm^{-1}[18]. It has been observed that the absorption peaks, which are characteristics of the aliphatic portion of the soap molecules, remain unchanged on going from soaps to the complexes. The absorption spectra in the region 710-725 cm^{-1} may probably be due to the rocking vibration of a chain of methylene group–$(CH_2)_n$. The peaks in the region 1575-1590 cm^{-1} and

1400-1410 cm^{-1} are due to COO$^-$, C-O anti-symmetric and symmetric stretching respectively. Presence of bands in the region 810 ± 20 cm^{-1} are due to C-H deformation (in plane) present in the tri-substituted benzene ring of benzothiazole segment, where two hydrogens are adjacent [1, 2 and 4 position]. The C-S stretching bands present in the region of 700-600 cm^{-1} also confirm the structure of molecule [19]. The IR bands of pure ligand 2-amino-6-chloro benzothiazole show a clear peak in the region around 1095 cm^{-1} which is due to Ar-C-Cl vibrations, which shifts to 1110 ± 5 cm^{-1}on complexation (Table 3)

Table 2: Infrared absorption spectral wave number (cm^{-1}) of soap, and ligand and their assignment

Assignment	**Soap**			**Ligand**
	Cu-Caprylate	**Cu-Caprate**	**Cu-Laurate**	**2-chloro-6-chloro benzothiazole**
N-H stretching in amino group	---	---	---	3450.6
CH_3, C-H antisym. Stretching	2900.0	2950.0	2965.0	---
CH_2, C-H symmetric stretching	2820.0	2840.0	2855.0	---
COO^-, C-O antisym. Stretching	1575.0	1590.0	1605.0	---
C-H deformation vibration of $-CH_3$ group	---	---	---	---
C=N stretching	---	---	---	1446.5
CH_2, deformation	1430.0	1430.0	1430.0	---
COO^-, C-O symmetric	1410.0	1410.0	1410.0	---
C-N stretching (aryl tertiary)	---	---	---	1320.0
CH_2 twisting and wagging	1295.0	1275.0 1230.0	1260.0	---
CH_3 rocking	1105.0	1110.0	1115.0	---
Ar-C-Cl stretching	---	---	---	1095.9
C-H deformation (in plane) due to benzene ring	---	---	---	813.4
CH_2 rocking	710.0	718.0	726.0	---
C-S stretching	---	---	---	620.0

Table 3: Infrared absorption spectral wave number (cm^{-1}) of complexes and their assignment

Assignment	**$CC_{pl}ACB$**	**$CC_{pr}ACB$**	**$CL_{rt}ACB$**
N-H stretching in amino group	3419.2	3424.4	3414.0
CH_3, C-H antisym. Stretching	2927.3	2953.5	2922.1
CH_2, C-H symmetric stretching	2859.3	2869.8	2848.8
COO^-, C-O antisym. Stretching	1587.8	1587.8	1582.6
C-H deformation vibration of $-CH_3$ group	---	---	

C=N stretching	1415.1	1425.6	1446.5
C-N stretching (aryl tertiary)	1320.9	1347.1	1315.7
CH_3 rocking	1125.0	1130.0	1110.0
Ar-C-Cl stretching	1116.9	1105.0	1106.4
C-H deformation (in plane) due to benzene ring	820.0	813.4	840.0
CH_2 rocking	729.7	710.0	740.1
C-S stretching	682.6	672.1	635.5

3.2 NMR Studies

The ^1H NMR spectra of the complexes were collected in C_6D_6 and examined to determine bonding and tautamerism present in the structure. A perusal of the spectra of the complexes shows aliphatic –CH_3 proton signal at δ - 0.9 and -CH_2 proton signal at δ - 1.2 of soap segment, indicating the presence of soap segment in the complex molecule. A broadened peak is observed in all the spectra of the complexes at δ 3.2 – 4.2 due to presence of aromatic –NH_2 protons. In the given spectra of $CC_{pl}ACB$ (Figure 8). The above peak is clear which indicates the coordination through the –NH_2 group of the benzothiazole segment to the metal atom of soap segment. The proton present on the nitrogen atom may undergo rapid, intermediate or slow exchange. The broadening of the observed peak is suggestive of a slow exchange because the electrical quadripole moment of the nitrogen nucleus induces a moderately efficient spin relaxation. A very weak signal is observed at δ - 8.5 in the spectra, which may be due to tautamerism present in the molecule of the complex similar to the pyrrol and indole. The triplets at δ 6.9 – 7.2 are may be due to tri-substituted aromatic protons of benzothiazole segment indicating that the environment of all the three protons are different [20].

