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Synthesis, spectroscopic and fungicidal studies of Cu (II) soaps derived from groundnut and sesame oils and their urea complexes

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Abstract
The application of surfactants in daily life increases due to large number of useful applications in industries and pharmaceuticals. The synthesized copper soaps and their urea complexes have been confirmed by UV, IR, NMR and ESR spectral analysis. The antifungal activities of copper soaps derived from groundnut and sesame oils and their urea complexes have been evaluated by testing against *Alternariaalternata* and *Aspergillusniger* at different concentrations by agar plate technique.

Keywords: Copper surfactants, Anti-fungal studies, IR, NMR, ESR, Edible oils.

1. INTRODUCTION

Copper soaps having a tendency of complex formation with the compound containing donor atoms like N, O, S etc. which are biodegradable and eco -friendly, because of this they play a very important role in wood preservation and other biological activities [1,2]. The deeper understanding of the role of metal ion in bio-system has led to the awareness that metal complexation is useful in the treatment of bacterial, fungicidal and viral diseases [3]. Most of the antimicrobial agents are obviously organic compounds, which behave as good chelating agent [4].Their pathogenic activity is enhanced on complexion with various transition and toxicmetals [5].The biological effect of these derivatives depends upon the nature and structure of ligands and their metal complexes and also on the presence of particular element [6].

Many copper compounds are employed as pesticides either alone (as Cu (I) oxide and Cu (II) sulfate) or in mixtures. Examples of the latter are the pesticide Cupronil which contains 35% Cu (II) carbonate hydroxide and 15% zinc N, N-chloride hydroxide and 15% Zineb (zinc ethylene-bis- dithiocarbamate); Bordeaux mixture (copper sulphate and hydrated lime) was the first fungicide to be used in large scale [7].

Nitrogenous ligands have been found to be effective against many metal enzymes, bacteria and number of fungi. Large number of compounds containing nitrogen and sulphur atoms in the heterocyclic ring shows different types of activities. Biological, pharmacological and medicinal significance of many co-ordination complexes has been studied by several workers [8,9].

Recently, wood has developed metabolic and antagonism theory of drug action and Fields led to the synthesis of several co- ordination compounds, which have been used for antibacterial and anti-tubercular activity [10].

The present work reveals that synthesis of copper soaps from various edible oils (i.e. groundnut, sesame) and their complexes with urea for comparative studies, spectral studies (IR ESR and NMR) and magnetic studies has been carried out to understand the structural insight of the soaps and complexes synthesized. In order to understand their biological aspects with special reference to fungicidal activities, the study has also been done which may be of utmost use in the field of biochemistry.

2. EXPERIMENTAL

Copper soaps derived from groundnut and sesame oils and their urea complexes have been synthesized and studied for structural aspects. All chemicals used were of LR/ AR grade. Oils were procured directly from the seeds oils. The fatty acid compositions of these edible oils were confirmed through GLC of their methyl esters, Table 1. Copper soaps were prepared by refluxing the edible oils i.e. groundnut and sesame oils in their pure form with 2N KOH solution and alcohol for about 3 h (direct metathesis). Copper soap so obtained was then washed with hot water and dried. The soap was recrystallized using hot benzene. Metal was analyzed by standard procedure (Iodometrically). The purified copper soaps derived from edible oil were refluxed with ligand urea in 1: 1 ratio using benzene as a solvent for one hour. It was then filtered, dried and then recrystallized and purified in hot benzene [11].

Table 1: Fatty Acid Composition of Oils Used For Copper Soap Synthesis

Name of oil	% Fatty Acids					
	16:0	18:0	18:1	18:2	18:3	Other Acids
Groundnut Oil	10	4	61	18	-	C_{20}-C_{24} 7%
Sesame Oil	8	4	45	41	-	-

The formation of copper soaps and complexes was confirmed by using UV, IR, NMR techniques and elemental analysis. TLC using silica gel was used to check the purity of the soaps and complexes. In general, copper soaps and complexes are green in colour and soluble in benzene and other organic solvents but insoluble in water. All the soaps and complexes are stable at room temperature. Their physical parameters are reported in Table 2. On the basis of their elemental analysis, 1: 1 (Metal: ligand) type of stoichiometry has been suggested.

