International Journal of Scientific Research in Science, Engineering and Technology

Print ISSN - 2395-1990
Online ISSN : 2394-4099

OPEN ACCESS

Available Online at : www.ijsrset.com
doi : https://doi.org/10.32628/IJSRSET2411245

Characterization, Comparative Assessment and Antibacterial Potential of Copper(II) Soya Complexes against *Staphylococcus Aureus*

Dr. Vandana Sukhadia

Department of Chemistry, S.D. Govt. College, Beawar , MDSU Ajmer, Rajasthan, India

ARTICLEINFO

Article History:

Accepted: 05 April 2024
Published: 20 April 2024

Publication Issue :
Volume 11, Issue 2
March-April-2024
Page Number :
346-354

ABSTRACT

Copper(II) soap complexes have been proven their activity against bacteria very effectively. Herein, the influence of biophysical and biomechanical parameters on the activity of Copper(II) soya thiourea complex was evaluated. To this aim, liquid as well as solid growth media were developed by Kirby-Bauer disc diffusion method. The antibacterial activity of Copper(II) soya thiourea complex against the Gram-positive bacterium *Staphylococcus aureus* was assessed in various concentration of Copper(II) Soya complexes. Copper (II) Soya complexes also resist bacterial growth at higher concentration. This review provides a board overview of *Staphylococcus aureus* with an emphasis on the Copper(II) soya thiourea complex

Keywords : Copper (II) Soya complexes, photocatalytic degradation, *Staphylococcus aureus*, antibacterial activity, inhibition zone, Kirby-Bauer disc diffusion method

I. INTRODUCTION

Staphylococcus aureus is Gram-positive bacteria (stain purple by Gram stain) that are cocci-shaped and tend to be arranged in clusters that are described as “grape-like.” The antimicrobial properties of Copper have been identified for a lot of years while it present as a metal ion or as in complex form.

The bacteriostatic consequences of Copper was reported by Dr. Phyllis J. Kuhn [1], who investigated that bacterial augmentation on Copper and brass strips showed slight or no growth, whereas the aluminum and stainless steel strips produced a intense growth of all the different types of microorganisms.

The bacteriostatic efficacy of Copper worn in paints to render surfaces self-disinfecting has also been confirmed.[2] A platform technology in textile industries has recently been developed to binds Copper ion with fibres[3] which may boast a considerable ramifications.

It has been suggested that in some compounds, microbiocidal effectiveness is greatly enhanced by small amounts of Copper which form chelates. Copper sulphate has been found to be a compelling molluscicide [4] and mainly used in the production of agricultural fungicides and wood preservatives. Copper naphthenate is as well used in efficient control of decompose fungi and excellent manage or

improvement of wood destroying insects like termites, beetles carpenter ants and other host organisms.

In fact Copper (II) chelates have been initiate to cooperate with organic systems and to illustrate anti-neoplastic activity [5-7], anticancer activity [8] and antibacterial, antifungal.[9,10]Several Copper(II) N,S,O/N,N donor chelators are excellent anticancer agents since they have tough binding capability with DNA base pair. [11]

In recent years various electroplated coating like Copper ,Zinc, cobalt etc. are examined to inhibit the growth of pathogenic bacteria(Enterococcus faecal sand methicillin-resistant S. aureus E. coli, Pseudomonas aeruginosa and Kl e b s i e l l apneumonia), they may be gram-positive bacteria and gram-negative bacteria.[12]Likewise, it was found that the antibacterial potency of numerous compounds is considerably higher when they form a complex with Copper. [13,14]

For the research of bactericide to be studied, edible oil such as Soyabean oil were used as unprocessed objects due to its easy availability, specific medicinal properties and biodegradability.

Hence, in this study we report the synthesis of Copper(II) soap complex (Copper (II) Soya complexes) which are proceed like an organic agent to control the growth of *Staphylococcus aureus*. The obtained Cu (II) complexes were tested *in vitro* against the bacterial strains *S. aureus*, which is some of the main pathogens causing nosocomial infections worldwide.

II. METHODS AND MATERIAL

2.1. Chemicals

2.1.1. Copper (II) soap complexes and Benzene

Copper(II) Soya complexes like Copper(II) Soya Urea (CSU) and Copper(II) Soya Thiourea (CST) have been synthesized in laboratory and discussed in previous study. (CPC refe.) Pure benzene is used as solvent. Purification of benzene is discussed earlier.

