Synthesis, Photophysical Property and Antibacterial Activity of Cu(II) and Co(II) Porphyrins: Role of Ligand Modification

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Research article

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Abstract

Many synthetic porphyrins are modified at the meso-position to achieve target properties in biomedical applications. The metalloporphyrins; CuTPP, CuTPPCOOMe, CuTPPNH 2 , CoTPPOMe and CoTPPCOOH were synthesized from their free base porphyrins and characterized by spectroscopic techniques. The Uv-Vis spectra show the decrease in number of Q bands from four to one/ two peaks and a blue shift when the metal is inserted in to the porphyrin core. They are capable of effecting bacterial and viral pathogens through the large number of different mechanisms. The antibacterial activity of the metalloporphyrins and their ligands were evaluated by using two gram positive and two gram negative bacteria by disc diffusion method. Among the complexes under study, CoTPPCOOH showed highest inhibition zone (15.5-16.5) vs 25-28 for the famous standard antibacterial drug and therefore, highest antibacterial activities as compared to other porphyrins. The study also indicates that metalloporphyrins with electron withdrawing groups have highest inhibition zones than complexes which possess electro donating group at para position of the meso-phenyl ring.

Introduction

The growth of infectious diseases and resistance of various antibiotics to such sever infectious diseases is one of the most serious public health concern globally. Taking into account a great number of infections resulting from different bacterial species, the development of compounds with high antibacterial activities with novel mechanism of action is an urgent need. As a consequence, researchers are designing a novel, convenient, robust and inexpensive strategies for combating microorganisms with minimal invasive consequences.[1, 2] Natural and synthetic porphyrins are among relatively low toxic molecules (either in vitro and in vivo) and are capable of effecting microbial and viral pathogens through the large number of different mechanisms.[3] In addition, their ability for numerous chemical modifications place porphyrins into a group of compounds that present an outstanding source for discovery of novel procedures, materials and agents active against a wide range of pathogenic microorganisms.[3] Moreover, porphyrins, metalloporphyrins, and related molecules have assumed extraordinary importance in recent years as the opportunities for using these compounds in photodynamic therapy, optoelectronic devices, sensors, molecular logic devices, and artificial solar energy harvesting and storage schemes have become apparent. Studies of the spectroscopy and photophysical dynamics of these compounds and their multichromophoric derivatives have grown exponentially in number in the last 10 years.[4] During this period, increasing attention has been paid to the behavior of higher electronic states accessed by pumping in the strong Soret absorption band located in the violet region.[5] Most of the porphyrins show two sets of distinct region or bands in their electronic absorption spectrum. The range of the first and second band sets are 350-500 nm and 500-750 nm respectively. The first set of bands (*Soret*- or B-band) with molar absorption coefficient of 10^5 M^{-1} cm^{-1} and involves the electronic transitions from the ground state to the second single excited state( ).The second set of band(the *Q-band*) with molar absorption coefficient of 10^4 M^{-1} cm^{-1} and involves the transition from the ground state to the first singlet excited state( ). The conjugation of 18-π electrons gives advantageous spectroscopic features to porphyrins,

which is supportive to monitoring the binding of diverse hosts to porphyrin by using UV-Visible spectroscopy. [6-8] The UV-Visible absorption spectrum is an important spectral phenomenon to distinguish between the free-base porphyrins and their metalloporphyrin derivatives. The spectrum changes from four-band to a two-banded spectrum on metallation. This dramatic effect is attributed to the enhancing of the D_2h symmetry of the free-base porphyrin to D_4h on metallation. [9]

Structurally, porphyrins have a planar aromatic structure with extended and conjugated 22 π-electron system and a size-limited coordination cavity for binding of different metal ions.[10,11] They are the strongest light-absorbing materials in nature, therefore are called as “the pigments of the life”.[6,12,13] The introductions of suitable substituents at the peripheral positions of the porphyrin core provokes tunable shape, size and symmetry and are found in material and therapeutic applications.[7] Many synthetic porphyrins are modified at the meso-position to achieve target properties in biomedical applications involving photo diagnosis and cancer therapy, with the most dominant use in photodynamic therapy (PDT).[15] The increase in the mortality rate linked with infectious diseases is directly related to microorganisms that demonstrate multiple resistances to antibiotics. The lack of effective treatments is the main cause of this problem. Hence, development of new antibacterial agents with novel and more efficient mechanisms of action is an urgent medical need. To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds against new targets is a matter of urgency. Based on this, pharmaceutical industries are looking for synthesizing alternative compounds to medicinal preparations originating from wild growing plants. However, plant based drugs have shortened the life span of the source (plants and animals). To alleviate these problems, synthesis and investigation of different metal complexes have been carried out and promising results are being found. The actions of these complexes against the microbes were reported to be via non covalent interactions with the DNA.[16-20] In addition to the non-covalent interactions with the DNA, the enhanced activity of the complex against the microbes is due to covalent interactions. In this work we, therefore report synthesis, characterization, photophysical and antibacterial activity study of Cu (II)-5, 10, 15, 20–Tetrakis-(para-X phenyl) porphyrins, where X= H, NH2 and COOMe and Co (II)-5, 10, 15, 20–Tetrakis-(para-X phenyl) porphyrins, where X= COOH and OMe). Because of teradentate nature of anionic porphyrinato ligand, in metalloporphyrins the minimum possible coordinatin number of metals is four, a divalent metal ion giving a neutral complex with square planer geometry. Porphyns as ligands have the ability to stabilize different metal ions in their unusual oxidation states.[21-23]

