American Journal of Microbiology and Biotechnology
2019; 6(1): 1-8
http://www.aascit.org/journal/ajmb
ISSN: 2375-3005

American Association for Science and Technology

Spectroscopic and Antimicrobial Activities of Biologically Active Agent Derived from Neem *(Azadirechta indica)* Oil

Priyanka Kumawat[1, *], Rashmi Sharma[1], Nitin Sharma[2]

[1]Department of Chemistry, Samrat Prithviraj Chouhan Government College, Ajmer, India
[2]Department of Biochemistry, Dr. Sampurnanand Medical College, Jodhpur, India

Email address
priyankakumawat.india@gmail.com (P. Kumawat)
[*]Corresponding author

Citation
Priyanka Kumawat, Rashmi Sharma, Nitin Sharma. Spectroscopic and Antimicrobial Activities of Biologically Active Agent Derived from Neem *(Azadirechta indica)* Oil. *American Journal of Microbiology and Biotechnology.* Vol. 6, No. 1, 2019, pp. 61-68.

Received: February 24, 2019; **Accepted**: April 7, 2019; **Published**: November 28, 2019

Abstract: In this study, we evaluated the antimicrobial potency of copper (II) soap and copper soap complex synthesized / formulated in our laboratory. The solid copper (II) soap derived from Neem (Azadirachta indica) oil and its complex with ligand containing nitrogen and sulphur atoms thiourea has been synthesized and characterized by elemental analysis, IR spectroscopy and biological studies. Elemental analysis was done for soap and complex for their metal content following standard procedures. The complex under study was prepared in two steps. In the first step, copper soap was prepared and in the second step complexation of copper soap was done with ligand thiourea. The absorption bands were found corresponding to the absorption bands of pure copper neem soap. Apart from these absorption bands the bands were also observed corresponding to the ligand moiety. In this study ESR Spectra, NMR Spectra, IR Spectra and Antimicrobial Activities against five bacteria related with skin disease have been done. Neem (Azadirechta indica) oil is widely used as insecticides, lubricant, drugs for variety of diseases. The synthesized biocidal molecule from neem oil and copper metal has been confirmed by spectroscopic studies. The antimicrobial studies and findings have high medical, industrial and economic significance as copper (II) soap and copper (II) soap complex could be hamessed in the formulation of medicated soaps and other externally applicable ointments and pharmaceuticals related with skin disease.

Keywords: Copper (II) Soap, Complex, Spectroscopic, Biological Studies

1. Introduction

Copper (II) soap derived from non-edible neem *(Azadirechta indica)* oil play a vital role in various fields due to its surface active properties. This oil was particularly chosen as it is easily available commercially and biodegradable in nature. Copper soap have a tendency of complexation with 'nitrogen' and 'sulphur' containing ligands. Using thiourea as a ligand, complexation of synthesized copper soap has been done to obtain its complex. Since copper metal is toxic in nature, literature survey reveals that the synthesized copper soap and its thiourea complex may play a significant role in biological activities. The interest in co-ordination chemistry is increasing continuously with the preparation of organic ligands containing a variety of donor groups and it is multiplied many folds when the ligand have biological importance. [1–4]

Microbiological monitoring of the environment in health facilities is part of preventing the transmission of nosocomial infections. The microbiological controls of the environment are one of the measuring tools that make it possible to evaluate a starting situation and the effectiveness of corrective measures, they must be implemented in a relevant way and obey very precise objectives while avoiding the inflation of useless analyses, consuming time and financial means. These heterocyclics have shown different pharmacological activities such as gram-positive/negative antibacterial agents, antibiotics, anti-parasitic, anti-inflammatory, lactase inhibitors, anti-stress, ulcer and anti-cancer agents. [5]

Bio-prospecting studies of endophytic microorganisms for pharmaceutical and biotechnological purposes are fundamental for the discovery of new substances for human therapeutics including antibiotics, anti-malarials, and anticancer. Metal-based complexes have been extensively investigated mainly for the development of anticancer agents and many of them have encouraging results. Microorganisms are important sources of bioactive natural products with enormous potential for the discovery of new molecules for drug discovery, industrial use and agricultural applications. [6-7]

Micro-Organisms taken for study are as follow:

Micro-oraganisms employed to study and explain the bactericidal processes of complexes synthesized in our laboratory were as follow:-

a. Staphylococcus aureus (S. aureus)

Staphylococcus aureus is a gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a member of the normal flora of the body, frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. [8]

b. Coagulase-negative staphylococci (CoNS)

