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DOI: 10.2174/1874844901805010036, 2018, *5*, 36-44

RESEARCH ARTICLE

Antimicrobial Studies and Characterization of Copper Surfactants Derived from Various Oils Treated at High Temperatures by P.D.A. Technique

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Received: July 26, 2018 Revised: October 14, 2018 Accepted: October 15, 2018

Abstract:

Introduction:

Biologically potent compounds are one of the most important classes of materials for the upcoming generations. 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. 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. Chemistry of present era aims to build a pollution free environment. For the same, it targets to create some alternativeswhich are eco-friendly and nature loving. Present research work is a step towards achieving such alternatives.

Method:

For this the metallic soaps of copper (derived from common edible oils) were synthesized. The synthesized copper soaps have been confirmed by elemental analysis, UV, and IR spectroscopic technique. The fungicidal activities of copper soaps derived from soyabean, sesame oils have been evaluated by testing against Alternaria alternate and Aspergillus niger by P.D.A. technique.

Result:

The fungi toxicity results indicate that the strain of fungal species are susceptible towards these soaps and suggests that with the increase in concentration of copper soap it may increase further. The transition metallic soaps showed good antifungal activity because chelation increases the anti-microbial potency.

Keywords: Soyabean oil, Sesame oil, Copper Soaps, Antifungal, *Alternaria alternate*, *Aspergillus niger*.

1. INTRODUCTION

Surface active agents are very useful in biological systems, as well as play an important role in many industrial processes [1]. Exact information about micellar feature of copper (II) surfactants play a vital role in its selection in various fields such as foaming, wetting, detergents, emulsifier, herbicides, pesticides, paints, varnishes, wood preservatives, lubricants etc [2]. Anionic soaps containing copper ions play a vital role in various fields such as rubber industries, paints, varnishes, lubrication, protection of crops, stabilization of nylon threads, preservation of wood etc [3]. Inspite of all these applications, copper surfactants derived from various edible oils have not been thoroughly investigated. Many copper complexes are found to have significant anti-tubercular, fungicidal and antitumor activities [4]. Several workers described the uses of copper soaps as stabilizers for nylon threads, synthetic polyamides and

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1874-8449/18 2018 Bentham Open

polyesters [5, 6]. The protection of fabrics, nets, cordage etc from fungi and decay by impregnating them in ammonical solution of copper soaps was described by several workers [7, 8]. The effectiveness of copper soaps as fungicides, bactericides, insecticides and herbicides were also studied. Recent development in metallic soaps preservation shows that zinc and copper napthanates, exhibiting no specific fungal weakness, can be used to prevent attack by wood boring insects the use of copper soaps as driers for the preparation of paints, varnishes and other protective coating. It was observed that the addition of copper soaps to fuel oil reduces the smoke and fumes of burning oil [9]. The use of copper linoleate as heavy-duty wood preservative and many other biological activities of copper metal containing surfactants have also been studied [10]. These facts led us to synthesize copper soaps of sesame and soyabean oils (Fresh and treated at high temperature at different times) and fungicidal activities was planned to study for exploring their applications.

2. EXPERIMENTAL

2.1. Synthesis

Soyabean and sesame oils are easily available in India and chosen for the investigation. Their compositions are recorded in Table **1**. Three samples of each oil have been prepared as fresh (untreated), treated oil at high temperature for 15 minutes and for 60 minutes. Copper soaps were prepared by Direct Metathesis process as earlier reported [11] and characterization was done by using elemental analysis, UV, IR methods.

Table 1. Composition of fatty acid in oils used for copper soap synthesis.