Table 2: Analytical and Physical Data of Copper Soaps Derived From Various Edible Oils

Name of copper Soap	Colour	Melting point (^{0}C)	Yield %	Metal %		S.V.	S.E.	Average Mol. Wt.
				Observed	Calculated			
CG	Green	97	75	9.58	9.60	188.70	297.29	656.08
CSe	Green	100	85	10.05	9.81	191.70	292.64	646.78

In order to study the structure of soaps and complexes, the infra-red (IR) absorption spectra of compounds were obtained on a Perkin-Elmer 5100, 4367 spectrophotometer(4000-200 cm-1) at RSIC, IIT Powai, Mumbai. ESR data were also obtained from RSIC, IIT Powai Mumbai. ^{1}H NMR is recorded at CSMRI, Bhavnagr, Gujrat using C_6D_6 as reference. The structures of their urea complexes have been confirmed by using IR, NMR, and ESR technique [12]. The antifungal activities of copper soaps and their urea complexes have been evaluated by testing against two easily available fungi *Alternaria alternate* and *Aspergillusniger,* which was isolated from natural habitat (plants debris) and then purified, characterized and identified. The antifungal activity of copper soaps and their urea complexes were checked by the agar plate technique. The culture medium used for the growth of the organism was natural media i.e. P. D. A. The solution was prepared in ppm of different concentrations (i.e. 10^3 and 10^4 ppm) in methanol-benzene mixture. The effect of solvent has been excluded by complete evaporation. After the solidification the above medium and evaporation of solvent, single fungal hypha/spore were isolated and transferred on agarslants (1%) for multiplication. After the period of incubation i.e. for 72 h at 30 ± 1^{o}C, the growth of the fungus was measured [13].

The data was statistically analyzed according to the following formula
% Inhibition = C –T / C x 100 (1)

Where
C = Total area of fungal colony in control plates after 72 h and
T = Total area of fungal colony in test plate after 72 h

3. RESULTS AND DISCUSSION

(A) SPECTRAL STUDIES OF COPPER SOAP

(I) INFRARED SPECTRA

The infrared spectra of these compounds in the present study were recorded in KBr disc by making use of Perkin Elmer infrared spectrometer. Some important characteristic absorption bands with relative intensities and assignments, wherever, possible are given in Table 3.

Table 3: Analytical and Physical Data of Copper Soap Urea Complexes

Compound	Colour	Melting point (^{0}C)	Metal %		Mol. Wt.
			Observed	Calculated	
CGU	Dark Green	85	9.31	8.86	716.08
CSeU	Dark Green	90	9.16	8.98	706.78

The detailed infrared absorption spectral studies reveal that there is a marked difference between the spectra of oils and that of corresponding copper soap. In the IR spectra of edible oils three distinct bands appear at 3008, 2925 and 2854 cm^{-1}dueto =C-H stretching, -C-H symmetrical and –C-H antisymmetric stretching vibration respectively. Apart from these, oils show characteristic absorption bands of esters (because oils are esters of long chain fatty acids). In IR spectra of oil, two bands are observed at 1745 and 1164 cm^{-1}. These bands may be assigned to C=O stretching and C-O stretching vibration of ester group.

In the spectra of copper soaps, strong band in the region 2970-2840 cm^{-1} are due to C-H symmetrical and antisymmetrical stretching vibration of methyl and methylene groupwhich is similar to oil Fig. 1