CoNS, as typical opportunists, represent one of the major nosocomial pathogens, having a substantial impact on human life and health. They are particularly associated with the use of indwelling or implanted foreign bodies, which are in dispensable in modern medicine. Colonization of different parts of the skin and mucous membranes of the host is the key source of endogenous infections by CoNS. However, they are transmitted mainly by medical and/or nursing procedures. Once inserted, foreign bodies can become colonized by CoNS and the success of there spective medical procedure is significantly impaired, resulting in enormous medical and economic burdens. [9]

c. Acinetobacter baumanii

Acinetobacter baumannii is a typically short, almost round, rod-shaped (coccobacillus) Gram-negative bacterium. It can be an opportunistic pathogen in humans, affecting people with compromised immune systems, and is becoming increasingly important as a hospital derived (nosocomial) infection. While other species of the genus Acinetobacter are often found in soil samples (leading to the common misconception that A. baumannii is a soil organism, too), it is almost exclusively isolated from hospital environments. [10]

d. Pseudomonas aeruginosa (P. aeruginosa)

This blue-green pigment is a combination of two metabolites of *P. aeruginosa,* pyocyanin (blue) and pyoverdine (yellow), which impart the blue-green characteristic color of cultures. Another assertion is that the word may be derived from the Greek prefix ae- meaning "old or aged, and the suffix ruginosa means wrinkled or bumpy. [11]

e. Micrococcus

*Micrococcus luteus*is a gram-positive, to gram-variable, non-motile, coccus, tetradarranging, pigmented, saprotrophic bacterium that belongs to the family Micrococcaceae. It is urease and catalase positive. An obligate aerobe, *M. luteus*is found in soil, dust, water and air and as part of the normal flora of the mammalian skin. The bacterium also colonizes the human mouth, mucosae, oropharynx and upper respiratory tract. [12-13]

2. Experimental

All the chemicals used were of LR/AR grade. Solvent was purified according to standard procedures before use. Elemental analysis was done for soap and complex for their metal content following standard procedures. The complex under study was prepared in two steps. In the first step, copper soap was prepared and in the second step complexation of copper soap was done with ligand like thiourea. [14] Benzene was dehydrated by storage over sodium wire for 2–3 days and by refluxing for about twenty hours, it was then distilled and redistillation was carried out azeotropically with ethanol. [15]

Copper soap was prepared by refluxing the non edible oil i.e. Neem *(Azadirechta indica)* oil, in its pure form, of a available in the Indian market, with alcohol and 2N KOH solution for 3 hours (Direct metathesis). The neutralization of excess KOH present was done by 1N HCl. Saturated solution of copper sulphate was then added to it for conversion of neutralized soap into copper soap. Copper soap so obtained was then washed with hot water and dried. The soap was recrystallized using hot benzene.

The fatty acid composition of the non edible oil was confirmed through gas liquid chromatography [GLC] of its methyl esters and is given in Table 1. [16-18]

Table 1. *Fatty acid composition of oil used for copper soap/complex.*

Name of oil	**% fatty acid**				
	16:00	**18:00**	**18:01**	**18:02**	**Other acid ($C_{20} – C_{24}$)**
Neem oil	14.9	14.4	61.9	7.5	1.3

The ligand thiourea was taken directly. The purified copper soap derived from non edible oil was refluxed with ligand (thiourea) in 1:1 ratio using benzene as a solvent for one hour. It was then filtered hot, dried, recrystallized and purified in hot benzene. Thin layer chromatography [TLC] using silica gel was used to check the purity of the complex. [19-20]

The complex obtained was light brown in color. The complex was soluble in benzene and other organic solvents but insoluble in water. The complex was stable at room temperature, its physical parameters like saponification value (S. V.), saponification equivalent (S. E.) and molecular weights are recorded in Table 2.

Table 2. *Analytical and physical data of copper soap and its complex derived from neem oil.*

Name of soap/complex	Colour	M. P.	Yield (%)	Metal Content%		S. V.	S. E.	Average M. W.
				Observe	Calculate			
Copper Neem Soap (CN)	Dark Green	50°C	85	10.16	10.07	198	283.3	630.16
Copper Neem Soap Thiourea Complex (CNT)	Light Brown	82°C	90	9.31	8.99	-	-	706.28

On the basis of the elemental analysis, 1:1 (metal: ligand) type of stoichiometry has been suggested. [21-23]

In order to study the structure of soap and complex, the infra–red (IR) absorption spectra of compounds were obtained on a ABB Horizon MB 3000 series instrument spectrophotometer (4000 – 600 cm^{-1}) from SPC Govt. College, Ajmer. In order to study the structure of soap and complex, the NMR and ESR Spectra of compounds were obtained from Saif CDRI Lucknow and Saif IIT Bombay.

The biological activities of copper soap and its corresponding complex with ligand thiourea have been screened against *Staphylococcus aureus, Coagulase-negative staphylococci (CoNs), Acinetobacter baumanii, Pseudomonas aeruginosa* and *Micrococcus* bacteria at 3 * 10^4 ppm, 1.5 * 10^4 ppm, .75 * 10^4 ppm and .375 * 10^4 ppm using disc of these solutions by Mueller Hinton Agar plates.