Name of oil	% Fatty Acids					
	16:0	18:0	18:1	18:2	18:3	Other Acids
Sesame Oil	8	4	45	41	-	-
Soyabean Oil	12	4	24	51	9	-

These soaps are abbreviated as follows:

A. From untreated oils
 1 Copper soap of soyabean oil CSo
 2 Copper soap of sesame oil CSe

B. From treated oils for 15 minutes
 1 Copper soap of soyabean oil CSo_{15}
 2 Copper soap of sesame oil CSe_{15}

C. From treated oils for 60 minutes
 1 Copper soap of soyabean oil CSo_{60}
 2 Copper soap of sesame oil CSe_{60}

2.2. Determination of Molecular Weight of Copper Soaps

Molecular weights of Cu (II) soaps are determined from S.E [12]. The values of saponification value and molecular weights are recorded in Table **2**.

Table 2. Various data of copper soaps derived from untreated and treated oils.

Name of Copper Soap	Color	M.P. (°C)	Yield %	Metal %		S.V.	S.E.	Av. Mol. Wt.
				Found	Calculated			
CSe	Green	104	71	10.05	9.870	191.70	292.64	646.78
CSo	Green	108	70	10.80	10.303	194.90	287.83	637.17
CSe_{15}	Green	97	73	13.01	13.174	252.40	222.26	506.02
CSo_{15}	Green	101	72	15.98	16.116	336.60	166.66	394.82
CSe_{60}	Green	80	75	20.15	20.352	448.00	125.22	311.94
CSo_{60}	Green	70	77	10.52	10.672	210.38	266.66	594.82

$$S.E = 56100/S.V \quad (1)$$

2.3. Reactions During Heating the Oils

2.3.1. Autoxidation

Literature independently suggested that the first reaction was between molecular oxygen and an ethylene bond with formation of peroxide which like hydrogen peroxide, was capable of oxidizing other compounds. It is further suggested that the initially formed peroxide changes by intra-molecular rearrangement to a tautomericenediol- ketohydroxide system. It is believed that the moloxide was the primary product of reaction and this rearranged to the peroxide and confirmed that the primary product of autoxidation of non-conjugated unsaturated acids or esters are hydroperoxides in which the double bond remains intact [13].

2.3.2. Thermal polymerization

When the esters of di and tri ethenoid acids are heated above 200°C, they undergo certain changes. It is confirmed from literature that thermal polymerization of non-conjugated and conjugated octadecadienoates have concluded that the first step in the polymerization of the non-conjugateddienes is isomerization to the conjugated esters and that after configurational change to the trans -trans diene, this enters into diels alder condensation with conjugated or non-conjugateddiene, preferably the latter since this is present in greater proportions [14]. As a result, average molecular weight of oil changes after heating. This fact is supported by several workers that the deterioration during frying is higher in the oils containing higher polyunsaturated fatty acids.

3. CHARACTERIZATION

3.1. Electronic Absorption Spectra

In order to confirm the formation of copper soaps derived from groundnut oil, the electronic absorption spectra was recorded on a Perkin-Elmer-Lambda-28 spectrophotometer.

3.2. Infrared Spectral Analysis

To study the structure of copper soaps derived from oils, the infrared spectra of these compounds in the present study were recorded in KBr disc by making use of Perkin Elmer infrared spectrometer. The IR absorption peaks are given in Table **3**.

Table 3. IR absorption spectral frequencies of copper soaps derived from untreated and treated oils.

Assignments	CSe	CSo	CSe_{15}	CSo_{15}	CSe_{60}	CSo_{60}
CH_3 andCH_2, C-H Antisym. Stretching	2975-2953	2975-2960	2970	2627	2920	2926
CH_3 andCH_2, C-H Sym. Stretching	2855-2840	2850-2840	2860	2855	2850	2854
COO^-, C-O Antisym. Stretching	1590	1580	1595	1590	1590	1590
CH_2 Deformation	1465	1465	1460	1460	1465	1461
COO^-, C-O Sym. Stretching.	1375	1385	1390	1418	1415	1417
CH_2 Twisting and Wagging	1310	1310	1320	1315	1320	1315
CH_3 Rocking	1170	1150	1172	1169	1170	1173
CH_2 Rocking	730	725	725	723	720	723
M-O Stretching	670	669	670	669	665	669

3.3. Fungicidal Activities

The fungicidal analysis procedure follows below steps as suggested by Booth and Hawks worth as follows:

3.3.1. Sterilization of Glassware's

For biological activity the glassware were thoroughly washed and cleaned with chromic acid, followed by washed with distilled water and keep them in hot air oven at 160 °C for 24 h. All operations concerning inoculation are done in a completely sterilized chamber.