Results And Discussions

UV–vis absorpition: It is known that absorption of free-base porphyrins and as synthesized metal complexes (metalloporphyrins) featured by two characteristic types of bands and are shown in Fig. 9. The λ_{max} values of these species are reported in Table 1 and compared to Co and Cu metalloporphyrins

Figure 1. UV–Vis absorption spectra of a) H_2TPP and CuTPP b) $H_2TPPCOOMe$ and

TPPCOOMe C) H_2TPPNH_2 and $CuTPPNH_2$

In all cases the absorption wavelength of copper metalloporphyrin is lower than that of the free base porphyrins. The number of Q bands decreases from four to a single band when going from the free base porphyrin to their metal complexes, CuTPP and CuTPPCOOMe, as well as four to two for the metal complex $CuTPPNH_2$. The decreases in number of Q bands and a shift on wavelength indicate the insertion of the copper in to the porphyrin cores. The Soret band is blue shifted up to 8 nm for CoTPPOMe and 7 nm for CoTPPCOOH, as well as 26 and 27 nm Hypsochromic shift for the Q band of CoTPPOMe and CoTPPCOOH from their free base porphyrins respectively. As the same as to copper metalloporphyrin, the decreases in the number of Q bands from four to a single band when going from the free base porphyrin to its cobalt complex indicates the insertion of the cobalt in the porphyrin core of $H_2TPPCOOH$ and $H_2TPPOMe$ and forms CoTPPCOOH and CoTPPOMe respectively. The Soret band is blue shifted up to 8 nm for CoTPPOMe and 7 nm for CoTPPCOOH, as well as 26 and 27 nm Hypsochromic shift for the Q band of CoTPPOMe and CoTPPCOOH from their free base porphyrins respectively.

Figure 2.UV–Vis absorption spectra of: a) $H_2TPPOMe$ and CoTPPOMe; b) $H_2TPPCOOH$ and CoTPPCOOH

Role of ligand substitution on UV–vis spectra: The absorption band of para-substituted CuTPPNH2 shows red-shift when compared with Cu-TPP and CuTPPCOOMe, suggesting the electronic effects from the electron releasing group NH_2. $CuTPPNH_2$ shows higher red-shift than Cu-TPP but CuTPPCOOMe shows lower blue shift than Cu-TPP due to the steric effect of methoxycarbonyl group in CuTPPCOOMe. CoTPPOMe shows a red shift when compared to CoTPPCOOH by 8 nm at the sort band and 12 nm at the Q band, which was also similar result in the free base porphyrins(shown in appendix A), suggesting the electronic effects from the electron releasing -OMe (methoxy) group. For clarity the UV-Vis data of porphyrins and metalloporphyrins are shown in supporting information (**Table SI-1**).

Figure 3.UV–Vis absorption spectra of: a) Copper complexes, b) Cobalt complexes

Mass spectrometry

The expected structure of all free base porphyrins had been confirmed by using mass spectrometry and the data is shown in **Table SI-2**. The H_2TPPNH_2 exhibited the molecular ion peak at m/z 676.54, as a major peak. The mass spectra of other porphyrin ligands are shown in Appendix. The mass spectra for all

show very intense molecular ion peak due to a great deal of energy requirement for fragmentation of porphyrins. Therefore, fragmentation of porphyrin structure was not observed at all and the molecular ion peak $[M+H]^+$ were observed. The ESI-Mass of CoTPPCOOH is displayed in Figure 11 and Appendix. The strong molecular ion peak $[M+H]^+$ at m/z 848.91 is assigned for $[CoTPPCOOH+H]^+$. In similar fashion, the mass spectra of all metalloporphyrins showed very intense molecular ion peaks due to the high energy from the delocalized electrons in the molecule. The mass spectra have been successfully received to confirm the expected corresponding structure of porphyrins as well as metal complexes.