The following Bacteria: *Staphylococcus aureus, Pseudomonas aeruginosa, CoNS, Micrococcus* and *Acinetobactor*. All bacterial strains were maintained on nutrient agar medium at ±37°C. These cultures are obtained from the Department of Microbiology, Dr. S. N. Medical College Jodhpur.

Antimicrobial Evaluation:-

The antimicrobial activity of newly synthesized compounds was evaluated using agar disc diffusion assay. Briefly, a 24 and 48 hours old culture of selected bacteria was mixed with sterile physiological saline (0.9%) and the turbidity was adjusted to the standard inoculum of Mac-Farland scale 0.5 (106 colony forming units (CFU) per ml). Petri plates containing 20 ml of Mueller Hinton Agar was used for antibacterial activity. The inoculum was spread on the surface of the solidified media and Whatman No. 1 filter paper discs (5 mm in diameter) impregnated with the test compound (20 µl/disc) were placed on the plates. Ampicillin (10 mg/disc) was used as positive control for bacteria. A paper disc impregnated with petroleum ether was used as a negative control. Plates inoculated with the bacteria were incubated for 24 hour at 37°C. The inhibition zone diameters were measured in centimeters. All the tests were performed in triplicate and the average was taken as final reading. Guidelines for evaluating a microbiological laboratory method usually include a list of desirable characteristics such as repeatability, reproducibility and ruggedness. A method is considered repeatable if independent repeats of the same experiment in the same laboratory produce nearly the same results. The conventional measure of repeatability is the standard deviation or some multiple of the standard deviation, std. error, variance etc. [24-27]

3. Results and Discussion

The copper soap and complex are abbreviated as follows-

1. Copper - Neem Soap (CN)
2. Copper – Neem Soap Thiourea Complex (CNT)

3.1. IR spectral Analysis

The absorption bands observed at 2924 cm^{-1} and 2854 cm^{-1} corresponds to asymmetric and symmetric stretching of methylene (-CH_2) group. The presence of absorption band sat 1465 cm^{-1} is representative of symmetric bending of nearly 3010 cm^{-1} corresponds to olefinic =C–H stretch. The strong absorption band at 1582 cm^{-1} was due to carboxylate ion COO-, C–O anti symmetric stretching respectively. Also>C=O stretching bands were observed at 1744 cm^{-1} Also peaks corresponding to –CH_3 and –CH_2 rocking have been seen at 1157 cm^{-1} and 725 cm^{-1} respectively. Copper–oxygen (Cu–O) stretching bands have been distinguished at 480 cm^{-1}. [14-16]

The above-mentioned absorption bands (Table 3) were found to be common with the absorption bands observed for pure copper soap of non edible oils. Apart from these absorption bands the following bands were also observed corresponding to the ligand moiety. [28-30]

The C–N stretching band of primary amide was observed at nearly 1543 cm^{-1} the absorption band 1744 cm^{-1} was found to be representative of amide>C=O group. A broad band near 3302 cm^{-1} was observed corresponding to N–H stretching of amides. An absorption band observed at 1396 cm^{-1} corresponds to N–C=S stretching. Also C–S stretching band was observed at 1311 cm^{-1}. C–H stretching band due to deformation out of plane (in benzene) was also observed at 679 cm^{-1}. (Table 3). [30-33]

Thus on the basis of above observations it can be safely assumed that complexation of copper soap has taken place with thiourea.

Table 3. *IR spectral data for copper (II) neem soap and its complex.*

Absorption bands	CN (cm^{-1})	CNT (cm^{-1})
Corresponding to soap moiety		
Olefinic =C–H stretching	3010	3009
CH_3 and CH_2, C–H Anti sym. stretching (v_{as})	2916	2924
CH_3 and CH_2, C–H sym. stretching (v_s)	2854	2854
C=C stretching	2300	2301
>C=O Stretching	1744	1744
COO^-, C–O Anti-sym. stretching	1582	1612
CH_2, C–H Bending (δ_s) (scissoring)	1465	1458
C–H, deformation, =C–H Rocking	1443	-
CH_3, C–H Rocking	1157	1157
CH_2, C–H Rocking	725	717
=C-H, Out of Plane Bending of C-H	679	609
Cu–O stretching	480	560
Corresponding to ligand moiety		
Asym. NH_2, N–H stretching	-	3672
NH_2, N–H stretching	-	3302
C–N stretching	-	1543
N–C–S stretching	-	1396
C=S stretching	-	1311
unconjugated C–N stretching	-	1095