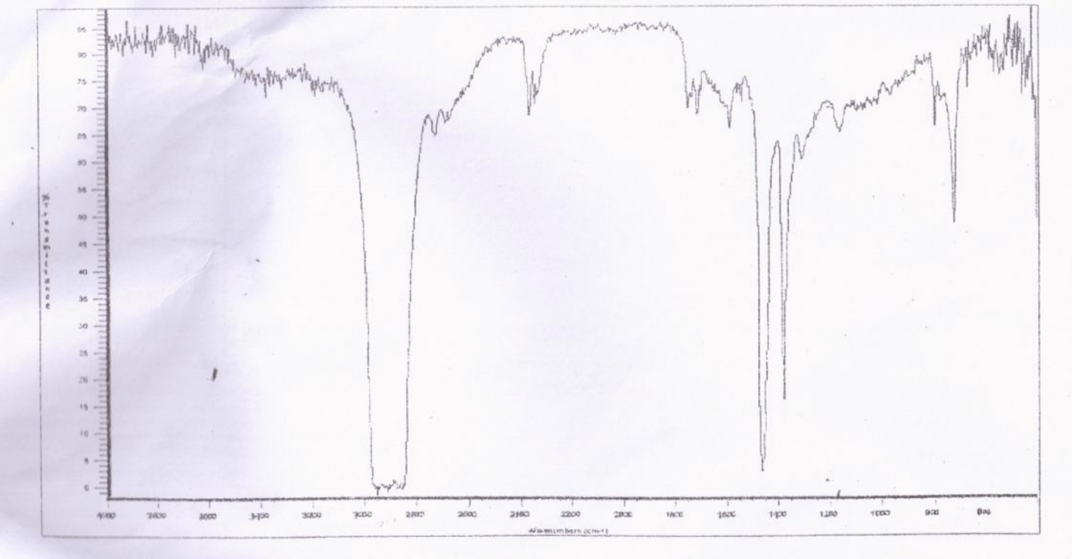

Fig.1: Infra -red spectra of CSe

There is complete disappearance of the characteristic bands of esters in the spectra of soap molecules and appearance of two new absorption bands in the region 1580-1610 cm^{-1} and 1380-1400 cm^{-1}. These bands may be assigned to a symmetric and antisymmetric vibration of carboxylate ion. The disappearance of the characteristic bands of esters and appearance of the bands of carboxylate ion indicate the formation of copper soaps.

The absence of C=O band in the IR spectra of soaps show that there is a resonance in the two C=O bonds of carboxylate group.

A number of progressive bands are observed for both oils and soaps in the region 1300-1120 cm^{-1}. Such progressive bands with medium or weak intensity are assigned to the wagging and twisting vibrations of the chain of successive methylene group of the soap molecule. Weak bands in the region 725-710 cm^{-1}may probably be due to methylene rocking vibrations of the straight carbon chain –(CH_2)-.The bands in the region 750-450 cm^{-1} in the infrared spectra of these soaps are due to metal to oxygen bond stretching vibration. These are called characteristic absorption of metal constituent of each soap molecule [14].

(II) ELECTRONIC ABSORPTION SPECTRA

In order to confirm the formation of copper soaps derived from various oils, the electronic absorption spectra were recorded on a Perkin-Elmer-Lambda-28 spectrophotometer. These spectra give information about copper-ligand binding. The electronic absorption spectra of all copper soaps show one broadband at about 670-680 nm (14925- 14706 cm^{-1}) and a sharp band at about 280 nm (35714 cm^{-1}). One broad band may be attributed to $^2E_g \rightarrow {}^2T_{2g}$ transitions arising from MLCT (metal-ligand charge transfer) absorption bands which confirms the formation of copper soaps and proposes a distorted octahedral stereochemistry around the metal ion Fig.2.

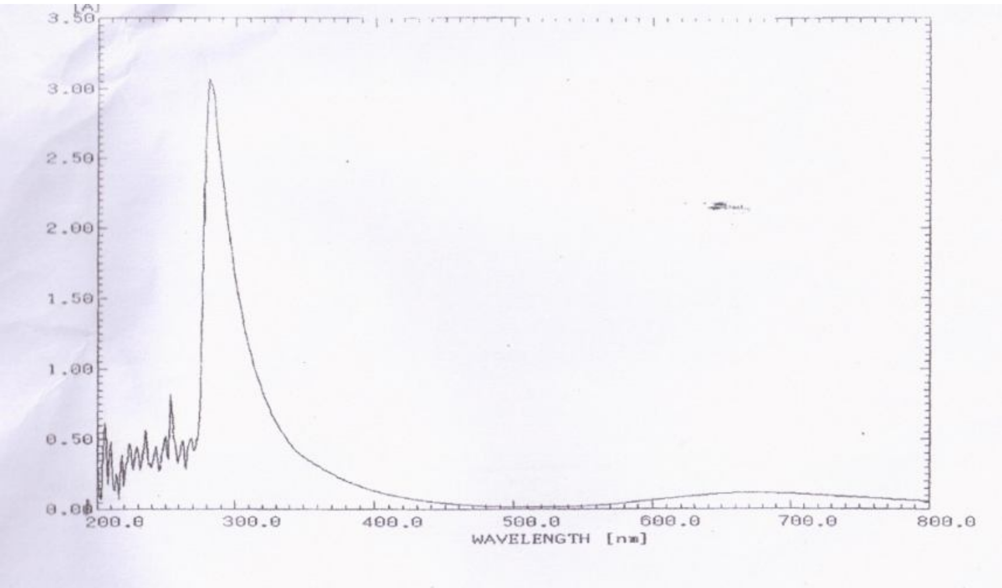

Fig.2: Electronic spectra of CG

(III) NMR STUDIES:

The ^{1}H NMR spectra of copper soap and their complexes were recorded in C_6D_6as reference. They were examined to determine bonding and tautamerism present in the structure. On comparison of NMR spectra of copper soap and complexes differences were found between two.