Figure 4. The mass spectrum of cobalt-5,10,15,20-tetrakis(4-carboxyphenyl) porphyrin

Antimicrobial Test Results

The compounds were tested for their in vitro antibacterial activity and were compared with the commercially available drug, gentamicin. They were tested against two Gram-positive (S. aureus and S. pyogenes) and two Gram-negative (E. coliandK. pneumoniae) bacteria. All the tasted metalloporphyrins were found active against all the tested pathogens. The commercial antibiotic drug(gentamicin) exhibited highest activities with inhibition zones ranging from 25 mm to 28 mm in all the four pathogens. The results of antimicrobial activities of the study compounds are reported as inhibition zone of diameter (mm) are showed in **Table 1**.

Table1. Antibacterial activity of Metalloporphyrins, Metal salt, and reference antibiotic drug gentamicin

Compounds	Antibacterial activity (mean IZ diameter(mm) ± SD)			
	S. aureus	S. pyogenes	*E. coli*	*K. pneumonia*
CoTPPCOOH	**16.5**	**15**	**16**	**16**
CoTPPOMe	**12**	**14**	**10**	**12**
CuTPPCOOMe	**16**	**15**	**13**	**13.5**
$CuTPPNH_2$	**13**	**13.5**	**12**	**12.5**
Co acetate	6.5	7	6.5	6.5
$CuCl_2.\ 2H_2O$	6.75	7	6.25	6.5
DMSO	0	0	0	0
Gentamicin	25	27	26	25

The synthesized compounds (metalloporphyrin) showed higher activities than free base porphyrins (ligands) against all bacteria's. This indicates that reaction of metal ions with the ligands plays an important role in antibacterial activity. This increased activity of metal complex can be explained on the basis of the overtone concept and chelation theory. According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials in which lipo-solubility is an important factor that controls the antimicrobial activity.[24] On chelation, the polarity of the metal ion will be reduced to a greater extent due to overlap of ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Furthermore, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of complexes. It is likely that the increased lipo solubility of the ligand upon metal complexation may contribute to its facile transport into the bacterial cell which blocks the metal binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism.[25]

Figure 5. Bar graph of CuTPPCOOMe and $CuTPPNH_2$ on the same concentrations

Figure 6. Bar graph of CoTPPCOOH and CoTPPOMe on the same concentrations

As we saw in the table and the bar graphs, the antibacterial results shows that the complexes were active and inhibit bacteria's even at the lowest concentration (31.25 mg/L).The complex CoTPPCOOH exhibited the greater antimicrobial activities than other metallporphyrins with inhibition zones 16.5 mm for *S. aureus.*

Figure 7. Gram positive Bacteria's, S. aureus and S. pyogenes*in CoTPPCOOH molecule*

Figure 8. Gram negative Bacteria's, E.*coli* andK. pneumonia*in CoTPPCOOH molecule*

Generally, as we saw in the bar graphs the metal complexs containing electro withdrawal group, CuTPPCOOMe and CoTPPCOOH showed better activities than the metal complex containing electro donating groups namely CuTPPNH_2 and CoTPPOMe.

Conclusion

In this report, metalloporphyrins were prepared from free base porphyrins and metal salts. The synthesized complexes were then purified by column chromatography and characterized by using Uv-Vis and mass spectrometry. Based on the UV-Vis data the *soert and Q* bands of metalloporphyrins showed a blue shift when Cu(II) and Co(II) is inserted in to their free base porphyrins. Also when the metal is inserted in to porphyrin ligand the Q bands are decreased from four to either one or two, which is an evidence for the formation of metalloporphyrin. Furthermore, the ESI-Ms spectrum indicates metalloporphyrins showed very intense molecular ion peaks due to delocalized electrons in the molecule. The antibacterial activity test revealed that the metalloporphyrins which possess electro withdrawing groups at para-positions have higher antibacterial activity than metalloporphyrins which possess electron donating group at para positions.

Declarations

Ethics approval and consent to participate

Since this study doesn't involve animals or human tissues, a statement on ethics approval is ***not applicable***.

Consent for Publication

Our manuscript does not contain data from any individual person; so consent for publication is "Not applicable" in this section.

Availability of data and material

All data generated or analyzed during this study are included in this manuscript and its supplementary information files.

Competing interests

We declare that no any competing interest at all.

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Authors' contributions

Getaneh A. did the synthesis of molecules (metalloporphyins only)

Belete B. synthesized the ligands, analyzed and interpreted the spectroscopic and Mass data, organized data and completed write-up of the manuscript

Authors' information (optional)

Dr. Belete B. is an expert in porphyrin chemistry and has published about 18 papers in international peer reviewed journals, currently serving as assistant professor and head of chemistry department in Bahir Dar University. He has international experience as Phd fellow (2011-2016), Postdoctoral researcher (2016-2017), visiting assistant professor.

Mr. Anteneh A. is Young and energetic lecturer at Haromaya University in Ethiopia.

