



## RESEARCH ARTICLE

## Fungicidal Activities and Characterization of Novel Biodegradable Cu (II) Surfactants Derived from Lauric Acid

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**Abstract:****Introduction:**

Colloidal systems are extremely widespread in nature and are of great practical importance in our daily life. Surfactants are very important in modern engineering and pharmaceutical soap and the complexes of soaps with different ligands are used in almost all sectors of national economy due to the formation of micelles in solutions and high surface activity *i.e.* the ability of their molecules to form surface adsorption layers. For this purpose, first time we thought about the synthesis of copper surfactants/soaps and their complexation by N/S donor ligands.

**Methods and Materials:**

In this paper, we report the synthesis of copper laurate thiourea by conventional methods and its characterization by elemental analysis, IR, NMR, ESR spectral studies. In order to understand their biological aspects and application of these surfactants/complexes as antifungal agents, a study has also been conducted in the field of biochemistry. In order to understand their biological aspects with special reference to fungicidal activities, three different fungi namely *Aspergillus alternaria*, *Aspergillus Fumigatus* and *Aspergillus niger* were taken and tested by different concentrations of copper laurate soap and its thiourea complex by P.D.A. (Potato dextrose agar) technique.

**Conclusion:**

Biological studies of these compounds will also provide an important account of information about their industrial utilization.

**Keywords:** Copper surfactants, Anti-fungal studies, IR, NMR, ESR, Lauric acid.

### 1. INTRODUCTION

The chemistry of macrocyclic N and S donor ligands and their complexes with transition metal ions has been an interesting and fascinating area of research activity all over the world since the last few decades. The continued interest to proliferate structural novelties of such complexes is due to their wide application in medicinal, biochemical, bioinorganic, environment, industrial and photochemistry [1]. Surface-active agents or surfactants are of great practical importance in our daily life, on account of their interesting behavior at surfaces and interfaces. They not only accumulate at the surface but also change the properties of surfaces [2]. Synthesis of Cu (II) soaps and their complexes derived from various edible and non-edible oils will provide fundamental information regarding their colloidal-chemical behavior and fungicidal activities [3]. Cu (II) soaps have gained importance due to their use in wood preservation, pesticidal and fungicidal activities, foaming, wetting, detergency, emulsification, plants lubrication *etc* [4]. Physical

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properties on Cu (II) soaps and their complexes such as ultrasonic studies [5, 6], density, molar volume, apparent molar volume [7, 8], viscometric studies [9], TGA analysis [10], photochemical degradation [11] and antifungal studies [12] were earlier reported to provide a fundamental and informative account of micellar features of Cu (II) soaps derived from various oils *i.e.* soyabean, mustard, sesame, groundnut, neem and karanj in pure benzene, varying concentration of benzene-methanol solvent system. The ligands used during the present investigation thiourea have been very well reported to possess biocidal activity [13]. For this purpose, the Cu (II) laurate soap and its thiourea complex have been synthesized by conventional methods and characterized by the various physicochemical and spectral analyses. Our continuing interest in the search for better fungicides and bactericides has led us to synthesize some new complexes derived from copper-laurate soaps with the above mention ligand and screen them for their fungicidal activities.

## 2. EXPERIMENTAL

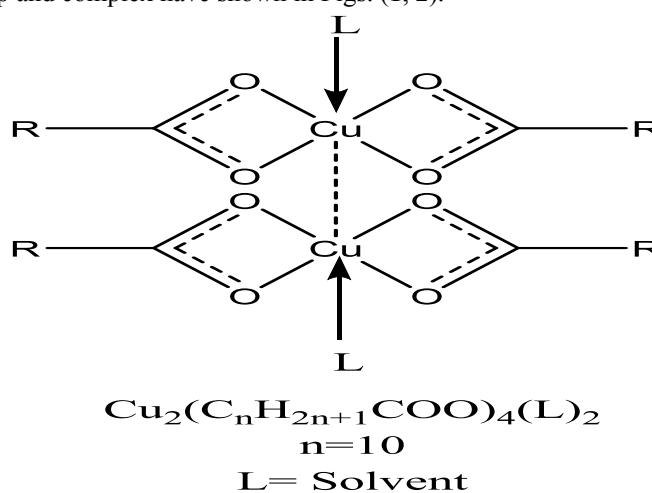
Copper laurate soap was prepared by direct metathesis of the corresponding potassium soap standard reported methods [14]. Copper soap-thiourea complex was prepared by taking copper soap and thiourea in a molar ratio (1:1). 0.005 moles of ligand (thiourea) was dissolved in 2-3 ml of ethyl alcohol and 0.005 moles of copper (II) soap derived from lauric acid was dissolved in 10-15 ml of benzene and solution of thiourea was added to it. The above reaction mixture was then heated for 1.5 h. Separated solid complex was filtered, washed with hot water and alcohol and dried in vacuum over fused calcium chloride. The dried sample was purified and re-purified with hot benzene. In general, the solid complex (90% yield) with bluish green periphery was obtained (Table 1). The compound is soluble in ethanol, methanol, benzene and other organic solvents and insoluble in water. The complex is quite stable at room temperature up to 170°C. On the basis of elemental analysis, the complex has been assigned with the composition and  $\text{Cu}_2[\text{C}_n\text{H}_{2n+1}\text{COO}]_4\text{L}_2$  suggested 1:1 type stoichiometry. Elemental analysis, was performed at RSIC, CDRI Lucknow, U.P. India. The newly synthesized agrochemicals are abbreviated as follows:

**Table 1. Analytical and physical data of the  $\text{CL}_n\text{S}$  and  $\text{CL}_n\text{T}$ .**

Molecular Formula	Color	Yield	M.P.	Cu	C	H	O	N	S	Av. M.W.
$\text{C}_{12}\text{H}_{23}\text{CuO}_2$ $\text{CL}_n\text{S}$	Dark Green	90%	182	12.06 [11.82]	62.41 [61.68]	9.96 [9.55]	13.87 [12.98]	–	–	526.4
$\text{Cu}_2(\text{C}_{11}\text{H}_{23}\text{COO})_4 \cdot (\text{CH}_4\text{N}_2\text{S})_2$ $\text{CL}_n\text{T}$	Blue	91%	205	10.53 [10.15]	61.18 [61.39]	8.59 [8.63]	10.15 [10.23]	4.34 [4.47]	5.03 [5.11]	602.4

1. Copper laurate soap ( $\text{CL}_n\text{S}$ )
2. Copper laurate thiourea complex ( $\text{CL}_n\text{T}$ )

The proposed structure of soap and complex have shown in Figs. (1, 2).



**Fig. (1).** Proposed structure of  $\text{CL}_n\text{S}$  ( $\text{Cu}_2(\text{C}_n\text{H}_{2n+1}\text{COO})_4(\text{L})_2$ ),  $n=11$ ,  $\text{L}=\text{solvent}$ .

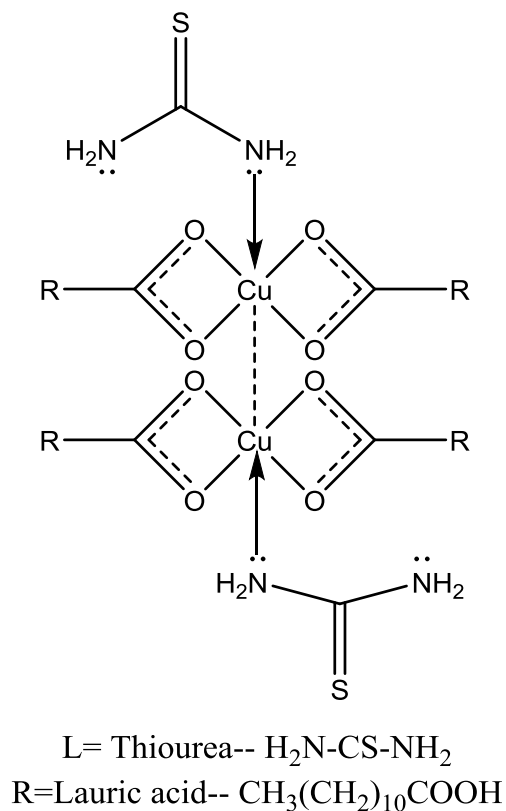


Fig. (2). Proposed structure of CL<sub>n</sub>T.

## 2.1. Materials and Instrumentation

A Perkin-Elmer spectrum-2000 Fourier transform IR spectrophotometer (USA) was used to obtain the IR spectra between 400 and 4000 cm<sup>-1</sup> CDRI Lucknow U.P. India. The samples were prepared in pellet form using spectroscopic grade KBr. <sup>1</sup>H-NMR spectra were recorded by multinuclear FTNMR spectrometer Model Advance-II (Bruker). The instrument was equipped with a cryo-magnet of field strength 9.4 T. Its 1H frequency was 400 MHz at CDRI Lucknow U.P. India. The ESR spectra of the complexes were recorded at X-Band at a modulation frequency of 100KHZ at liquid nitrogen temperature. TCNE was used as the field marker at RSIC, IIT. Powai, Mumbai, India. The g values for ESR signal are calculated by the formula-(Eq 1).

$$g = \frac{h\nu}{\beta H} \quad (1)$$

Where  $\nu$  = Frequencies of Band in KHz

B = Bohr magneton

H = Magnetic field, h = Planks constant.