2.1.2. Preparation of Copper (II) soap complexes solutions

The complexes are stable in atmospheric conditions and soluble in benzene. Both the complexes were analyzed for elemental analysis. Benzene is used for solution preparation and further dilution. All the Copper (II) Soya complexes are dissolved in Benzene. After dissolving in benzene final volume for all the complexes (CSU, CST) were 1 ml.

Two different dilutions were made from stock solution to get antimicrobial activity of complexes, two different dilutions C_1(50mg/ml) and C_2(25mg/ml) were made to get antimicrobial activity of all complex against *Staphylococcus aureus.* Streptomycin (300mcg/ml) was used as positive control for all complexes300mcg/ml. Composition of stock solution of complexes are shown in Table -1

Table – 1. Composition of stock solutions for Copper (II) Soya (CSU and CST) complexes.

S. No.	Name of the complex	Required concentration	Extract	Benzene	Final Volume
1.	CSU	50mg/ml	50 mg	1 ml	1ml
2.	CST	50 mg/ml	50mg	1ml	1ml

Table -2 Zone of inhibition for Copper(II) Soya (CSU and CST) complexes at two different concentrations against *Staphylococcus aureus.*

S. No.	Copper(II) soap complexes	PC	C_1(50mg/ml)		C_2(25mg/ml)		NC
			24hrs	48hrs	24hrs	48hrs	
1.	CSU	31mm	14mm	14mm	14mm	14mm	NZI
2.	CST	31mm	9mm	9mm	9mm	9mm	NZI

PC- Positive Control,
NC – Negative Control,
C_1 and C_2-two different concentrations of Copper (II) soap complexes,
NZI – No Zone of Inhibition

2.1.3. Media

Mueller-Hinton Agar plates and Peptone water

MHA plate is frequently used for the routine antimicrobial susceptibility testing of non-fastidious microorganism by the Kirby-Bauer disk diffusion technique. It is a standard, non-selective and non-differential medium for the Bauer Kirby method. It is a loose agar which provides a better diffusion with truer zone of inhibition for the antibiotics than most other plates.

MHA media contains beef extract, acid hydrolysate of casein, starch and agar. **Beef extract** and **acid hydrolysate of casein** supply all the essential nutrients such as Nitrogen, vitamins, carbon, amino acids, Sulphur. **Starch** used as absorber of any toxic metabolites produced as a source of dextrose, which provides energy. **Agar** is the solidifying agent.

Peptone Water is used as a growth medium and as a base for carbohydrate fermentation media.

III. EXPERIMENTAL

Kirby-Bauer disc diffusion method was operated for antibacterial study which is described here in brief.

3.1.Antimicrobial Susceptibility Testing (Kirby-Bauer disc diffusion method)

A test of the antibiotic sensitivity of bacteria is also known as Kirby–Bauer test (disc-diffusion antibiotic susceptibility test, disc-diffusion antibiotic sensitivity test, KB test). Mueller-Hinton agar medium was used for antimicrobial activity of given compounds on various concentrations by disk/ well diffusion susceptibility testing.

Antibiotic discs are used to test the level of bacterial resistance by preferred antibiotics. Wafers/plates containing antibiotics are positioned on an agar plate where bacteria have been sited and then the plate is moved out to incubate. There will be an region around the wafer is found, where the bacteria have not grown enough, if an antibiotic stops the bacterial growth or, kills the microbes is called a zone of inhibition.[16]

All the Copper soap complexes derived from Mustard oil and Soyabean oil were used for their antibacterial study. For this study *S. aureus* was selected as test organism. Antibacterial study was done by incorporating these steps.

3.2.Preparation of inoculums

Fresh Cultures of *Staphylococcus aureus* strain ATCC-25923were inoculated in Peptone water & kept for incubation for 30 minutes at 37°C. Inoculum size of bacteria was adjusted using McFarland turbidity standard as reference which is described in previous section. The bacterial suspensions were compared to 0.5 McFarland Turbidity Standard.

3.3.Swabbing of the liquid cultures of *Staphylococcus aureus*

Microbial cultures were swabbed onto the Mueller Hinton Agar surface through sterile Cotton swabs sticks.

3.4.Loading of different test solutions into the wells

After proper marking of plates, 0.1 ml extracts from different dilutions prepared from stock was loaded into the respective wells.

3.5.Incubation

The *Staphylococcus aureus,* plates were kept for incubation at 37°C for 24-48 hours and results were observed.