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References

1. Gholamreza, K.; Saeed, K.; and Asghar, N.; New Aminoporphyrins Bearing Urea Derivative Substituents: Synthesis, Characterization, Antibacterial and Antifungal Activity; Brazilian Archives of Biology and Technology, 2015,58(3): 431-442 2. Alenezi, K.;Tovmasyan, A.;Batinic-Haberle, I.;Benov, L.T.; Optimizing Zn porphyrin-based photosensitizers for efficient antibacterial photodynamic therapy. Photodiagnosis and photodynamic therapy, 2017, 17:154-159. 3. Amarnath, V.; Kranthi, K. G.; and Patri, S. V.; Synthesis of novel porphyrin-based lipids and their antibacterial activity; Medicinal chemistry research, 2011, 20:1068–1073. 4. Sujata,K.;Investigations on Substituted Porphyrins and their Axially Ligated Metal

Derivatives, Indian Journal of Chemistry, 2014,32:446. 5. Jerzy, K.; Dorota,K.;Adam, L.;Andrzej, M.; and Ronald,P.; Photophysical Studies of Porphyrins and Metalloporphyrins; J. Phys. Chem. A,2004,108:570-575 6. Valicsek, Z.;Horváth, O.; Application of the electronic spectra of porphyrins for analytical purposes: The effects of metal ions and structural distortions. Microchemical Journal. 2013 1:47-62. 7. Kadish, K. M.; Smith, K. M.; Guilard, R.; The Porphyrin Handbook, Academic Press, Burlington, 1999 8. Hodgson, M.J.; Preparation structure and spectra of meso-metalloporphyrins,2005 9. Sujata, k.;Investigations on Substituted Porphyrins and their Axially Ligated Metal Derivatives,Journal of Polymer Science,2014 10. Hambright, P.; The coordination chemistry of metalloporphyrins. Coordination Chemistry Reviews.1971, 6:247-268. 11. Berezin, B. D.; Karmanova, T. V.; Gromova, T. V.; Syrbu, S. A.; and Semeikin, A. S.; Study of Extra Coordination of Bromo-Substituted Porphyrins to Organic Bases; Russian Journal of Coordination Chemistry , 2002,28(9): 60–63. 12. Mitchell, R.T.; Synthesis of water soluble porphyrins and their applications; University of Wollongong research online, 2016:154-216. 13. Gilchrist T.L.; Heterocyclic Chemistry.; Longman Scientific and Technical John Wiley and Sons .Inc., New York; 2nd ed;1972. 14. Kooriyaden, F.;Subramaniam, S.;Chellaiah, A.; Synthesis, spectral, structural and antimicrobial studies of fluorinated porphyrins; Polyhedron,2015,97:66-74. 15. Alexandra, B. O.; and Harold ,S. F.; Effects of substituents on the photophysical properties of symmetrical porphyrins; Dyes and pigments,2013,96:440-448 16. Rothemund, P. J.; Am. Chem. Soc. 1939, 61, 912. 17. Parul, A.;syntheses of meso-substituted porphodimethenes and porphyrins with exocyclic ring systems; Journal of the American Chemical Society,2003 18. Geier, R. G.; Lindsey, J. S.;J. Chem. Soc., Perkin Trans. 2001, 2:687. 19. Dame, S. D.; and Emiliano,R.V.; Antimicrobial resistance in search of a collaborative solution, 2013, 49:394–400. 20. Gang, Z.; Qing S, S.; Xiaomo, H.; and Xiao, B. X.; Int. J. Mol. Sci. 2015,16, 11-33 21. Hoard, J.L.; Smith, K.M; Porphyrins and Metalloporphyrin; Elsevier, 1975:317. 22. Lee, C. H.; and Lindsey, J. S.;. One-Flask Synthesis of Meso-Substituted Dipyrromethanes and Their Application in Synthesis of Trans-Substituted Porphyrin Building Blocks, Tetrahedron. 1994 50(39): 27–44. 23. Rothemund, P.; J. Am. Chem. Soc.,1939, 61: 29. 24. Raman, N.;Kulandaisamy, A.; Thangaraja, C.; Manisankar, P.; Viswanathan,S.; and Vedhi, C.; Synthesis, structural characterisation and electrochemical and antibacterial studies of Schiff base copper complexes. Transition Metal Chemistry. 2004, 29(2):129-135. 25. Zemede, Y.B.; Nithyakalyani, D.; Kumar A.; Synthesis, Structural Characterization, Corrosion inhibition and in vitro antimicrobial studies of 2-(5-Methoxy-2-Hydroxybenzylideneamino) Phenol Schiff Base ligand and its transition metal complexes Synthesis. 2014, 6(11):4569-78.