On the basis of  $g_{||}$  and  $g_{\perp}$ , the  $g_{av}$  and G values were calculated using the following (Eq 2 and 3).

$$g(av) = (g_{||} + 2g_{\perp})/3 \quad (2)$$

$$G = g_{||} - 2/g_{\perp} - 2 \quad (3)$$

The general laboratory techniques followed in the course of this investigation are as suggested by Boothand Hawks worth [15] as follows:

## 2.2. Sterilization of Glasswares

For a biological activity, the glassware were thoroughly washed and cleaned with chromic acid, followed by washing with distilled water. Now they were sterilized by keeping them in a hot air oven at 160 °C for 24h. All

operations concerning inoculation are done in a completely sterilized chamber.

### 2.3. Inoculation

The artificial induction of micro-organism into a medium is called inoculation. The latter is the most fundamental technique for studying the growth characteristics of micro-organisms and for the transfer and maintenance of culture under aseptic condition.

### 2.4. Preparation of Slant

Agar slants were prepared to inoculate microbial culture. To prepare agar slant, the required number of culture tubes were taken and about 12 to 15 ml of liquefied agar medium was poured in each of them. The tubes were now cotton-plugged and sterilized in an autoclave. After the sterilization was over, the tubes were taken out and were placed in slanting (stopping) position for some time, the tubes got cooled and the medium in them was solidified resulting in a sloppy surface.

### 2.5. Culture Media Used

In preparing a Culture medium for any micro-organism, the primary goal is to provide a balanced mixture of the nutrient that will permit good growth. Additionally, the culturing of micro-organisms requires Careful Control of various environmental factors which normally are maintained within narrow culture media.

### 2.6. Preparation of PDA

The culture medium used for the growth of the organism in the present study was natural media Potato Dextrose Agar (Abbreviated as 'PDA'). For the preparation of culture media standard reported procedure [16].

### 2.7. Test Organism

The test organisms used in the present study were *Alternaria alternate*, *Aspergillus fumigatus* and *Aspergillus niger* which were isolated from their natural habitat (plants, debris) and then purified, characterized and identified.

### 2.8. Fungicidal Testing

The antifungal activity of the copper soaps and their complexes derived from saturated fatty acid under study was checked by the agar plate technique as reported in the literature [17]. The growth of fungus was measured by recording the total area of the fungal colony

The data were statistically analyzed according to the following formula [18].

$$\text{Fungal Growth Formula} = C - \frac{T}{C} \times 100 \quad (2)$$

(% Inhibition)

C = Total area of the fungal colony in control plats after 2 days.

T = Total area of the fungal colony in the test tube after 2 days.