IV. RESULTS AND DISCUSSION

Herein we report an account of microbial activity of Copper (II) complexes. The growth profile of *Staphylococcus aureus* strain ATCC-25923was monitored for 24 h of incubation. Each plate was examined and observed a resultant inhibition zones which were consistently clear and spherical, there was a lucid lawn of growth of the *Staphylococcus aureus*. From the Table -**2**, it could be seen that by a clear zone of inhibition around the each plate, all the complexes confirm high inhibition at higher concentration(50 mg/ml).

At higher concentration by comparing all the complexes of Copper (II) Mustard soap, results prove that CSU resist bacterial growth efficiently. Likewise in Copper (II) Soya complexes, CST also resists bacterial growth at higher concentration. Whereas at lower concentration, differently CSU complex shows highest resistivity and CST lowest resistivity at lower concentration.[17]

CSU=CSB>CST .....(1)

As shown in Table -**2** and Figure- **1** the zone of inhibition in different type of Copper(II) soap complexes at higher concentration is represented in (1) and (2).

CSU>CSB>CST .....(2)

Figure-1 Results of antimicrobial susceptibility test of Copper (II) Soya Thiourea complex against *Staphylococcus aureus.*

As well (3) and (4) represent the zone of inhibition in different type of Copper (II) soap complexes at lower concentration. Graphical presentation of bacterial resistivity (zone of inhibition) of all complexes is shown in Figure-**2**.

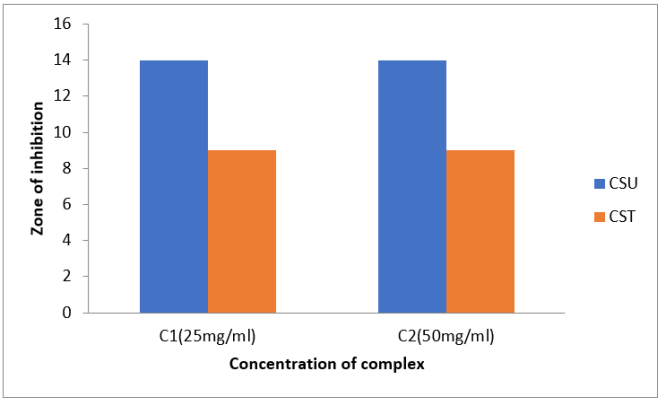

Figure-2 Zone of inhibition of Copper (II) Soya complexes (CSU and CST) against *Staphylococcus aureus.*

It is indicative that in Copper (II) Neem and Copper (II) Pongamia Benzothiazole complexes Candida species can be more susceptible and effective with increase in concentration whereas at lower concentration the inhibition of growth is less. [18]

An assessment of Table -**2** suggests to facilitate the entire complexes demonstrate higher activity due to Benzothiazole and other N,S (Urea, Thiourea) containing compounds to recover the appearance of Copper soaps complexes. The enhanced activity of newly synthesized complexes could possibly explained on the basis of incidence of donor atoms N and S as well as the structural compatibility with molecular nature of the poisonous moiety. Improved biocidal activity of complexes is in accordance with the chelation theory. According to this theory polarity of the metal atom is condensed by the partial sharing of its positive charge with donor groups and probably delocalization of π–electron above the entire ring. As a result lipophilic nature of the complex is amplified and favours the access of the complex in the course of the lipid layer of cell membrane. The molecules of the complex obstruct the metal binding sites in the enzymes of microorganisms. As a result, the complex disturbs the metabolism channel in cell and microbes die. [19]

4.1.Analysis of variance (ANOVA)

ANOVA is a compilation of statistical models and their related evaluation procedures (such as the "variation" among and between groups) used to analyze the differences among group means in a sample. The descriptive statics results of Copper (II) soap complexes are shown in Table- **3** confirm appropriate results. All the experiments were performed in triplicate and confirm the satisfactory results in triplet. [20] The results of ANOVA [21] for the antibacterial activities for Copper (II) Mustard and Copper (II) Soya complexes on two different concentrations are reported in Table -**4**.

Table-3 Descriptive statics results for antibacterial activities of Copper(II) Soya (CSU, CST) complexes.

Copper (II) soap complexes	Con. (ppm)	Count	Sum	Average	Variance	Std. Dev.	Std. Dev. Error	Coff. variance
CSU	5 x 10^4	3	42	14	0.25	0.5	0.2886	0.1666
	2.5 x 10^4	3	39	13	0.25	0.5	0.2886	0.1666
CST	5 x 10^4	3	27	9	0.75	0.8660	0.5000	0.5
	2.5 x 10^4	3	24	8	0.75	0.8660	0.5000	0.5

Table-6 ANOVA results for antibacterial activities of Copper (II) Soya (CSU, CST and CSB) complexes.