3.3.2. 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 transfer and maintenance of culture under aseptic condition.

3.3.3. Preparation of Slant

Agar slants were prepared to inoculate microbial culture. To prepare agar slant, a 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 sometimes, the tubes got cooled and the medium in them was solidified resulting in a sloppy surface.

3.3.4. 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.

3.3.5. Preparation of PDA

Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) are common microbiological media from potato infusion and dextrose (corn sugar) it was prepared by earlier reported method [15].

3.3.6. Preparation of Sample Solutions

The calculated amount of the copper surfactants derived from sesame and soyabean were weighed in a standard flask and 10^3 and 10^4 ppm concentration of solutions prepared by serial dilution method.

3.4. Test Organism

The test organism was *Alternaria alternate,* and *Aspergillus niger* which was cultured and isolated from its natural habitat and identified morphologically .

3.5. Fungicidal Testing

The fungicidal testing procedure was exactly same as reported by Sharma *et al.* [16].

The data were statistically analyzed according to the following formula [17].

$$\%Inhibition = C - T / C \times 100 \quad \textbf{(2)}$$

(% Inhibition)

C- Total area of fungal colony in plat without copper surfactants after 2 days.

T- Total area of fungal colony in plate with copper surfactants after 2 days.

4. RESULTS AND DISCUSSION

4.1. Electronic Absorption Spectra

The spectra give information concerning copper-ligand binding. The electronic absorption spectra of copper sesame soap show that one broad band 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 MLCTabsorption bands which confirms the formation of copper sesame soap and proposes a distorted octahedral stereochemistry around the metal ion. Absorption peaks (λ<300nm) belong to $\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ orbital transition of the ligand [18, 19].

4.2. IR Spectra

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 sesame oil, three distinct bands appear at 3008, 2925 and

2854 cm^{-1} due to =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) [20]. 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 group. 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}(symmetricvibration of carboxylate ion) and 1380-1400 cm^{-1}(anti-symmetric vibration of carboxylate ion). 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 [21, 22]. 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 [23].In the IR spectra of CSe_{60} also, bands are present at about 3500 cm^{-1} (very weak), 1750 cm^{-1} (strong), 1625 cm^{-1} (weak) and 1100 cm^{-1} (weak). Appearance of these bands may be due to formation of various autoxidized products such as ene-diol, keto-hydroxide or carbonyl degradation products. In IR spectra of CSo_{15} and CSo_{60} bands are present at 3450 cm^{-1} and 1745 cm^{-1} which shows the formation of keto- hydroxide during the autoxidation reactions.

4.3. Fungicidal Activities

Copper soaps derived from untreated and treated oils have been screened for their anti-fungicidal activity against *Alternaria-alternata* and *Aspergillus-niger* at 1000 ppm and 10000 ppm by agar-plate technique [24]. Copper soaps showed moderate activities against both the fungi.

A perusal of Fig. (**1**) reveals that all the copper soaps have significant fungitoxicity at 10000 ppm but their toxicity decreases markedly on dilution (at 1000 ppm). It is apparent that their efficiency increases with their concentration. Thus it is evident that concentration plays a vital role in increasing the degree of inhibition [25, 26]. Fungicidal screening data revealed that at lower concentration the inhibition of growth is less as compared to higher concentration. From comparison of the results for both the fungi, it is found that all copper soaps are more potent (more toxic) against *Aspergillus niger* than against *Alternaria- alternata* i.e. inhibition of growth is higher for *Aspergillus niger* than inhibition of growth for *Alternaria alternate* (Figs. **2**, **3**).