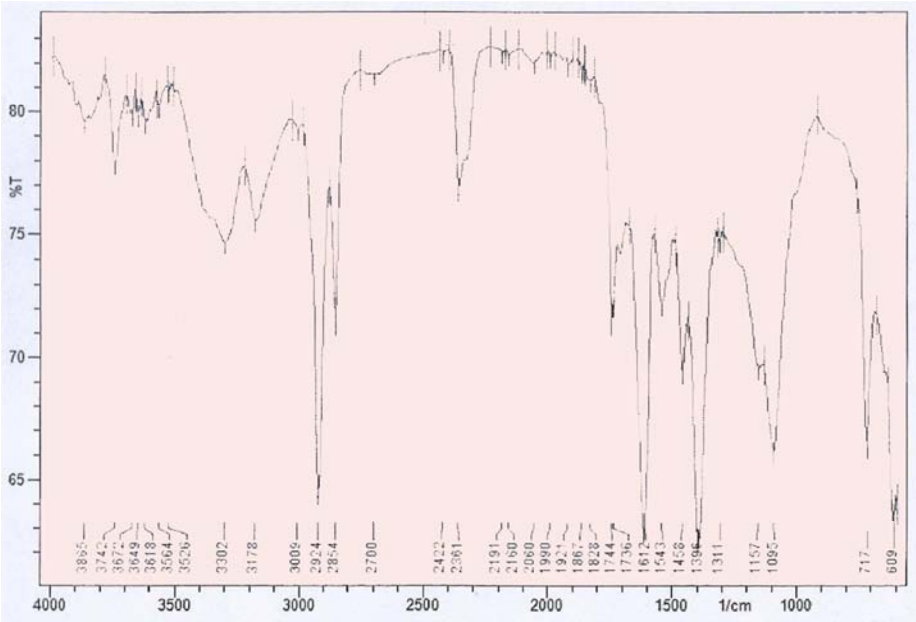

Figure 1. *IR spectra of copper neem soap thiourea complex (CNT).*

3.2. NMR Spectra

A perusal of the spectra of copper soaps shows signal of aliphatic $–CH_3$ proton attached to $–CH_2$-R group at nearly -0.95. $–CH_2$ proton attached to CH_2-R group shows signal at -1.20. Other signal observed are corresponding to $–CH_2$ attached to one –C=C- at -2.10, while $–CH_2$ attached to two –C=C- is observed at -2.86. Vinylic proton gives signal at -5.50. Other signal observed are corresponding to -C=O-NH at 4-4.15. For NMR peaks at -5.502, the peak area for neem soap has been found to be larger then that of neem soap thiourea complex owing to lesser number of –C=C- group of 18:1, 18:2 and 18:3 types in neem oil as clear from their composition in Table 1. All the above peak (Table 4) are due to the long chain fatty acid content (R-) of the Soap molecule $[(R\text{-}COO)_2Cu]$. A broadened peak is observed at -4.3-4.35 corresponding to $–NH_2$ Proton. [22] This peak indicates the co-ordination through the $–NH_2$ group of thiourea segment to the metal atom of the soap segment. In case of thiourea complex, the proton present on the nitrogen atom may under – go rapid, intermediate or slow exchange. The broadening of the observed peak is suggestive of a slow exchange because the electrical quadripole moment of nitrogen nucleus induces a moderately efficient spin relaxation. [23, 34-35]