Figures

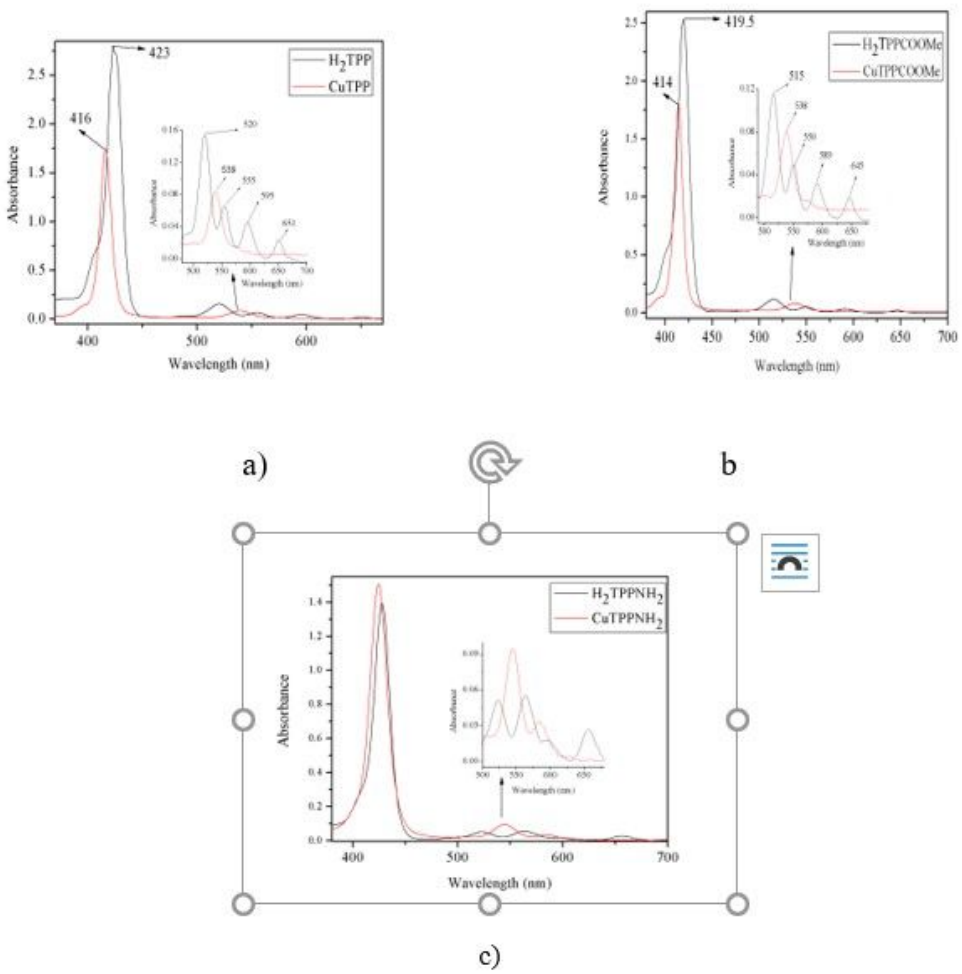
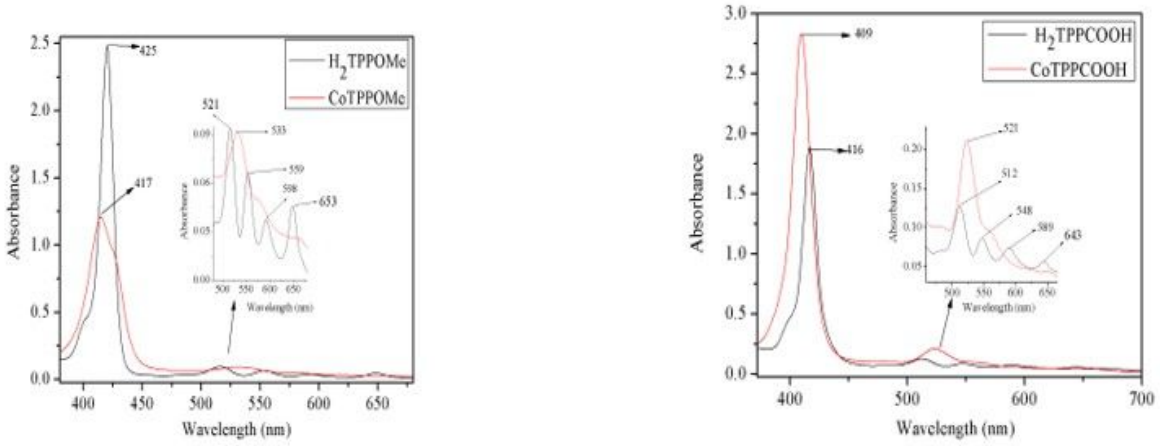