## 3. RESULTS AND DISCUSSION

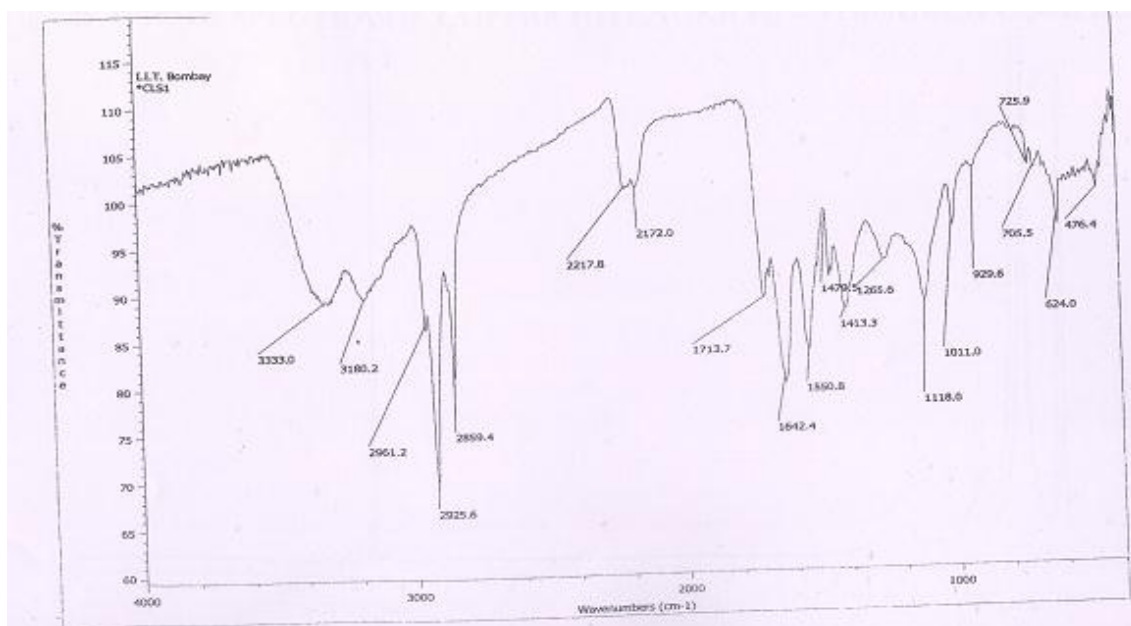
### 3.1. IR Spectra

The absorption bands observed at 2926  $\text{cm}^{-1}$  and 2859  $\text{cm}^{-1}$  are assigned to the antisymmetric and symmetric stretching for  $-\text{CH}_2$  group (methylene) of the soap segment present in the complex. The absorption bands observed at 2961  $\text{cm}^{-1}$  are due to  $-\text{CH}_3$  antisymmetric stretching of lauric acid. A strong absorption band at 1551  $\text{cm}^{-1}$  is due to carboxylate ion  $\text{COO}^-$ , C-O antisymmetric respectively. The peaks corresponding to  $-\text{CH}_3$  and  $-\text{CH}_2$  have been seen at 1118  $\text{cm}^{-1}$  and 726  $\text{cm}^{-1}$  respectively. Cu- O stretching bands have been shown in the region of 476.4  $\text{cm}^{-1}$  similar observation was reported by Khan *et al.*, [19]. The absorption bands observed at 3333  $\text{cm}^{-1}$  and 3180  $\text{cm}^{-1}$  are due to the N-H antisymmetric stretching and N-H symmetric stretching of  $\text{NH}_2$  group. Other strong peaks in the region of 1479  $\text{cm}^{-1}$  are due to the  $\text{CH}_2$  bending group. The absorption band 1714  $\text{cm}^{-1}$  was found to be representative of amide group of  $>\text{C}=\text{O}$ . The C-N stretching band of primary amide was observed at 1413  $\text{cm}^{-1}$ . Apart from these absorption

corresponding to the ligand moiety, a peak in the region of  $1266\text{ cm}^{-1}$  N=C=S stretching of ligand thiourea in the complex was observed. Due to the C=S stretching group in an absorption in the region of  $624\text{ cm}^{-1}$  (Table 2). On the basis of the above observations, it can be safely assumed that complexation of copper laurate soap has been done with thiourea ligand (Fig. 3).

**Table 2. Infrared absorption spectral frequencies ( $\text{cm}^{-1}$ ) of complex ( $\text{CL}_{\text{rt}}\text{T}$ ).**

<u>PHYSICO-CHEMICAL STUDIES OF Cu (II) SURFACTANTS IN NON-AQUEOUS MEDIA.</u> Assignment	Complex $\text{CL}_{\text{rt}}\text{T}$
-NH <sub>2</sub> , -NH antisym. stretching	3333.5
-NH <sub>2</sub> , -NH sym. stretching	3180.2
-CH <sub>3</sub> , -C-H antisym. Stretching	2961.4
-CH <sub>2</sub> , -C-H antisym. stretching	2926.7
-CH <sub>2</sub> , -C-H sym. stretching	2859.3
-C=N stretching	2217.1
>C=O stretching	1714.8
-COO <sup>-</sup> , -C-O antisym. stretching	1551.2
-CH <sub>2</sub> -CH (twisting and wagging) bending	1479.9
-C-N stretching	1413.1
N-C=S stretching	1266.9
-CH <sub>3</sub> , C-H rocking	1108.0
-CH <sub>2</sub> rocking	726.4
C=S stretching	624.0
Cu-O stretching	476.4



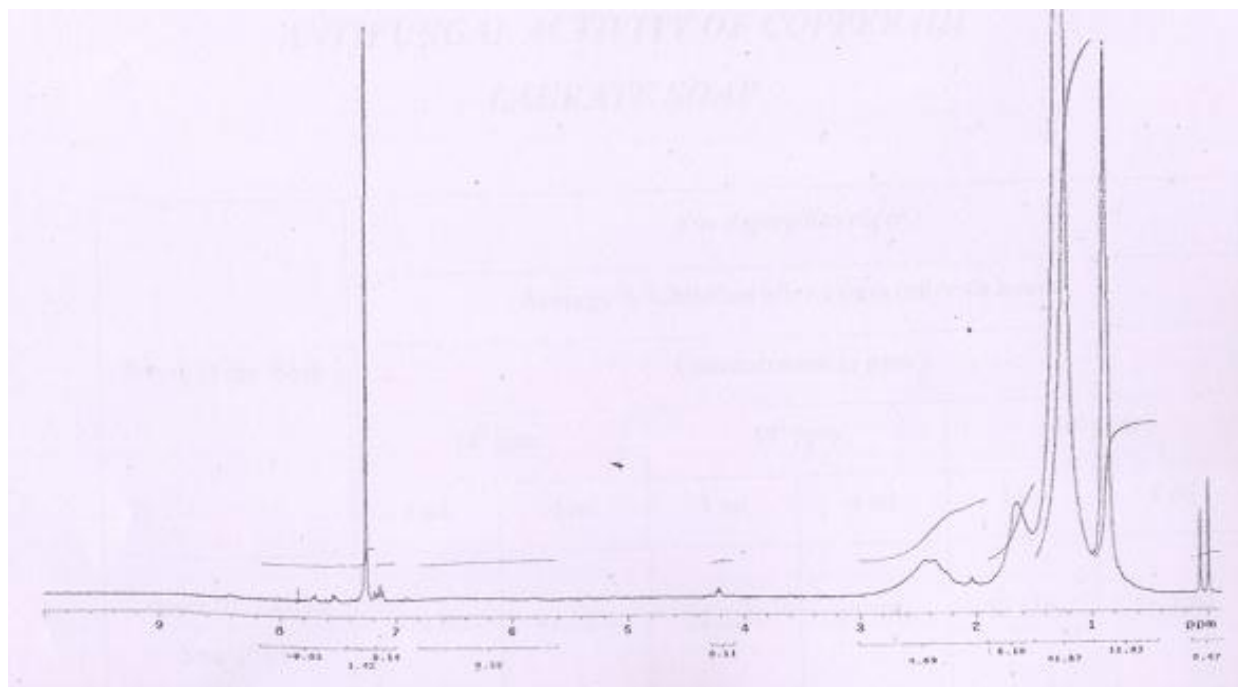
**Fig. (3).** IR Spectra of complex ( $\text{CL}_{\text{rt}}\text{T}$ ).