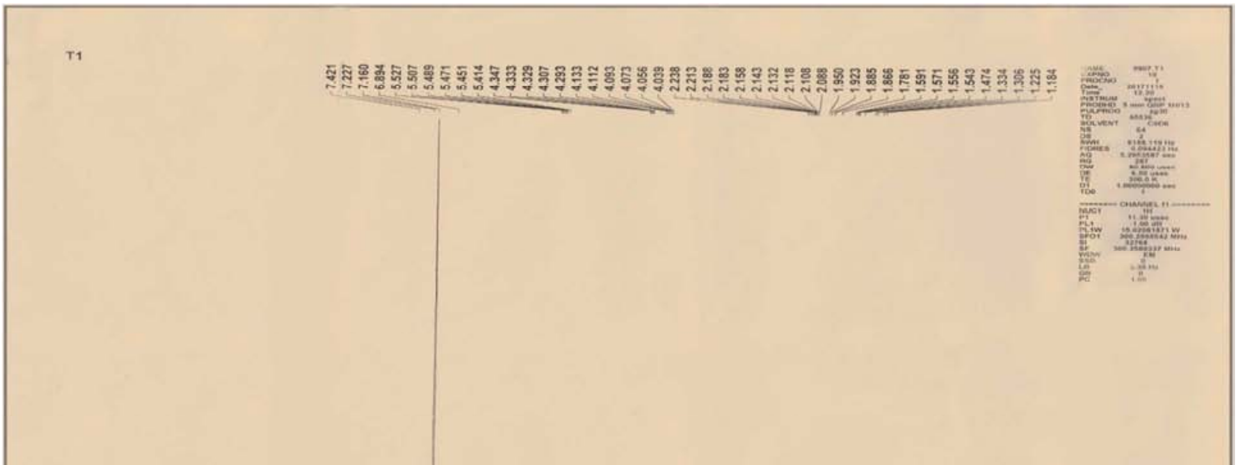

Figure 2. *NMR spectra of copper neem soap thiourea complex (CNT).*

Table 4. *NMR spectra data copper neem soap thiourea complex.*

Peak/Signal	CNT ( )
CH_3–CH_2-R	.95 (DOUBLET)
CH_2–CH_2-R	1.29 (SINGLET)
–CH_2- C=C-	2.1 (MULTIPLET)
–C=C-H (Vinylic proton)	5.5 (TRIPLET)
-C=C-CH_2-C=C-	2.89 (TRIPLET)
NH_2 broadened peak	4.3 -4.35 (DOUBLET)
R-C=O-NH	4-4.15 (MULTIPLET)
C≡N OR NH_2	1.6 (QUARTET)

3.3. ESR Spectra Analysis

ESR spectroscopy provides a powerful tool for the detection and characterization of free radical and paramagnetic transition metal complex. An ESR spectrum contains several kinds of information. The spectral intensity is proportional to radical concentration and the electron nuclear hyperfine pattern is a fingerprint for radical identification. The values of $g_{\parallel}$ and g are greater than the value of g_0 ie 2.00277. This indicates that distortion from regular octahedron complex has taken place in the shape of the complex. Also the complex, the trend $g_{\parallel}$>g indicate that the unpaired electron is most likely to be in $d_{x^2-y^2}$ orbital of Cu (II) giving $^2B_{1g}$ as the ground state. This fact supports its complex possess elongated octahedral geometry. Kivelson and Neiman showed that $g_{\parallel}$ is a moderately sensitive function for indicating covalency. Thus the value of $g_{\parallel}$ is suggestive of covalent character of metal – ligand bond. Since the value of G is a greater than four, it is suggestive of negligible exchange coupling interaction between the two copper centers in the complex. Here $A_{\parallel}$ is the parallel coupling constant expressed in gauss. [36-37]

3.4. Biological Activities

Neem thiourea soap complex show higher antibacterial activity than pure soap suggesting that complex is more powerful antibacterial agent and other N & S etc. containing compounds are able to enhance the performance of copper soap. The enhanced activity of newly synthesized complex as compared to the soap can possibly be explained on the basis of presence of donor atoms N and S as well as the structural compatibility with molecular nature of the toxic moiety. [38]

The activity of copper neem soap and copper neem thiourea soap complex derived from Neem oil were found in the follow order soap and complex respectively:

For soap

Staphylococcus aureus= Coagulase-negative staphylococci (CoNs) >Acinetobactor baumanii= Pseudomonas aeruginosa >Micrococcus

For complex

Staphylococcus aureus>Coagulase-negative staphylococci (CoNs)>Acinetobactor baumanii> Pseudomonas aeruginosa>Micrococcus

Table 5. *Descriptive statistics bactericidal data for copper (II) soap and its complex derived from neem oil.*