Copper (II) soap complexes	SS	Df	MS	F	P-value	F crit
CSU	1.5	1	1.5	6	0.0704	7.7086
CST	1.5	1	1.5	2	0.2303	7.7086

SS= sum of squares,

MS= mean square,

df= degree of freedom,

$p < F$(level of significance)

According to the results of ANOVA, the standard deviation has been deliberated by the predictable

evaluation of repeatability and the average was preferred as ultimate elucidation. The consequence is statistically significant, by the standards of the study, due to $F > p$.[22,23] Thiazole a heterocyclic nucleus performed a considerable pose in the succession of diverse medicinally essential moiety. They are effectively noxious to microbes mainly to fungi and bacteria. [24]

On studying the experimental data it reveal that Copper (II) Mustard Thiourea complex show highest resistivity with compare to Copper (II) Soya Thiourea complex. CSU and CSB show more reactivity than CMU and CMB complexes.

From this analysis, it is inferred that the entire Copper (II) soap complexes have higher activity than solitary ligand or individual soap, on the other hand through complexation resistivity of all complexes amplify at a level.

V. CONCLUSION

The work described here shows that the synthesized metal complexes have higher biological activities compared to free ligands. Copper (II) Soya complexes (CSU, CST) are bactericidal for *S. aureus*. Our results point out that Copper (II) soap complexes may be appealing to release novel paths against different bacterial genus since a few of these complexes have been found to reveal an outstanding antibacterial activity. Copper (II) complexes show higher resistivity at higher concentration. Thus, it is evident that concentrations enclose an essential position in increasing the extent of inhibition. By formation of chelate, complexes enhance their activity towards S. aureus bacteria and so the vicinity is still untie for advance-research.

VI. Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

VII. Conflict of interest

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

VIII. Acknowledgements

The authors pay their sincere gratitude to Dr. Lal Clinical laboratory Pvt. Ltd., Malviya Nagar, Jaipur for providing analytical data and Principal, S.D.Govt College Beawar and Principal, S.P.C Govt College Ajmer for laboratory facilities.

IX. REFERENCES

[1]. P. J. Kuhn, Doorknobs: a source of nosocomial infection? Diagnostic Medicine,1983.

[2]. T. E. Cooney, Bactericidal Activity of Copper and NonCopper Paints, Infect.Control Hosp. Epidemiol, 1995, 16(8), 444-450. doi: 10.1086/648361.

[3]. G. Borkow, Gabbay, Putting Copper into action: Copper-impregnated products with potent biocidal activities,FASEB Journal, 2004, 18(14), 1728-1730.

[4]. A. R. Manley ,The effects of Copper on the behaviour, respiration, filtration and ventilation activity of Mytilusedulis, JMBAUK, 1983,63(1),205-222. doi: https://doi.org/10.1017/S0025315400049900

[5]. D. T. Minkel,C.H.Chanstier, D. H. Petering, Reactions of 3-Ethoxy-2-oxobutyraldehyde Bis(N4-dimethylthiosemicarbazonato)- Zinc(II) withtumor cells andmitochondria, MolecularPharmacology, 1976, 12(6),1036–1044.

[6]. V. Rajendiran, R. Karthik, M. Palaniandavar,Mixedligand Copper(II)-phenolate complexes: effect of coligand on enhanced DNA and protein binding, DNA cleavage, and anticancer activity, Inorganic Chemistry, 2007,46(20), 8208–8221.

[7]. A. E. Liberta , D. X. West, Antifungal and antitumor activity of heterocyclicthiosemicarbazones and their metal complexes:current status, Biometals, 1992, 5(2), 121–126.

[8]. C. Marzano, M. Pellei, F. Tisato, C. Santini, Copper complexes as anticancer agents, Anti-Cancer Agents in Medicinal Chemistry, 2009, 9(2), 185–211.

[9]. Y. Harinath, D. H. K. Reddy, B. N. Kumar, C. Apparao, K. Seshaiah, Synthesis, spectral characterization and antioxidant activity studies of a bidentate Schiff base, 5-methyl thiophene-2- carboxaldehyde-carbohydrazoneand its Cd(II), Cu(II), Ni(II) and Zn(II) complexes, SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy, 2013, 101, 264–272.