Figure 1

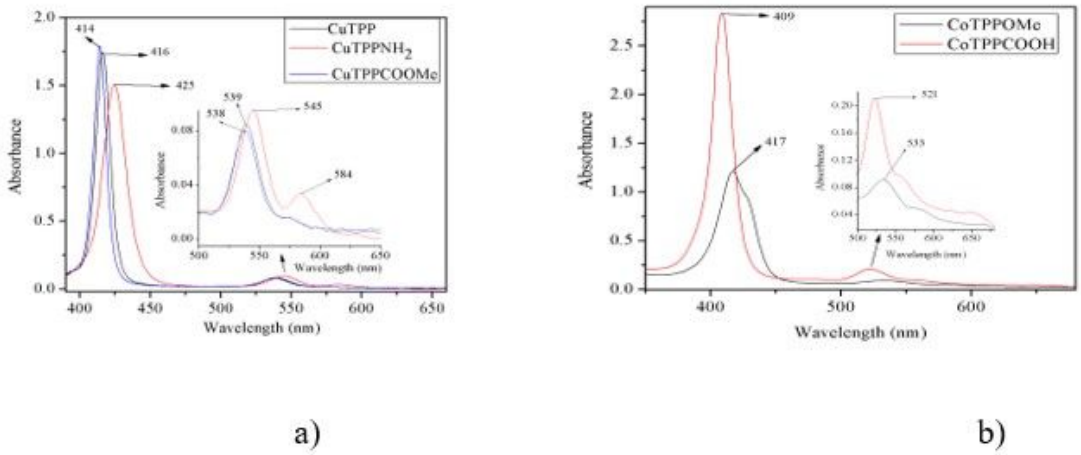
UV–Vis absorption spectra of a) H2TPP and CuTPP b) H2TPPCOOMe and TPPCOOMe C) H2TPPNH2 and CuTPPNH2



a) b)

Figure 2

UV–Vis absorption spectra of: a) H2TPPOMe and CoTPPOMe; b) H2TPPCOOH and CoTPPCOOH



a) b)