Bacteria	Soap/Complex	Group	Count	Sum	Average	Variance	Std. Deviation	Std. Error	Coff. Variance
S. aureus	CN	$3*10^4$	3	5	1.67	0.003	0.058	0.033	0.035
		$1.5*10^4$	3	4.4	1.47	0.003	0.058	0.033	0.039
		$.75*10^4$	3	3.8	1.27	0.003	0.058	0.033	0.046
		$.375*10^4$	3	3.1	1.03	0.003	0.058	0.033	0.056
	CNT	$3*10^4$	3	5.3	1.77	0.003	0.058	0.033	0.033
		$1.5*10^4$	3	4.7	1.57	0.003	0.058	0.033	0.037
		$.75*10^4$	3	4.1	1.37	0.003	0.058	0.033	0.042
		$.375*10^4$	3	3.5	1.17	0.003	0.058	0.033	0.049
Cons	CN	$3*10^4$	3	5	1.67	0.003	0.058	0.033	0.035
		$1.5*10^4$	3	4.4	1.47	0.003	0.058	0.033	0.039
		$.75*10^4$	3	3.7	1.23	0.003	0.058	0.033	0.047
		$.375*10^4$	3	3.1	1.03	0.003	0.058	0.033	0.056
	CNT	$3*10^4$	3	5	1.67	0.003	0.058	0.033	0.035
		$1.5*10^4$	3	4.4	1.47	0.003	0.058	0.033	0.039
		$.75*10^4$	3	3.8	1.27	0.003	0.058	0.033	0.046
		$.375*10^4$	3	3.2	1.07	0.003	0.058	0.033	0.054
A. baumanii	CN	$3*10^4$	3	4.7	1.57	0.003	0.058	0.033	0.037
		$1.5*10^4$	3	4.1	1.37	0.003	0.058	0.033	0.042
		$.75*10^4$	3	3.5	1.17	0.003	0.058	0.033	0.049
		$.375*10^4$	3	2.9	0.97	0.003	0.058	0.033	0.060
	CNT	$3*10^4$	3	4.7	1.57	0.003	0.058	0.033	0.037
		$1.5*10^4$	3	4.1	1.37	0.003	0.058	0.033	0.042
		$.75*10^4$	3	3.5	1.17	0.003	0.058	0.033	0.049
		$.375*10^4$	3	2.9	0.97	0.003	0.058	0.033	0.060
P. aeruginosa	CN	$3*10^4$	3	4.7	1.57	0.003	0.058	0.033	0.037
		$1.5*10^4$	3	4.1	1.37	0.003	0.058	0.033	0.042
		$.75*10^4$	3	3.5	1.17	0.003	0.058	0.033	0.049
		$.375*10^4$	3	2.9	0.97	0.003	0.058	0.033	0.060
	CNT	$3*10^4$	3	4.4	1.47	0.003	0.058	0.033	0.039
		$1.5*10^4$	3	3.8	1.27	0.003	0.058	0.033	0.046
		$.75*10^4$	3	3.2	1.07	0.003	0.058	0.033	0.054
		$.375*10^4$	3	2.6	0.87	0.003	0.058	0.033	0.067

Bacteria	Soap/Complex	Group	Count	Sum	Average	Variance	Std. Deviation	Std. Error	Coff. Variance
Micro coccus	CN	$3*10^4$	3	4.4	1.47	0.003	0.058	0.033	0.039
		$1.5*10^4$	3	3.8	1.27	0.003	0.058	0.033	0.046
		$.75*10^4$	3	3.5	1.17	0.003	0.058	0.033	0.049
		$.375*10^4$	3	2.9	0.97	0.003	0.058	0.033	0.060
	CNT	$3*10^4$	3	4.1	1.37	0.003	0.058	0.033	0.042
		$1.5*10^4$	3	3.5	1.17	0.003	0.058	0.033	0.049
		$.75*10^4$	3	2.9	0.97	0.003	0.058	0.033	0.060
		$.375*10^4$	3	2.3	0.77	0.003	0.058	0.033	0.075

Table 6. *Anova table bactericidal data for copper (II) soap and its complex derived from neem oil.*

Bacteria	Soap/Complex	SS	df	MS	F	P-value	F crit
S. aureus	CN	0.66	3	0.22	66.25	5.43E-06	4.067
	CNT	0.6	3	0.2	60	7.93E-06	4.067
Cons	CN	0.68	3	0.23	68.33	4.82E-06	4.067
	CNT	0.6	3	0.2	60	7.93E-06	4.067
A. baumanii	CN	0.6	3	0.2	60	7.93E-06	4.067
	CNT	0.6	3	0.2	60	7.93E-06	4.067
P. aeruginosa	CN	0.6	3	0.2	60	7.93E-06	4.067
	CNT	0.6	3	0.2	60	7.93E-06	4.067
Micrococcus	CN	0.39	3	0.13	39	4.02E-05	4.067
	CNT	0.6	3	0.2	60	7.93E-06	4.067