### 3.2. NMR Spectra

NMR spectra of Cu (II) laurate thiourea complex show a signal of aliphatic  $-\text{CH}_3$  proton attached  $-\text{CH}_2\text{-R}$  group at nearly  $\delta$ -0.979 and  $-\text{CH}_2$  proton attached to  $-\text{CH}_2\text{-R}$  group which shows a signal at  $\delta$ -1.256. Other signal observed is co-responding to the  $-\text{CH}_2$  proton attached to  $-\text{CH}_2(\text{COO})_2\text{Cu}$  group and is observed at  $\delta$ -2.354. All these peaks are due to the saturated fatty acid content of the soap in the complex. A broad peak is observed in the spectra of the complex at  $\delta$ -2.354 due to the presence of  $-\text{NH}_2$  protons (Table 3). This peak indicated co-ordination through the  $-\text{NH}_2$  group of thiourea segment to the metal atom of the soap segment. A very weak signal is observed at  $\delta$ -7.84 in the spectra, which may be due to tautomerism [20] present in the complex (Fig. 4).

**Table 3.** NMR spectral signals ( $\delta$ ) of complex ( $CL_nT$ ).

Peak/signal	$CL_nT(\delta)$
$-\underline{CH}_3-CH_2-R$	0.979
$-\underline{CH}_2-CH_2-R$	1.256
$-\underline{CH}_2-C(=O)OCu$	2.354
$-\underline{CH}_2-CH_2-C(=O)OCu$	1.646
$-NH_2$ (broadend peak)	4.22
$-NH_2$ (tautomeric and weak signal)	7.84

**Fig. (4).** NMR Spectra of complex ( $CL_nT$ ).

### 3.3. ESR Spectra

The value of ESR parameters for the complex is given in the Table 4. A perusal of Table 4 shows that the values of  $g_1$ ,  $g_{11}$ ,  $g$  are greater than the value of  $g$  *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  $g_{11} > g$  for complex indicates that the unpaired electron is most likely in the  $d_{x^2-y^2}$  orbit of Cu (II) giving the ground state. This fact supports that complex possesses elongated octahedral geometry. It is well known that  $g_{11}$  is a moderately sensitive function for indicating co-valency [21]. Thus the values of  $g_{11}$  are of the covalent character of the metal-ligand bond. Earlier magnetic studies about Cu (II) soap confirmed the binuclear configuration in solid state [22] (Fig. 5).

**Table 4.** ESR spectral values of  $CL_nT$ .

Name of Complex and Molecular Formula	$g$	$g_{11}$	$g_{\perp}$	$g_{av}$	$G$
$CL_nT$ $Cu_2(C_{11}H_{23}COO)_4(CH_4N_2S)_2$	2.0270	2.2402	2.1292	2.1662	1.859