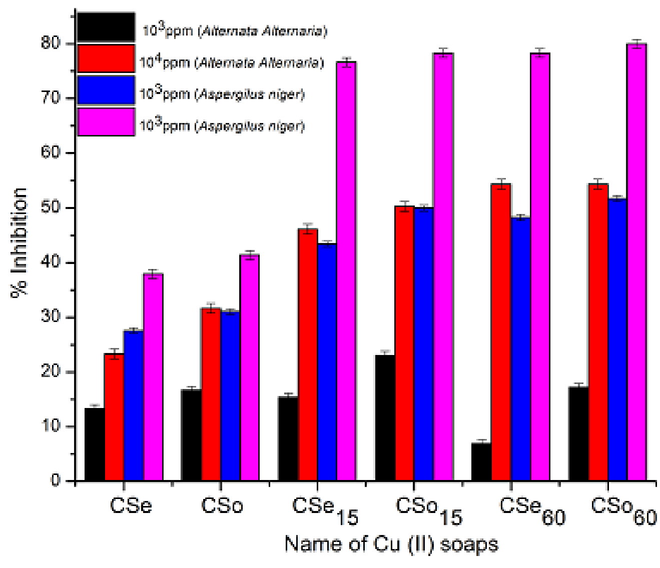

Fig. (1). Comparative germ inhibition for Cu (II) soaps derived from treated and untreated oils.

Fig. (2). Presence of *Alternaria Alternata* on tomato.

Fig. (3). Presence of *Aspergillus Niger* on bread.

It reveals that CSe is the least fungi toxic (% inhibition lowest) whereas CSo is the most toxic against both fungi. The activity (toxicity) of copper soaps derived from untreated oils is found to increase in the order:

$$CSo > CSe$$

For copper soaps derived from treated oils for 15 and 60 minutes, the results are same as copper soaps derived from untreated oils. $CSe_{15,}$ CSe_{60} is the least active and $CSo_{15}CSo_{60}$ is the most active against both fungi. The order of activity of copper soaps derived from treated oils for 15 minutes is as follows:

$$CSo_{15} > CSe_{15}\ CSo_{60} > CSe_{60}:$$

From comparison of copper soaps derived from untreated and treated sample of oil, it is found that fungitoxicity increases with the increase of time of heating for oils. All the tests were performed in triplicate the standard deviation has been measured by the conventional measure of repeatability and the average was taken as final reading. The results of ANOVA for the antifungal activities for all sops complexes are shown in Table **4** [27, 28].The predicted R^2 are in reasonable agreement and closer to 1.0 [29, 30]. This confirms that the experimental data are well satisfactory. The descriptive statics results of Cu (II) soaps shown in Tables **5**, **6** confirm satisfactory results in triplet. The result is statistically significant, by the standards of the study, due to $p < F$.

$$CSe_{60} > CSe_{15} > CSe$$

$$CSo_{60} > CSo_{15} > CSo$$

Table 4. ANOVA results for antifungal activities of Cu (II) soaps.

Fungi	*Cu (II) soap*	*SS*	*df*	*F*	*P-value*	F_{crit}	R^2
Alternaria alternata	CSe	104	1	4162	0.000240206	18.51	0.995
	CSo	228	1	9120	0.000109626	18.51	0.992
	Cse_{15}	964	1	29665	3.37084E-05	18.51	0.996
	CSo_{15}	729	1	16200	6.17227E-05	18.51	0.997
	CSe_{60}	2255	1	82822	1.20739E-05	18.51	0.991
	CSo_{60}	1362	1	54464	1.83601E-05	18.51	0.992
Aspergilus niger	CSe	110	1	4410	0.00022668	18.51	0.992
	CSo	102	1	4080	0.000244984	18.51	0.996
	Cse_{15}	1086	1	33406	2.99332E-05	18.51	0.997
	CSo_{15}	784	1	17422	5.7393E-05	18.51	0.991
	CSe_{60}	900	1	45000	2.22215E-05	18.51	0.992
	CSo_{60}	841	1	18689	5.35034E-05	18.51	0.995

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

Table 5. Descriptive statics results for antifungal activities of Cu (II) soaps.