A perusal of the spectra of copper soaps shows signal of aliphatic $-CH_3$ proton attached to $-CH_2R$ group at nearlyδ-0.91 $-CH_2$ proton attached to CH_2-R group shows signal atδ-1.28. Other signals observed are corresponding to $-CH_2$attached to one –C=C- atδ-2.10, while $-CH_2$ attached to two–C=C- is observed atδ-2.86. Vinylic proton gives signal atδ-5.49. All the above peaks Table 4 are due to the long chain fatty content (R^-) of the soap molecule [$(RCOO)_2Cu$] [15].

Table 4: Infrared Absorption Spectral Frequencies (In cm^{-1}) Of Copper Soaps Derived From Various Edible Oils

Assignments	CG	CSe
CH_3 andCH_2, C-H Antisym. Stretching	2975-2960	2975-2953
CH_3 andCH_2, C-H Sym. Stretching	2850-2840	2855-2840
COO^-, C-O Antisym. Stretching	1590	1590
CH_2 Deformation	1465	1465
COO^-, C-O Sym. Stretching.	1385	1375
CH_2 Twisting and Wagging	1295	1310
CH_3 Rocking	1160	1170
CH_2 Rocking	730	730
M-O Stretching	665	670

(B) SPECTRA OF COMPLEXES

(I) INFRARED SPECTRA

In order to study the structure of copper soap-urea complexes, the infrared spectra of these compounds were recorded in Table 5.The detailed infrared absorption spectral investigation reveals that there are marked differences between the spectra of copper soap than those of corresponding complexes.

The absorption bands observed in the region 2849.2 and 2930.7 cm^{-1} shows the confirmation of symmetric stretching methyl ($-CH_3$) group and asymmetric stretching methylene ($-CH_2$)group of the soap segment present in the complex. Symmetric stretching bands for methyl group has been assigned in the region 2849.2 cm^{-1} while 2930.7 cm^{-1} are assigned to the antisymmetric stretching for methyl group of the soap segment present in the complex.

Table 5: Infrared Absorption Spectral Frequencies (In Cm-1) Of Copper Soap Urea Complexes and Their Assignments

Assignments	CGU	CSeU
CH_3, C-H Antisym. Stretching	2940	2945
CH_3 , C-H Sym. Stretching	2850	2855
CH_3 Scissoring band	1474	1474
CH_2 Sym. bending	1475	1474
C-O Sym. Stretching.	1408	1418
-N-H Bending	1591	1591
C=O Stretching	1708	1708
-N-H Wagging	731	731
C-N Stretching	1220	1150

A clear peak exhibitsat 1474 cm^{-1}in the spectra confirming the presence of $-CH_2$as well as $-CH_3$ group. The peaks in the region 1418 cm^{-1} are due to C-O symmetric stretching. The asymmetric vibration generally overlaps the scissoring vibration of the methylene group. Two distinct bands are observed, however compounds such as keto groups is present in which the methylene scissoring band has been shifted to a lower frequency, 1439 -1399 cm^{-1}. The absorption band observed in the region 1418 cm^{-1}indicate the above condition in the present compound.

The C=O stretching vibrations with five or more member rings exhibit stretching peak in the region 1750-1700 cm^{-1}.a sharp peak clearly shows at 1708 cm^{-1}indicate the presence of amide C=O group.

Apart from this absorption a peak appears in the region 1591 cm^{-1}dueto N-H bending vibration in dilute solutions confirms that the complexation between copper soap and NH_2groupcontaining ligand has been occurred successfully. Primary amides show a sharp peak in dilute solutions resulting from NH_2 bending at a somewhat lower frequency than the C=O band. The nature of R group also effect upon the amide band.