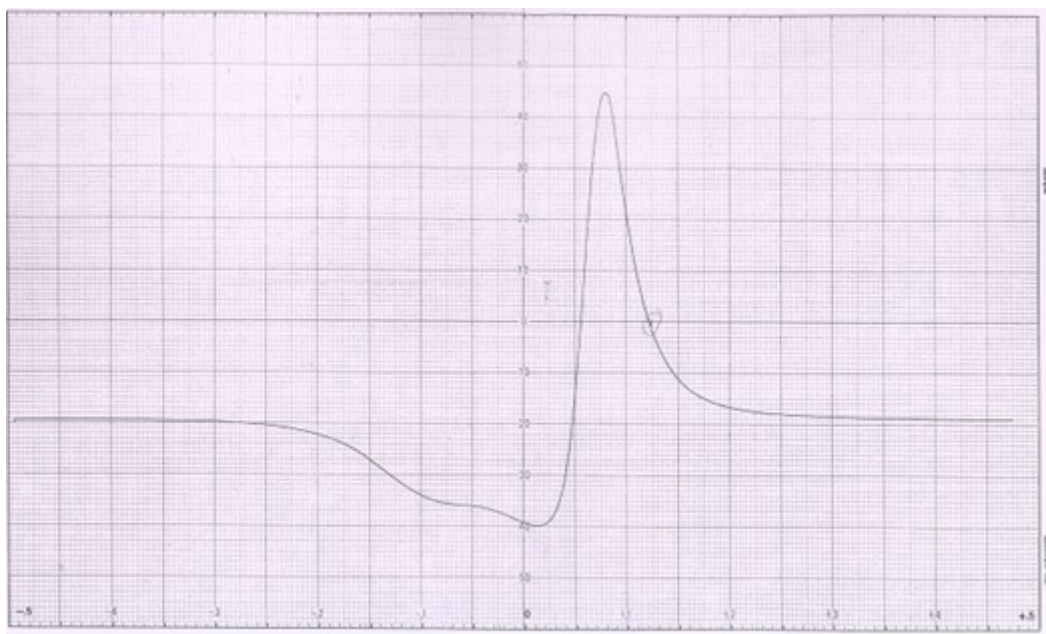


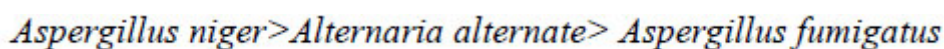
Fig. (5). ESR Spectra of complex (CL<sub>π</sub>T).

### 3.4. Fungicidal Activities

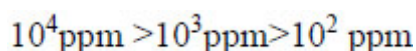
A perusal of Figs. (6-8) reveals that the complex shows higher activity than pure soap suggesting that complex is more powerful antifungal agent. Thiourea and other N,S,O *etc.* containing compounds are able to enhance the performance of copper soap. These results show that the Cu (II) soap complex of ligands is much toxic than the copper-soap themselves. The enhanced activity of synthesis of the complex as compared to copper soap can possibly be explained on the basis of chelate formation in the presence of donor atoms, basicity as well as the structural compatibility with molecular nature of the toxic moiety [23, 24]. The final conclusion suggests that the appearance of enhanced activity may be due to a synergistic mechanism *i.e.* the soap is less active but on complexation shows more activity in combination with thiourea. The studies suggest that the Cu (II) ions in soaps may be responsible for the enhancement of the activity against fungi. The evaluation of anti-fungal studies further revealed that fungitoxicity of the complex also depends on the nature of metal ions [25]. The chelating reduces the polarity of central metal ion mainly because of the partial attaining of its positive charge with the donor groups and possible ring. Such chelating increases the lipophilic character of the central atom, which subsequently favors its permeation through the lipid layer of the cell membrane [26]. 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 a lower concentration of the inhibition of growth is less as compared to higher concentration [27 - 29]. On the basis of fungicidal screening data, it was revealed that the complex shows lower activities for *Alternaria alternate* and *Aspergillus fumigatus* in comparison with *Aspergillus niger* (Figs. 9a-c). For *Aspergillus niger*, the complex's efficiency increases with its concentration. Thus it is evident that the concentration plays a vital role in the degree of inhibition. The study suggests that copper laurate soap is the least fungi toxic (% inhibition lowest) whereas its thiourea complex which shows the highest inhibition. The activity of copper soap and complex derived from lauric acid is found to increase in the order.



It has also been observed that, in general, copper complex derived from lauric acid shows higher % inhibition for *Aspergillus niger* as compared to *Aspergillus fumigatus* and *Alternaria alternate*.

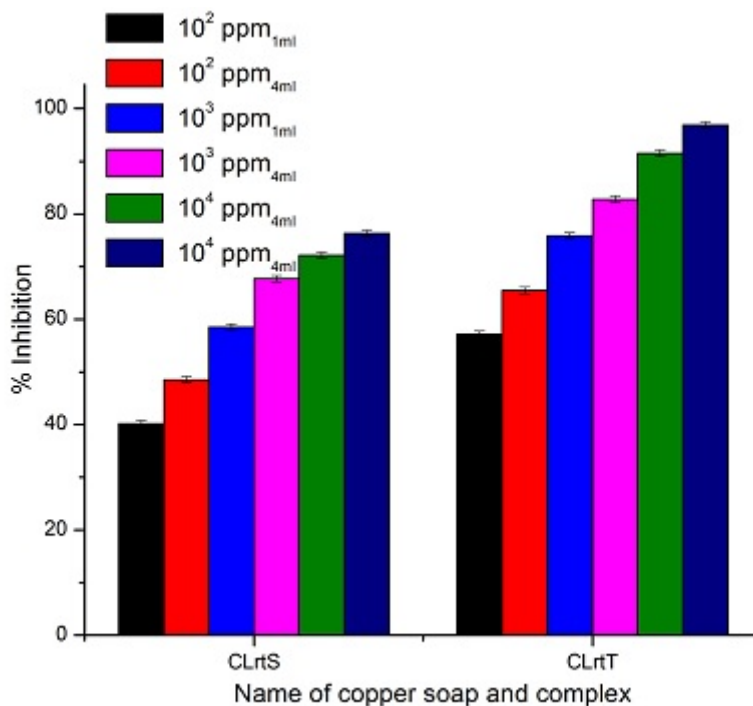


Higher concentration shows higher % inhibition when compared to the lower concentration.