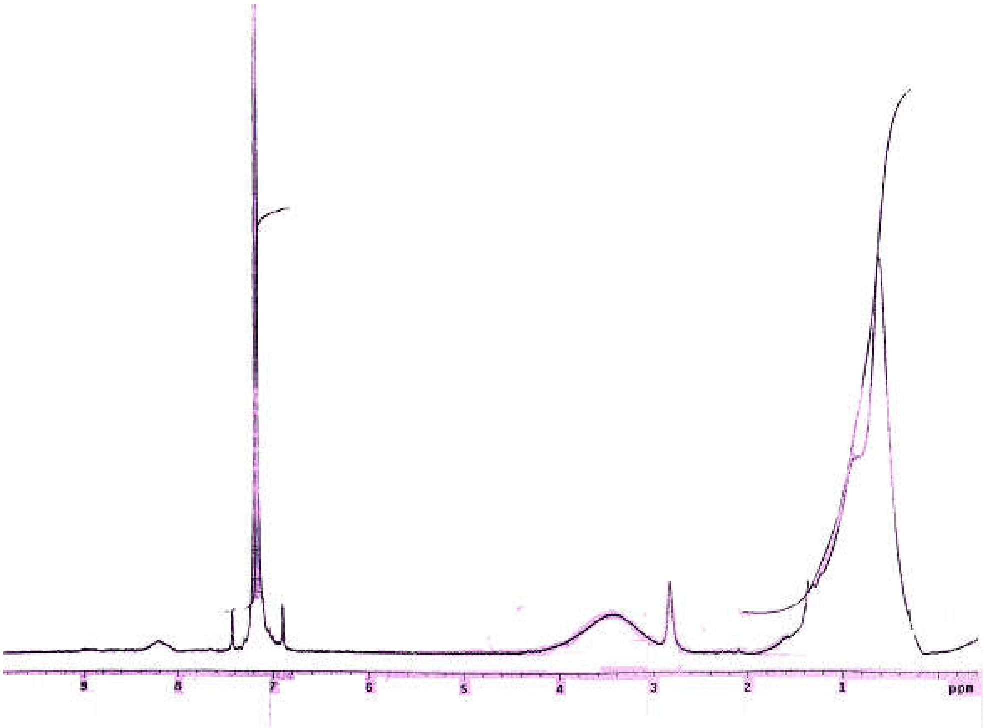

Figure 8: NMR spectra of ligand (2-amino-6-chloro benzothiazole)

3.3 ESR Studies

Literature survey suggests that magnetic conductivity and spectral studies of complexes of some first row transition elements with pyrazine carboxylic acid have been made, where it acts as bidentate ligand through O and N donor atoms [21,22]. The ESR spectra of the complexes synthesized by us were recorded on X-band at frequency 9.07 GHz under the magnetic field strength of 2000±4000 and 3000±2000 gauss at room temperature. The value of ESR parameters for all the complexes is given in the Table 4. A perusal of Table 4 shows that the values of g, g_{II}, $g_{\perp}$ are greater than the value of g_0 i.e. 2.0027. This indicates that the distortion from the regular octahedron has taken place in the shape of the complex. Also the trend $g_{II}>g_{\perp}$ for all the complexes indicates that the unpaired electron is most likely in d_{x2-y2} orbital of Cu (II) giving $^2B_{1g}$ as the ground state [23]. This fact supports that complexes possess elongated octahedral geometry. It is well known that g_{II} is a moderately sensitive function for indicating covalency. Thus, the values of g_{II} are suggestive of covalent character of metal-ligand bond. Earlier magnetic studies, about copper (II) soaps confirm the binuclear configuration in solid state. However the low values of the magnetic moments for copper ion present in copper (II) soaps in non-aqueous solvent like alcohol and hydrocarbon confirm that the binuclear configuration exist in the soap solutions and the dispersion unit is a binuclear molecule having the structure proposed in Figure 9.