The C-C bending vibrations occur at very low frequency (below 500 cm^{-1}) and therefore do not appear in our spectra. The bands assigned to C-C stretching vibrations are weak and may appear in the broad region of 1200-800 cm^{-1} but they are generally of little value of identification. The C-C band with –N-H wagging vibration shifted towards 1080-700 cm^{-1}. The –N-H out of the plane also wagging in hydrocarbons with amides causes broad absorption in the 800-700 cm^{-1} which in spectra observed at 731 cm^{-1}[16].

(II) NMR SPECTRAL STUDIES

NMR spectral data for Copper soap urea complexes are recorded in Table 6.A perusal of the spectra of complexes show signals for $-CH_3$ protons at δ-.91 and for $-CH_2$ protons atδ-1.28 indicating the presence of soap segment in the complex molecule.

Table 6: NMR Spectral Data for Copper Soap and Their Urea Complexes

Assignments	CG (δ)	CSe(δ)	CGU (δ)	CSeU(δ)
CH_3-CH_2-R	0.91	0.970	0.93	0.957
-CH_2-CH_2-R	1.28	1.293	1.29	1.290
-CH_2-C=C-	2.10	2.062	2.10	2.100
-C=C-H	5.49	5.480	5.50	5.500
-C=C-CH_2-C=C-	2.86	2.870	2.87	2.870
-NH_2 (Broadened)	-	-	3.2-4.2	3.2-4.2

Aliphatic $-CH_3$and $-CH_2$ protons attached to $-CH_2R$ groups shows signals at nearly δ-0.93 and δ-1.29 respectively. Another signal observed atδ-2.10 corresponds to $-CH_2$ proton attached to –C=C- group, while vinylic proton gives signal atδ-5.5.A broadening peak is observed atδ-3.2-4.2 corresponds to $-NH_2$ proton. This peak indicates the co-ordination through the $-NH_2$ group of urea segment to the metal atom of the soap segment [17].

(III) ESR SPECTRAL STUDIES

Literature reveals 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.

The value of ESR parameters for all the complexes is recorded in Table 7.A perusal of Table shows that for all the complexes of copper soaps, the values of ESR parameters$g_{\|}$, $g_{\perp}$and$g_{av}$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 $g_{\|} > g_{\perp}$ for all the complexes indicates that the unpaired electron is most likely in dx^2-y^2 orbital of copper (II) giving $^2B_{1g}$ as the ground state.

Table 7: ESR Spectral Data for copper Soap–Urea Complexes

Name of Complex	$g^{\perp}$	gII
CGU	2.0505	2.318
CSeU	2.0270	2.316

This fact supports that complexes possess elongated octahedral geometry. Thus, the values of $g_{\|}$ are suggestive of covalent character of metal-ligand bond. Earlier magnetic studies, about copper soaps confirm the binuclear configuration in solid state.

However the low value of the magnetic moments for copper ion present in copper soap in non-aqueous solvent like alcohol and hydrocarbon confirms that the binuclear configuration exist in soap solution [18].

(C) Antifungal activities

The antifungal activities of copper soaps and their corresponding urea complexes have been evaluated by testing them against *Alternaria-alternata* and *Aspergillus-niger* at 10^3 ppm and 10^4 ppm using 2ml and 5ml of these solutions by agar plate technique. The results of the fungicidal screening data are recorded in Table 8 and 9.

A perusal of Table 8 and 9reveals that all the urea complexes of copper (II) soaps show higher activity than pure soaps, suggesting that complexes are more powerful anti-fungal agents: and N, S, O etc. containing compounds are able to enhance the performance of copper soaps. The organic compounds containing amino group play important role in biology.

From the (Tables 8-9) it is apparent that all the copper soaps and their complexes with urea have significant fungitoxicity at 10^4 ppm but their toxicity decreases markedly on dilution (at 10^3 ppm). Their comparative order could be as follow: 1000ppm < 10000ppm. It is evident that their efficiency increases with increase in concentration Fig 3-4.