	Soap	*Concentration (ppm)*	*Count*	*Average % Inhibition*	*Variance*	Coefficient Variance	*Std. Deviation*	*Std. Error*
Alternaria alternata Fungi	CSe	10^3	3	13.2	0.04	0.23	0.21	0.12
		10^4	3	23.3	0.02	0.22	0.15	0.14
	CSo	10^3	3	16.5	0.01	0.22	0.36	0.11
		10^4	3	31.2	0.02	0.36	0.21	0.24
	CSe_{15}	10^3	3	15.3	0.02	0.22	0.25	0.32
		10^4	3	46.2	0.02	0.16	0.11	0.22
	CSo_{15}	10^3	3	23.4	0.01	0.11	0.15	0.33
		10^4	3	50.4	0.01	0.10	0.22	0.22
	CSe_{60}	10^3	3	6.70	0.02	0.22	0.15	0.10
		10^4	3	54.2	0.01	0.11	0.11	0.30
	CSo_{60}	10^3	3	17.4	0.01	0.22	0.22	0.22
		10^4	3	54.2	0.02	0.16	0.14	0.10

Table 6. Descriptive statics results for antifungal activities of Cu (II) soaps.

	Soap	*Concentration (ppm)*	*Count*	*Average % Inhibition*	*Variance*	Coefficient Variance	*Std. Deviation*	*Std. Error*
Aspergilus niger Fungi	CSe	10^3	3	27.35	0.02	0.11	0.15	0.33
		10^4	3	37.85	0.01	0.10	0.22	0.22
	CSo	10^3	3	31.35	0.01	0.22	0.15	0.10
		10^4	3	41.45	0.02	0.11	0.11	0.30
	CSe_{15}	10^3	3	43.40	0.02	0.22	0.22	0.22
		10^4	3	76.35	0.02	0.16	0.14	0.10
	CSo_{15}	10^3	3	50.35	0.01	0.21	0.15	0.33
		10^4	3	78.35	0.01	0.26	0.22	0.15
	CSe_{60}	10^3	3	48.40	0.02	0.22	0.15	0.22
		10^4	3	78.40	0.01	0.11	0.11	0.22
	CSo_{60}	10^3	3	51.35	0.01	0.22	0.22	0.22
		10^4	3	80.35	0.02	0.16	0.14	0.22

CONCLUSION

From the comparison between IR spectra of Cu (II) soaps of untreated oils and Cu (II) soaps of treated oils, it is found that there is no band in 3200-3600 cm^{-1} region in Cu (II) soaps derived from untreated oils. But in the IR spectra of copper soaps derived from treated oils, there are bands at about 3400-3500 cm^{-1}, 1750 cm^{-1}, 1625 cm^{-1}, 1100 cm^{-1}. These bands may be due to formation of various autoxidized products such as enediol, keto-hydroxide or carbonyl degradation products. The antifungal activities of copper soaps derived from various edible oils have been evaluated by testing these against *Alternaria alternata* and *Aspergillus niger* at different concentrations by agar plate technique. It has been suggested that copper soaps derived from the oils treated for longer period show maximum activity (inhibition of the growth) against both the fungi. Copper soaps derived from oils treated for lesser period show lesser activity and soaps derived from untreated oils show minimum activity against both fungi. The activity (inhibition of the growth) also increases with the increase in concentration of soap. These studies suggested that used oils available in the Indian market can be used as fungicidal, pesticidal or herbicidal agents as they show positive result.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

The authors pay their sincere gratitude to Principal, S.D. Govt. college Beawar and S.P.C. Govt. College Ajmer, Rajasthan (India) for providing necessary research facilities to accomplish this study. IIT, Mumbai is gratefully acknowledged for providing spectral data.

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