Figure 3

UV–Vis absorption spectra of: a) Copper complexes, b) Cobalt complexes

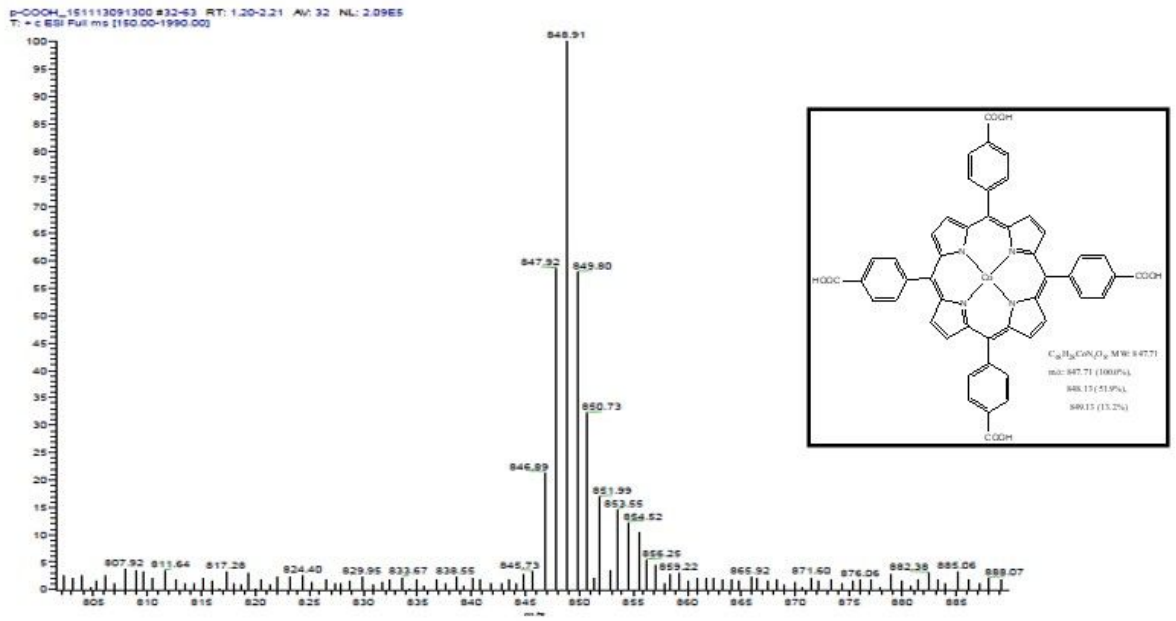


Figure 4

The mass spectrum of cobalt-5,10,15,20-tetrakis(4-carboxyphenyl) porphyrin

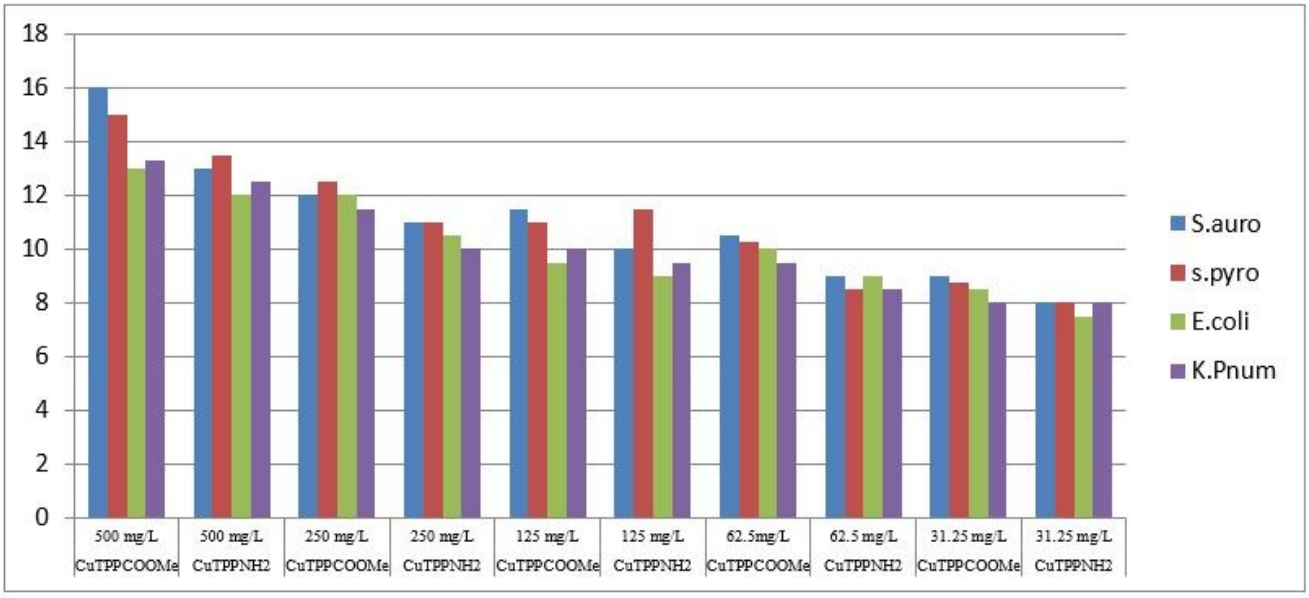


Figure 5

Bar graph of CuTPPCOOMe and CuTPPNH2 on the same concentrations

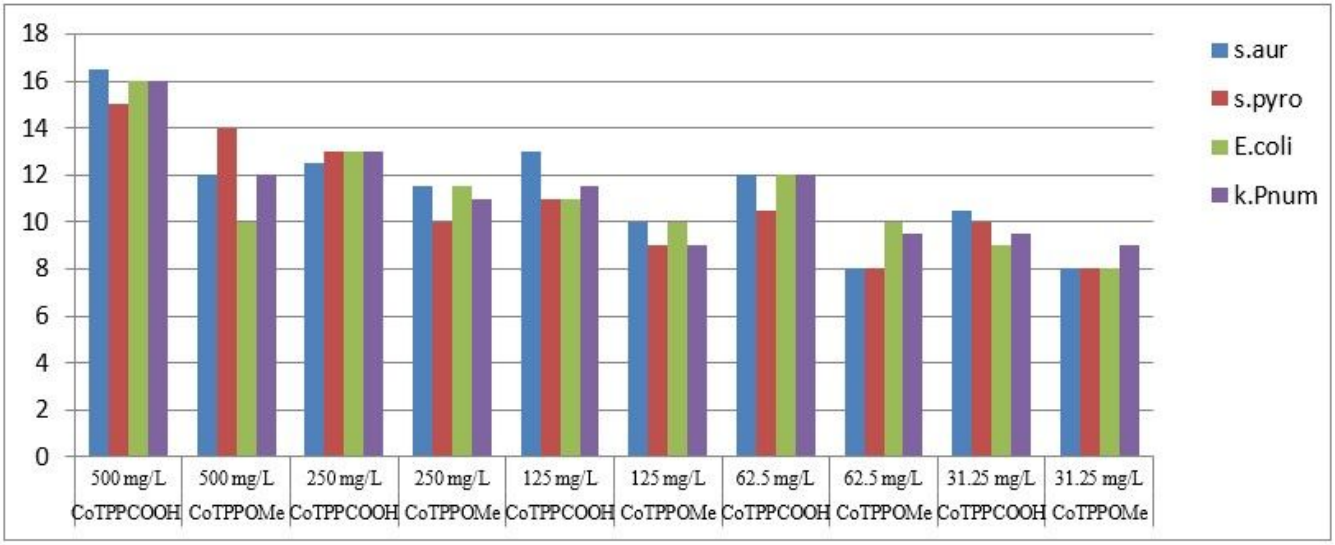


Figure 6