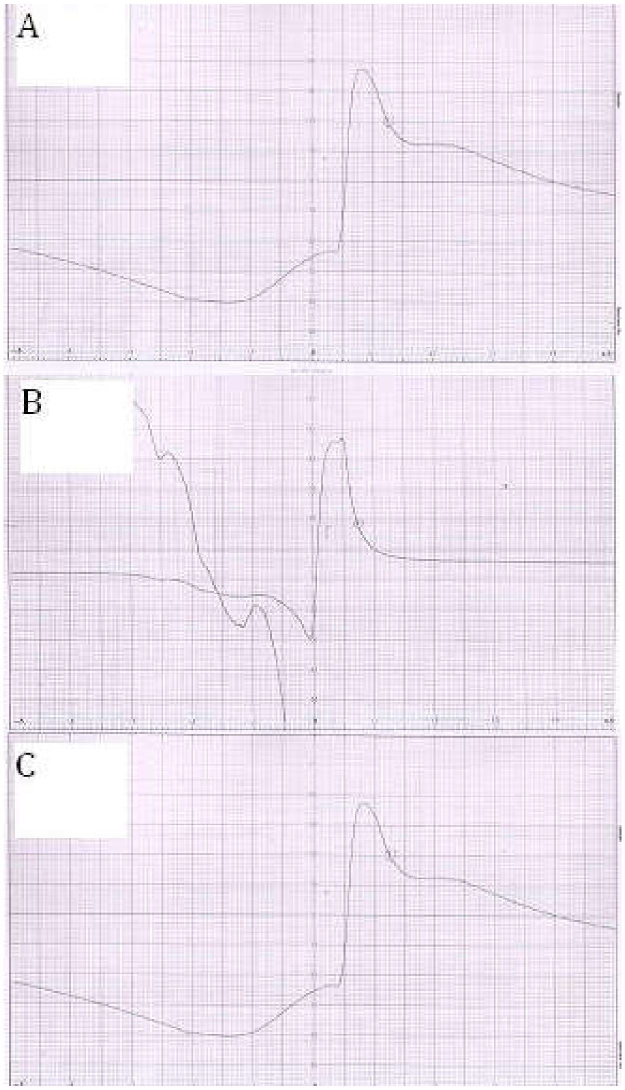

Figure 9: ESR spectra of complex (A) $CC_{pl}ACB$, (B) $CC_{pr}ACB$, (C) $CL_{rt}ACB$

Table 4: ESR spectral values of complexes

Name of Complex and Molecular Formula	**g**	**g_{II}**	**$g_{\perp}$**
$CC_{pl}ACB$ $Cu_2(C_7H_{15}COO^{-})_{4*}(C_7H_5N_2SCl)_2$	2.0372	2.2316	2.0930
$CC_{pr}ACB$ $Cu_2(C_9H_{19}COO^{-})_{4*}(C_7H_5N_2SCl)_2$	2.1180	2.2840	2.0768
$CL_{rt}ACB$ $Cu_2(C_{11}H_{23}COO^{-})_{4*}(C_7H_5N_2SCl)_2$	2.0505	2.2639	2.0970

4. RESULTS AND DISCUSSION

The antifungal activities of the pure ligands, pure soaps and their corresponding complexes have been evaluated by testing against *Alternaria-alternata* at different concentration by Agar plate technique [24,25]. A perusal of Fig. 10 suggests that all complexes show higher activity than pure soaps and ligands suggesting that complexes are more powerful antifungal agents. Benzothiazole and other N, S, O etc. containing ligands are able to form complexes with metal soaps and enhance the performance of copper soaps against fungi. The organic compounds containing amino group play important role in biology, as it constitutes the repeating unit of the polypeptide macromolecules [26,27]. These results show that the Cu (II) soap-complexes of ligands are much more toxic than the ligands themselves. The enhanced activity of newly synthesized complexes as compared to those of the ligand can possibly be explained on the basis of chelate formation, presence of donor atoms, basicity as well as the structural compatibility with molecular nature of the toxic moiety. Enhanced biological activity of complexes is in accordance with the chelation theory [28].

The results of ANOVA (Analysis of variance) [29] for the antifungal activities for all soaps complexes are shown in Table 5. The predicted R^2 are in reasonable agreement and closer to1.0. This confirms that the experimental data are well satisfactory. The descriptive statics results shown in Table 6 confirm satisfactory results in triplet. In final conclusion, that the appearance of enhanced activity may be due to synergistic mechanism i.e. the free ligands are less active but on complexation show more activity in combination with copper (II) soaps [30].

The evaluation of anti-fungal studies further revealed that fungi toxicity of the complexes also depends on the nature of metal ions [31]. The chelation reduces the polarity of central metal ion mainly because of partial attaining of its positive charge with the donor groups and possible π-electron delocalization over the whole chelate ring. Such chelation increases the lipophilic character of the central atom, which subsequently favors its permeation through the lipoid layer of the cell membrane [32]. Their efficiency increases with their concentration. Thus it is evident that concentration plays a vital role in increasing the degree of inhibition. So fungicidal screening data revealed that at lower concentration the inhibition of growth is less as compared to higher concentration. In all complexes toxicity increases with the domination of methanol in the solvent mixture of methanol and benzene [40% methanol–benzene and 80% methanol–benzene]. Suggesting that complexes are more effectively playing their role to check the growth of fungi in the domination of polar solvent in the ternary system: complex + methanol + benzene.