[10]. J. Sheikh,H. Juneja, V. Ingle, P. Ali, T. B.Hadda, Synthesis and in vitro biology of Co(II), Ni(II), Cu(II) and Zinc(II) complexes of functionalizedbeta-diketone bearing energy buriedpotential antibacterial and antiviral O,O pharmacophore sites, J. Saudi Chem. Soc., 2013, 17(3), 269–276.

[11]. S. M. Saadeh, Synthesis, characterization and biological properties of Co(II), Ni(II), Cu(II) and Zn(II) complexes with an SNO functionalized ligand, Arab. J. Chem., 2013, 6(2), 191–196.

[12]. Z. H. Zhao, Y. Sakagami ,T.Osaka, Toxicity of hydrogen peroxide produced by electroplated coatings to pathogenic bacteria. Can. J. Microbiol.,1998, 44, 441-447.

[13]. Z. H. Chohan, H. Pervez, A. Rauf, A. Scozzafava, C. T. Supuran, Antibacterial Co (II), Cu (II), Ni (II) and Zn (II) complexes of thiadiazole derived furanyl, thiophenyl and pyrrolyl Schiff bases, Enzyme Inhib. Med. Chem.,2002, 17, 117-122.

[14]. D. K. Sau, R. J. Butcher, S. Chaudhuri, N. Saha,Synthesis and spectroscopic characterization of new cobalt(III) complexes with 5-methyl-3formylpyrazole 3-hexamethyleneiminyl thiosemicarbazone (HMPz3Hex): X-ray crystallographic identification of HMPz3Hex about the azomethine double bond of the ligand on complexation with cobalt(III), Polyhedron,2004,23(1),5-14.

[15]. V.Sukhadia , R. Sharma,A. Meena, Study of Photocatalytic degradation, Kinetics and Microbial Activities of Copper (II) Soya Urea Complex in Non Aqueous Media. Letters in Organic Chemistry, 2021, 18, 912-923.

[16]. D F Brown, D. Kothari, Comparison of antibiotic discs from different sources, J. Clin. Pathol. 1975, 28 (10), 779–783. doi:10.1136/jcp.28.10.779. PMC 475859. PMID 1214010

[17]. R. Subramaniyam, F. Aafreen , R.R. Jonnalagadda, U.N. Balachandran , Antibacterial activity of Copper(II) complexes against Staphylococcus aureus, RSC Adv., 2014, 4, 32004–32012.

[18]. S. Khan, R.Sharma, A.K. Sharma, Antifungal Activities of Copper Surfactants derived from Neem (Azadirecta indica) and Karanj (Pongamiapinnata) Oils: A Pharmaceutical Application, Glob J Pharmaceu Sci. ,2017, 3(4), 555616.

[19]. S. Chandra , D. Jain , A. K. Sharma ,P. Sharma, Coordination Modes of a Schiff Base Pentadentate Derivative of 4- Aminoantipyrine with Cobalt(II), Nickel(II) and Copper(II) Metal Ions: Synthesis, Spectroscopic andAntimicrobial Studies , Molecules ,2009, 14, 1 74-190.

[20]. H.H. Horowitz, A. Metzger, New analysis of thermogravimetric traces. G. Anal. Chem., 1963, 35(10), 1464-1468. http://dx.doi.org/10.1021/ac60203a013

[21]. A. Broido, A simple, sensitive graphical method of treating thermogravimetric analysis data, J. Polymer Sci., Part B-2, 1969, 7(10), 1761-1773. http://dx.doi.org/10.1002/pol.1969.160071012

[22]. R. Bhutra, R. Sharma, A. K. Sharma, AntimicrobialStudies and Characterization of

Copper SurfactantsDerived from Various Oils Treated at High Temperaturesby P.D.A. Technique. Open Pharm. Sci. J.,2018, 5, 36. http://dx.doi.org/10.2174/1874844901805010036

[23]. N. Mathur, N.Jain, A. K. Sharma, Biocidal activities of substituted Benzothiazole of Copper surfactants over candida albicans & trichoderma harziamunon on muller hinton agar. Open Pharm. Sci. J., 2018, 5, 24-35. http://dx.doi.org/10.2174/1874844901805010024

[24]. P.Tank, R.Sharma, A. K. Sharma, A Pharmaceutical Approach & Antifungal Activities of Copper Soaps with their N & S Donor Complexes Derived from Mustard and Soya bean Oils, Glob J Pharmaceu Sci. 2017,3(4), 555619. doi: 10.19080/GJPPS.2017.03.555619