Figure 3. *Skin disease of Staphylococcus aureus.*

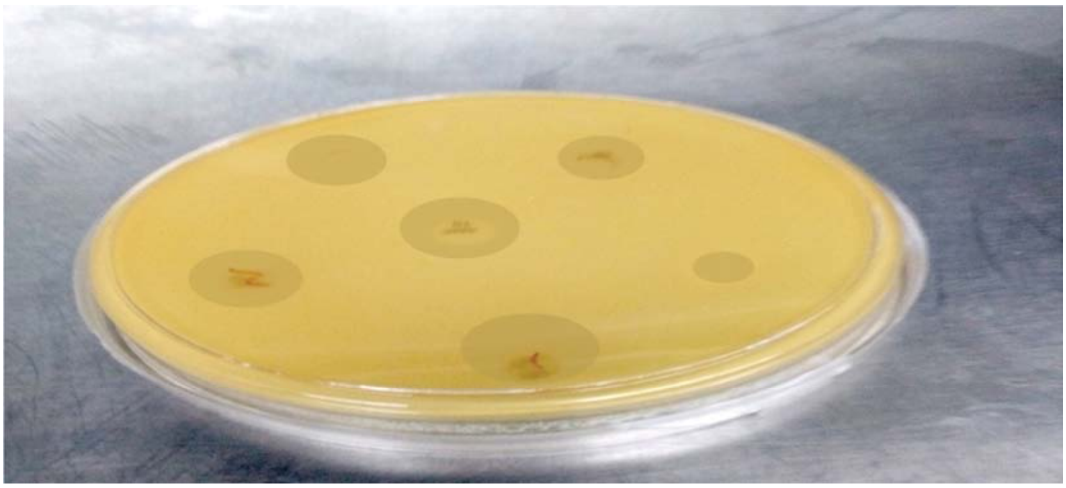

Figure 4. *Zone of copper neem soap thiourea complex for Staphylococcus aureus.*

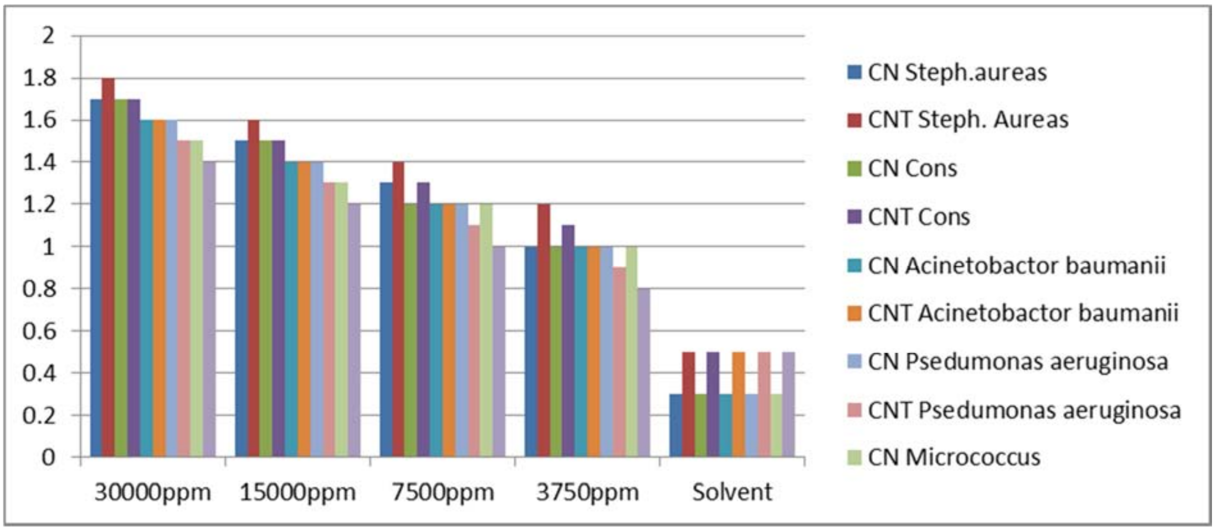

Figure 5. *The Plot data for copper (II) soap and its complex derived from neem oil.*

4. Conclusions

Copper neem soap and its complex has been synthesized and evaluated for their anti-microbial activity against Gram-positive and Gram-negative bacteria. The synthesized compound was confirmed by IR, NMR and ESR spectra. Further evaluation of antimicrobial activity was carried out for the synthesized molecule and it was found active against skin disease causing bacteria. Many of pharmaceuticals and related chemicals, however, are toxic to humans and hard to degrade within the environment. Biomedical and bio-degradable products will be in the near future largest application of antimicrobial studies, which must be the area of interest for the scientists to explore the new drug formulation.. The shortcomings of antiseptic soap (treated with synthetic antiseptics) and other non biodegradable chemicals generate the need to develop more environment friendly Pharmaceuticals and other biologically active molecules.

Acknowledgements

The authors are thankful to UGC for JRF and Principal and Head of Department (SPC Govt. College, Ajmer) for laboratory facilities. We are also thankful to Dr. P. K. Khatri (Head of Department Microbiology, Dr. S. N. Medical, Jodhpur) Dr. Archana Bora and Dr. R. S. Parihar for their kind cooperation. We are also thankful to Saif CDRI Lucknow and Saif IIT Bombay for NMR and ESR Spectra.

References

[1] R. Sharma and S. Khan, RPMP Natural Products, 18 (2007) 429.

[2] M. R. K. Sherwani, R. Sharma, A. Gangwal and R. Bhutra, *Indian J. Chem.,* 42A (2003) 2527.

[3] N. Singh, N. K. Sangwan and K. S. Dhindsa, Pest Management, Sci. 56 (2000) 28.

[4] G. N. Mukherjee and A. Das, *J. Indian Chem. Soc.,* 78 (2001) 78.

[5] C. O. Ebongue, C. M. Nguatcheussi, J. P. N. Mefoo, J. Bollanga, D. Adiogo, H. N. Luma, Scientific Research Publishing, 8 (2018) 125-137.