Bar graph of CoTPPCOOH and CoTPPOMe on the same concentrations

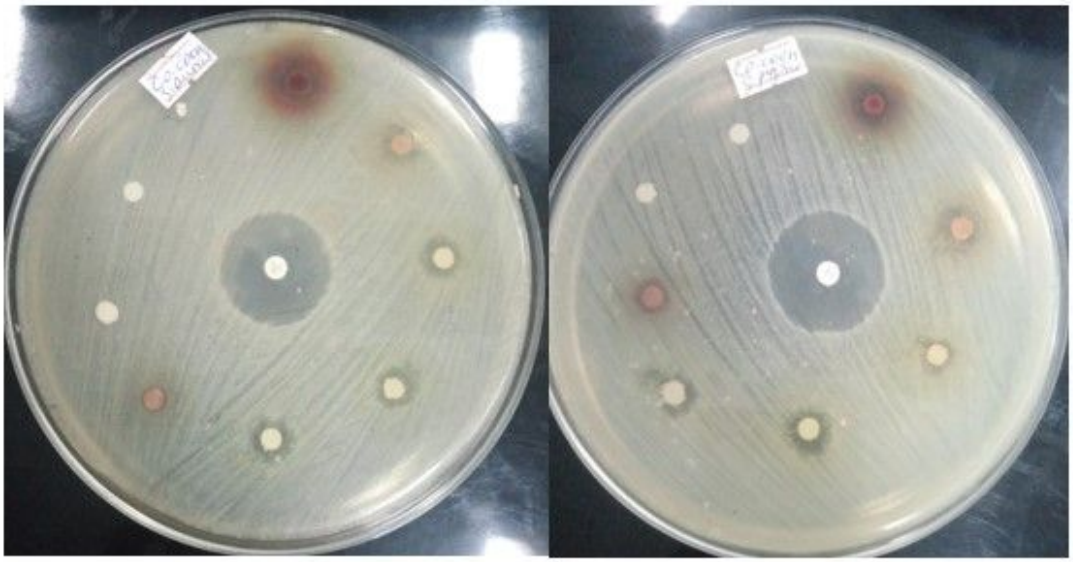


Figure 7

Gram positive Bacteria's, S. aureus and S. pyogenesin CoTPPCOOH molecule

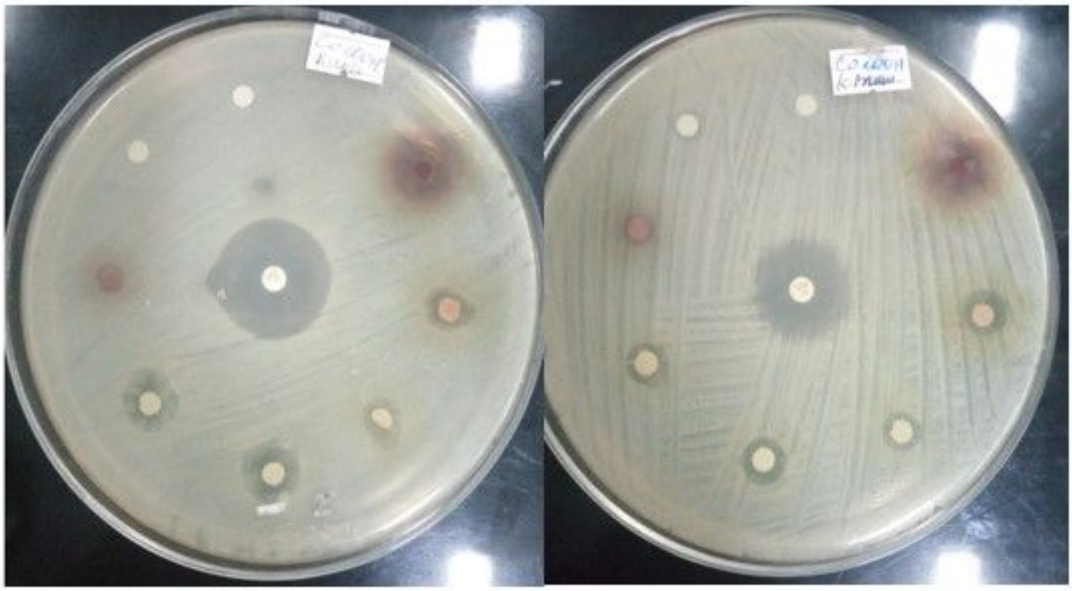


Figure 8

Gram negative Bacteria's, E.coli andK. pneumoniain CoTPPCOOH molecule

Supplementary Files

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