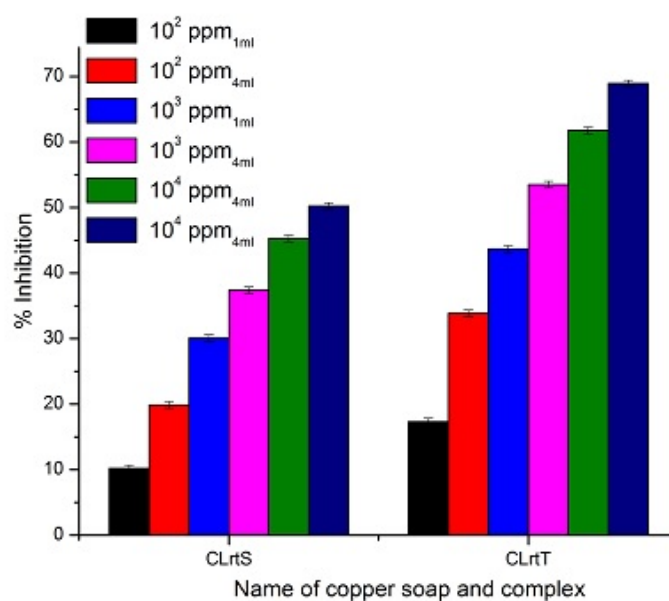


4ml > 1ml

The results of ANOVA for the antifungal activities for all sops complexes are shown in Table 5. The predicted  $R^2$  is in reasonable agreement and closer to 1.0. This confirms that the experimental data are well satisfactory. The descriptive statistics results of  $CL_{it}S$  and  $CL_{it}T$  showed in Tables 6-8 confirm satisfactory results in triplet for all the fungi studied earlier in our laboratory and other studies conducted by scientists [30]. The findings are similar to our studies on different systems [31]. The result is statistically significant, by the standards of the study, due to  $p < F$ .



**Fig. (6).** Antifungal activity of  $CL_{it}S$  and  $CL_{it}T$  for fungi *Aspergillus Niger*.



**Fig. (7).** Antifungal activity of  $CL_{it}S$  and  $CL_{it}T$  for fungi *Aspergillus fumigatus*.



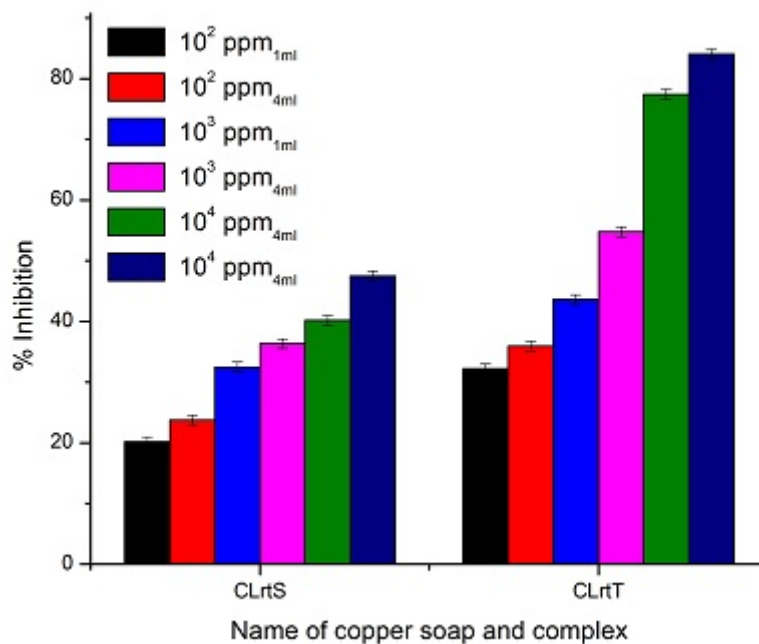


Fig. (8). Antifungal activity of CL<sub>r</sub>S and CL<sub>r</sub>T for fungi *Alternaria Alternata*.

Table 5. ANOVA results of Cu (II) laurate soap and thiourea complex.

Fungi	SS	df	MS	F	P-value	F <sub>crit</sub>	R-SQUARE
<i>Aspergillus Niger</i>	2898	5	580	4261	4.66E-19	3.11	0.995
	10985	5	2197	7800	1.2E-20	3.11	0.992
<i>Aspergillus Fumigatus</i>	3493	5	699	5444	1.07E-19	3.11	0.992
	5403	5	1081	5146	1.5E-19	3.11	0.996
<i>Alternata alternaria</i>	1609	5	322	4749	2.4E-19	3.11	0.997
	7198	5	1440	19482	5.1E-23	3.11	0.997

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



Fig. (9a). Presence of *Aspergillus fumigatus* on papaya fruit.