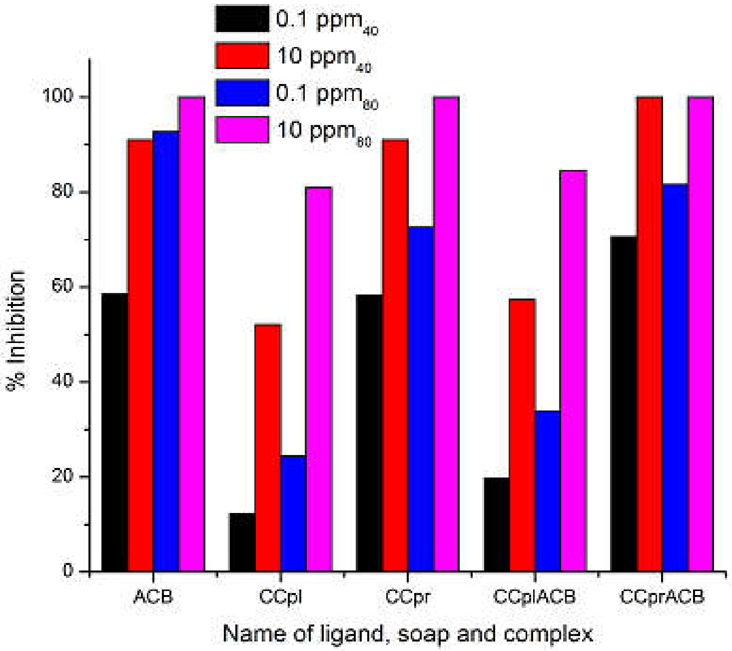

Figure 10: Antifungal activity of ligand, soap and complex (chloro) for fungi *Alternaria alternata*

Table 5: Descriptive statics results for antifungal activities of Cu (II) soaps and its complexes

Compound	**conc. (ppm)**	**Count**	**Average % Inhibition**	**% Variance**	**% Coefficient Variance**	**% Std. Deviation**	**% Std. Error**
CCplS_{40}	0.1	3	12.40	0.015	0.12	0.072	1.007
	10	3	52.22	0.012	0.11	0.063	0.210
CCplS_{80}	0.1	3	24.36	0.043	0.20	0.120	0.854
	10	3	80.50	0.160	0.04	0.230	0.496
CCprS_{40}	0.1	3	58.20	0.010	0.17	0.100	0.057
	10	3	90.76	0.023	0.16	0.152	0.088
CCprS_{80}	0.1	3	72.55	0.013	0.15	0.115	0.066
	10	3	99.99	0.015	0.01	0.010	0.005
ACB_{40}	0.1	3	58.20	0.010	0.17	0.100	0.057
	10	3	90.70	0.020	0.16	0.152	0.088
ACB_{80}	0.1	3	92.80	0.010	0.05	0.050	0.028

	10	3	99.99	1E-04	0.01	0.010	0.005
$CCplACB_{40}$	0.1	3	19.75	0.001	0.20	0.040	0.023
	10	3	57.43	0.043	0.36	0.208	0.120
$CCplACB_{80}$	0.1	3	33.70	0.041	0.60	0.202	0.117
	10	3	84.36	0.028	0.19	0.168	0.097
$CCplACB_{40}$	0.1	3	70.58	0.004	0.08	0.063	0.036
	10	3	99.93	0.010	0.10	0.101	0.058
$CCplACB_{80}$	0.1	3	81.64	0.0003	0.020	0.017	0.010
	10	3	99.99	3.33E-05	0.005	0.005	0.003

Table 6: ANOVA results for antifungal activities of Cu (II) soaps and its complexes

Name of compound	**SS**	**df**	**MS**	**F**	**P-value**	**F crit.**	**R^2**
CCplS	8408	3	2802	445508	3.16E-21	4.06	0.992
CCprS	3144	3	1048	96521	1.43E-18	4.06	0.995
ACB	3108	3	1036	115354	7.03E-19	4.06	0.995
CCplACB	7233	3	2411	84302	2.46E-18	4.06	0.993
CCprACB	1890	3	630.	172622	1.4E-19	4.06	0.995

SS= sum of squares, MS= mean square, df= degree of freedom, $p < F$ (level of significance)

5. CONCLUSION

The synthesized copper (II) complexes, which are biologically more active than pure copper soaps have been tested on *Alternaria-alternata* for antifungal activities. The results of this investigation show that the complexation increases the activity of the above metal complexes. The complexation can be used for innovating promising prospective antibiotic agents against some known pathogenic organism and can be used for pharmaceutical applications.

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