Table 8: Antifungal Activity of Cu (II) Soaps and Their Urea Complex in Methanol-Benzene Mixture (For *Alternaria- alternata*)

Name of the Soap &complex	Average % inhibition after 3 days			
	Concentration in ppm			
	1000		10000	
	2 ml	5 ml	2 ml	5 ml
CG	25.50	64.03	30.93	78.36
CSe	41.37	78.12	51.24	90.00
CGU	35.50	70.00	37.95	86.50
CSeU	50.00	86.50	55.36	96.60

Table 9: Antifungal activity of Cu (II) Soaps and their Urea complex in Methanol-Benzene Mixture (for *Aspergillus-niger*)

Name of the Soap &complex	Average % inhibition after 3 days			
	Concentration in ppm			
	1000		10000	
	2 ml	5 ml	2 ml	5 ml
CG	17.75	57.41	35.07	78.00
CSe	20.25	79.82	39.70	79.97
CGU	28.00	60.32	45.48	85.65
CSeU	32.00	85.40	50.50	95.76

Thus concentration plays a vital role in increasing the degree of inhibition. Also, on increasing the amount of solution in petri-plates from 2ml to 5ml, the % inhibition increases, suggesting that the % inhibition is affected by the increase in the concentration of the active fungicidal moiety in the analyzed system [19].

On the basis of earlier workers observations, it was observed that enhanced activity of complexes may be due to synergistic mechanism i.e. the free soaps are less active but on complexation they show more activity in combination with ligands containing N atom. The evaluation of anti-fungal studies further revealed that fungi-toxicity of the complexes and soaps also depends on the nature of metal ions

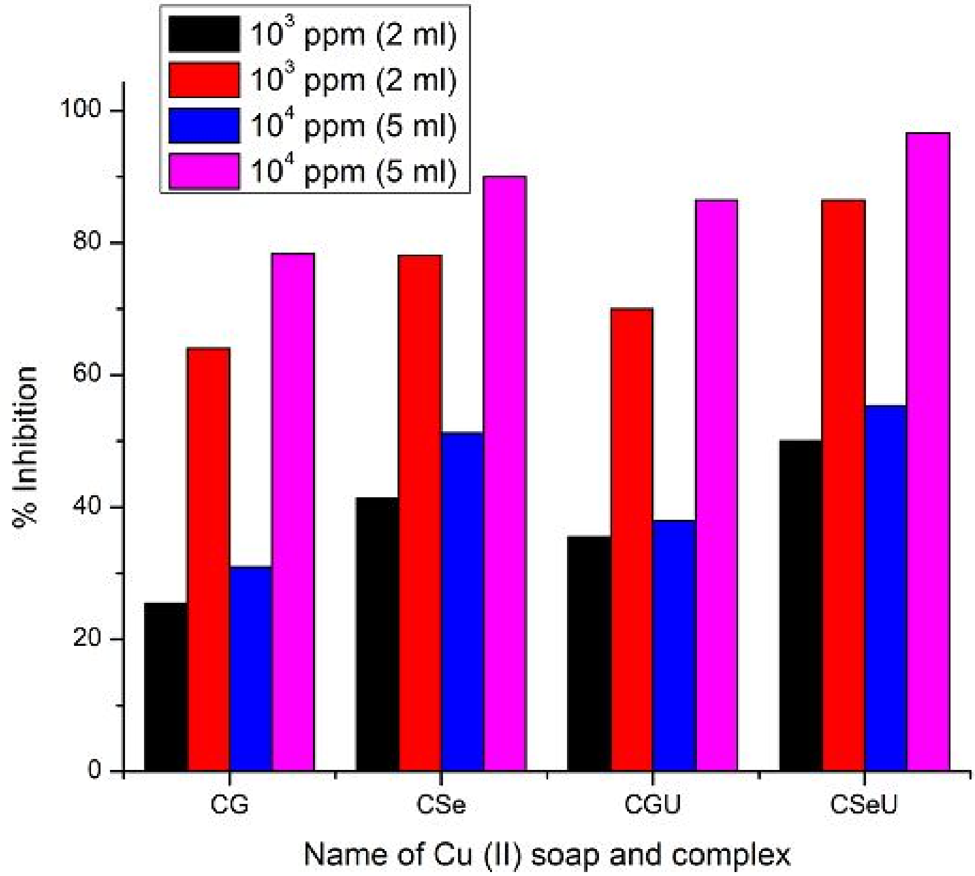

Fig. 3: Antifungal activity of Cu (II) soaps and their urea complex in methanol-benzene mixture (for A*lternaria- alternata*)

From comparison of the results for both the fungi it is found that Cu (II) soaps and their urea complexes show almost similar behavior for both the fungi [20].