[6] Alhazmi, H. A. Sci. Pharm. 87 (2019) 5.

[7] Kodzius R, Gojobori T. Mar Genomics 24 (2015) 21 -30.

[8] Masalha M, Borovok I, Schreiber R, Aharonowitz Y, Cohen G Journal of Bacteriology. 183 (24) (2001) 7260–7272.

[9] Pfaller MA, Herwaldt LA. Clin. Microbiol. Rev. 1 (1988) 281–299.

[10] Antunes, LCS; Visca, P; Towner, KJ Pathogens and Disease. 71 (3) (2014) 292–301.

[11] William Riley. In: Topley & Wilsons Microbiology & Microbial infections, 10th Edition Editors: S. Peter Borriello, PatrickMurray, Guido Funke. 2 (2005) 1301-1305.

[12] Madigan M; Martinko J, eds. Brock Biology of Microorganisms (11th ed.), (2005).

[13] T. V. Sivanandan, Minister: India Close to Setting U Petrochemicals Complex in Iran, (2016).

[14] Silver, S.: Membrane and Transport, Vol 2, edited by A. N. Martibisum 115 (1982) 7.

[15] Ojha, K. G., Jaisinghani, N. and Tahiliani, H.: *J. Indian Chem. Soc.* 79 (2002) 191.

[16] Sharma, V. K. and Shrivastava, S.: *Indian J. Chem.* 45A (2006) 1368.

[17] Basset, J., Denney, R. C., Jeffery, G. H. and Mendham, J,: Vogels Textbook of Quantitative Inorganic Analysis. 4th ed. Longman, London (1985).

[18] Riddick, J. R. and Bunger, W. B.: Organic Solvents: Physical Properties and Methods of Purification. Wiley Interscience, New York (1970).

[19] Gunstone, F. D.: An Introduction to the Chemistry of Fats and Fatty Acids. Chapman and Hall Ltd., London (1958).

[20] D. A. Kalashnikov, S. H. Tan, M. V. Chekhova, and L. A. Krivitsky, 19 (2011) 9352.

[21] D. J. Mac. Lowry, M. J. Jaqua, and S. T. Selepak, Appl. Microbiol., 20 (1970) 46-53.

[22] Portillo, R. Vila, B. Freixa, T. Adzet and S. Can-igueral., J. Ethnopharmacol., 76 (1) (2001) 93-98.

[23] Silverstein, Bassler, Morrill: Spectrometric Identification of Organic Compounds. 5th ed John Wiley and sons. Inc.(1991).

[24] Arun K. Singh, Raj K. Singh *J Surfact Deterg* 15 (2012) 399–409.

[25] Joseph J and Boomadevi JG: *Journal of Material and Environment Science* 5 (3) (2014) 693-704.

[26] Lindsey A. Torre, Britton Trabert, Carol E. Desantis, Kimberly D. Miller, GoliSamimi, Carolyn D. Runowicz, Mia M. Gaudet, AhmedinJemal and Rebecca L. Siegel, Ovarian cancer statistics, (2018).

[27] Sinkou H, Hurevich H, Rusovich V, Zhylevich L, Falzon D, de-Colombani P, *et al. EurRespir J.;* 49 (2017).

[28] Adams, R.: Organic Reactions. John Wiley and sons, New York 3240, (1959).

[29] Mehta, V. P., Hasan, M., Mathur, S. P. and Rai, G. L.: *Tenside Surf. Det.* 16 (1979) 2.

[30] Manhas, B. S., Kalia, S. B. and Sardana, A. K.: J. *Indian Chem. Soc.* 83 (2006) 652.

[31] Reddy, R. K., Reddy, K. M. and Mahendra, K. N.*: Indian J. Chem*. 45A (2006) 377.

[32] Lal, R. A., Basumalary, D., Chakraborty, J., Bhaumik, S. and Kumar, A.: *Indian J. Chem*. 45A (2006) 619.

[33] Patel, R. N., Singh, N. and Gundla, Y. L. N.: Indian J. Chem. 45A (2006) 614.

[34] Mijanuddin, M., Gangopadhyay, S., Mondal, S., Mukharjee, M. and Ali, M.: *Indian J. Chem.* 45A (2006) 858.

[35] Ali, A., Salunke, S. Gawali, Rao, P. and Linares, J.: *Indian J. Chem.* 45A (2006) 853.

[36] Erden, I., Demirhan, N. and Avciata, U.: *Indian J. Chem.* 45A (2006) 1395.

[37] Yamada, S.: Coord Chem. Rev. (1996) 415.

[38] Kivelson, D. and Neiman, R.: J. Chem. Phys. 35 (1961) 149.