Fig. (9b). Presence of *Alternaria Alternata* on tomato.



Fig. (9c). Presence of *Aspergillus Niger* on bread.

Table 6. Descriptive Statics results of Cu (II) laurate soap and thiourea complex for *Aspergillus Niger* fungi.

Fungi	Complex	Concentration	Amount(ml)	Count	Avg. % Inhibition	Std Error	Variance	Std Deviation	Coff. Var.
		(ppm)							
Aspergillus Niger	CLrTS	10 <sup>2</sup>	1	3	40.52	0.01	0.34	0.59	0.34
			4	3	48.64	0.03	0.02	0.14	0.08
		10 <sup>3</sup>	1	3	58.63	0.04	0.05	0.23	0.13
			4	3	67.63	0.01	0.26	0.51	0.3
		10 <sup>4</sup>	1	3	71.93	0.01	0.14	0.38	0.22
			4	3	76.87	0.05	0.12	0.35	0.2
	CLrTT	10 <sup>2</sup>	1	3	57.2	0.01	0.49	0.7	0.4
			4	3	65.17	0.01	0.12	0.35	0.2
		10 <sup>3</sup>	1	3	75.33	0.03	0.05	0.23	0.13
			4	3	82.57	0.03	0.04	0.21	0.12
		10 <sup>4</sup>	1	3	91.37	0.04	0.1	0.32	0.19
			4	3	96.27	0.02	0.04	0.21	0.12

Table 7. Descriptive Statics results of Cu (II) laurate soap and thiourea complex for *Aspergillus Fumigatus* fungi.

Fungi	Complex	Conc.	Amount (ml)	Count	Avg. % Inhibition	Std Error	Variance	Std Deviation	C off Var.
		(ppm)							
Aspergillus Fumigatus	CLrtS	10 <sup>2</sup>	1	3	10.47	0.01	0.02	0.15	0.09
			4	3	19.67	0.02	0.17	0.42	0.24
		10 <sup>3</sup>	1	3	30.63	0.02	0.26	0.51	0.3
			4	3	37.23	0.01	0.06	0.25	0.15
		10 <sup>4</sup>	1	3	45.23	0.01	0.12	0.35	0.2
			4	3	50.57	0.01	0.12	0.35	0.2
	CLrtT	10 <sup>2</sup>	1	3	17.43	0.01	0.04	0.21	0.12
			4	3	33.4	0.01	0.21	0.46	0.26
		10 <sup>3</sup>	1	3	42.93	0.02	0.54	0.74	0.43
			4	3	53.87	0.01	0.33	0.58	0.33
		10 <sup>4</sup>	1	3	61.5	0.05	0.09	0.3	0.17
			4	3	68.7	0.03	0.04	0.2	0.12

Table 8. Descriptive Statics results of Cu (II) laurate soap and thiourea complex for *Alternata*.

Fungi	Complex	Conc.	Amount (ml)	Count	Avg. % Inhibition	Std Error	Variance	Std Deviation	C off Var.
		(ppm)							
Alternata alternaria	CLrtS	10 <sup>2</sup>	1	3	20.27	0.01	0.04	0.21	0.12
			4	3	23.47	0.01	0.06	0.25	0.15
		10 <sup>3</sup>	1	3	32.4	0.01	0.03	0.17	0.1
			4	3	36.37	0.04	0.02	0.15	0.09
		10 <sup>4</sup>	1	3	40.87	0.01	0.12	0.35	0.2
			4	3	47.53	0.01	0.12	0.35	0.2
	CLrtT	10 <sup>2</sup>	1	3	32.43	0.02	0.24	0.49	0.28
			4	3	35.03	0.01	0.04	0.21	0.12
		10 <sup>3</sup>	1	3	43.17	0.01	0.09	0.31	0.18
			4	3	54.2	0.02	0.01	0.1	0.06
		10 <sup>4</sup>	1	3	77.23	0.02	0.02	0.15	0.09
			4	3	84.3	0.02	0.03	0.17	0.1

## CONCLUSION

Biologically potent compounds are one of the most important classes of materials for the upcoming generations. This initiates a task for current chemistry to synthesize compounds that show promising activity as therapeutic agents with lower toxicity. Therefore, a substantial research is needed for their discovery and improvement. Transition metal complexes share an important place in this regards. Further, it is evidenced that complexation of the above metal ions with nitrogen and sulfur donor ligands increases the efficiency of biocidal activity. The antifungal activities of copper soap and its complex have been evaluated by the P.D.A. method. A scrutiny of the results reveals that the transition metal complexes showed antifungal activity better than soap, suggesting that the complexes are more powerful agents. Thiourea and other N and S containing compounds are able to enhance the performance of copper soaps.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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