The activity (toxicity) of Cu (II) soaps derived from various edible oils is found in the order:
CSe> CG

And for complexes the order of activity is almost same as soaps.
CSeU> CGU

In general, CSe and its complex CSeU both are more potent (more toxic) than groundnut soap and its complex.

The observation suggests that the soap possessing maximum fungicidal activity has lowest molecular weight.

The activity of soap and complexes both follows the same order which is just reverse of their average molecular weights suggesting that the soaps and complexes derived from sesame oils containing more content of shorter fatty acids and more poly-unsaturation are more active than soaps and complexes derived from groundnut oil, containing longer fatty acid chain content and less unsaturation in their chemical composition.

The data clearly indicates that the inhibition power of soap has been increased on the complexation. Thus it may be suggested that nitrogen, and oxygen containing ligand enhance the activity of the soap molecule for all the referred systems. These studies will play a significant role in selection and promotion of ecofriendly and biodegradable fungicides, pesticides and insecticides.

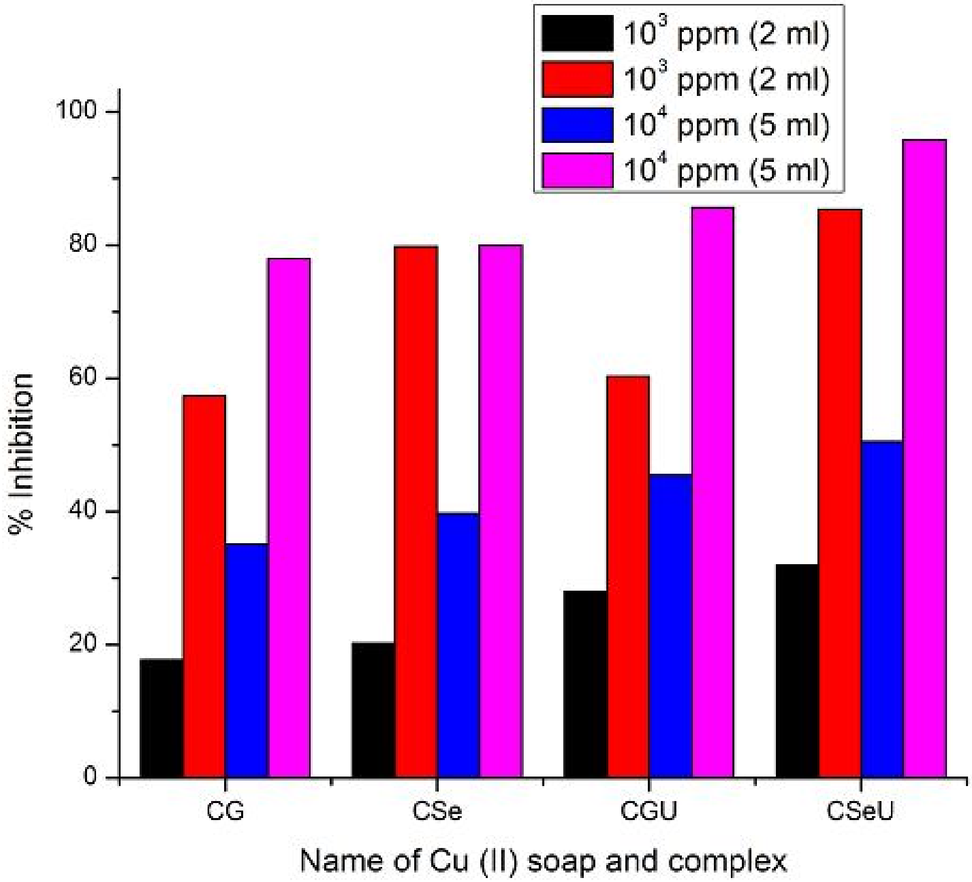

Fig. 4: Antifungal activity of Cu (II) soaps and their urea complex in methanol-benzene mixture (for *Aspergillus-niger*)

4. CONCLUSION

It has been observed that the antifungal activity increases with the increase in the concentration of the solution. It has been observed that complexes show higher activity than pure soaps, which suggests that complexes are more powerful antifungal agent. The toxicity of copper soaps and their urea complexes are found in the order:

CSeU>CSe
CGU > CG
CSeU>CGU>CSe>CG

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