Zinc, Tin and Silver Porphyrins (TPP, TCPP, TMPP, THPP, TPPS, TMPyP) as photosensitizers in antibacterial photodynamic therapy for chronic wounds: A screening study

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Abstract

Continuous proliferation of bacteria in a wound delays its healing process, and could further extend to becoming a chronic wound infection. The effectiveness of different porphyrins as a photosensitizer in antibacterial photodynamic therapy for the inactivation of some wound-colonizing bacteria was studied as a screening experiment. Meso-tetra(4-methoxyphenyl) porphyrin, (TMPP), meso-tetra(4-hydroxyphenyl) porphyrin (THPP), meso-tetra(4-carboxyphenyl) porphyrin, (TCPP), meso-tetra(N-methyl-4-pyridyl) porphyrin, (TMPyP) were synthesized, each complexed with zinc, tin and silver. The in-vitro and photo-toxicity properties of the porphyrins and their complexes were assessed on some selected wound colonizing multi-drug resistant bacterial strains (*Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis,* and *Escherichia coli*) using agar well diffusion method. Photo-toxicity of the compounds were investigated using a 100-Watt tungsten lamp while the in-vitro toxicity was carried out in the dark. The results were compared with previously reported work carried out by this group on meso-tetra(phenyl)porphyrin (TPP), meso-tetra(4-sulphonatophenyl) porphyrin, (TPPS) and their corresponding Zn, Sn and Ag complexes. Most of the porphyrins showed biocidal activities against three of the test isolates with an exception to *Proteus* sp. ZnTMPyP and ZnTHPP only showed photo-toxic activities against the four test isolates. While SnTHPP, ZnTPPS, ZnTCPP, and SnTCPP all exhibited both toxic and photo-toxic activities against all four bacterial isolates. The Ag-porphyrins had the poorest inactivation activity.

Keywords: Wound; Bacteria; Antibiotics; Porphyrins; Antibacterial; Photodynamic therapy
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INTRODUCTION

The porphyrin molecule is a macrocyclic compound usually comprises four pyrrole rings joined together by methine bridges. It has 22 π electrons, 18 are

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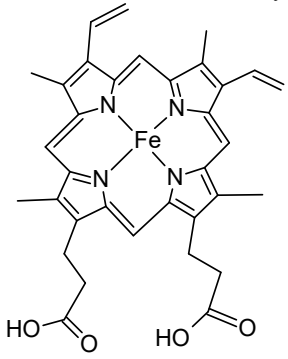
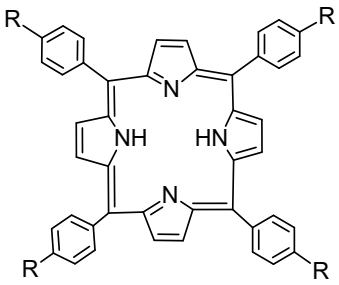
delocalized and due to their extensive conjugated systems, this class of compounds are coloured. The interest in porphyrins arose from the role they play in various biological processes – the electron-transfer (cytochromes), oxygen-transport (heme) and light-harvesting (chlorophyll). Natural occurring porphyrins are β-substituted and asymmetrical having different substituents on the ring while meso-substituted porphyrins have not been found in nature (Figure 1). Porphyrins are usually synthesized by the condensation reaction between an aldehyde and pyrrole in the presence of an oxidizing agent and an organic acid as catalyst (Rothemund and Menotti, 1941; Adler *et al.,* 1967; Lindsey *et al.*, 1986). Using pyrrole with substituents would produce β-substituted porphyrin while substituents on the aldehyde yields meso-substituted porphyrins. Synthesis of asymmetrical porphyrins often results in tedious purification process due to the production of large number of isomers, hence symmetrical porphyrins are more common.



Asymmetrical β-substituted porphyrin
(Heme b)



Symmetrical meso-substituted porphyrin

Figure 1. Structures of types of substituted porphyrins

The porphyrin molecule is unique because its electronic and optical properties can be altered by adding various metals into the central cavity, axial ligation of these metals, introducing several substituent at various positions on the ring which may extend the π-bond system, fusion of extra rings, replacing atoms on the ring with another, addition of hydrogen atoms across any double bond, ring expansion or ring contraction (Srivastava and Tsutsui, 1973; Herrman, *et al.*, 1978; Biffinger *et al.*, 2003; Ye and Naruta, 2003; La Deda *et al.*, 2004; Kral *et al.*, 2006; Rio *et al.*, 2008;). As a result, the porphyrin molecules are being investigated for a wide range of applications (Miragliotta, 1995; Chang *et al*., 2000; Wamser *et al*., 2002; Chou *et al*., 2003; Dolmans *et al*., 2003; Camerin *et al*., 2005; Huang *et al.,* 2015; Imran *et al.,* 2018; Lopes *et al.,* 2019; Shi *et al.,* 2021).

Porphyrins are one of the many photosensitizers currently being studied for Photodynamic Inactivation (PDI) of bacteria (or Antibacterial Photodynamic Therapy (aPDT)) which might be a cheaper and easier method to manage chronic wounds as this technique uses only light, a photosensitizer, and oxygen. The photosensitizers are organic compounds (dyes) which absorb light and then transfer energy or electron to neighbouring molecules usually molecular oxygen (Figure 2). When the molecule absorbs light it changes from its singlet ground state (S_0) to a singlet excited state (S_1). The molecule remains in this state (S_1) for a very short time (lifetime in nanoseconds (ns)) before returning to the ground state either by the fluorescence ($S_1 \rightarrow S_0$) or it undergoes intersystem crossing from S_1 to T_1. T_1 is a triplet excited state, and the duration of the molecule in the triplet state (microseconds (ms)) is long enough to facilitate the transfer of energy or electron to molecular oxygen, producing various reactive oxygen species (ROS). The ROS are responsible for the destruction of microorganisms. Phosphorescence is the radiative relaxation process from the triplet state to the singlet ground state ($T_1 \rightarrow S_0$) (Lakowicz, 1999).

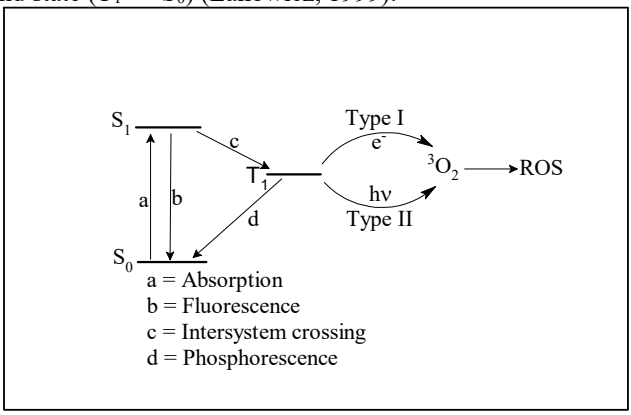


Figure 2. A schematic diagram of the production of reactive oxygen species.

In the electron transfer route (Type I), the triplet state of the photosensitizer (^{3}PS) is reduced by the electron-donating molecules within the biosystems (BS) generating anionic radicals $PS^{-\bullet}$ and in turn, it becomes a cation radical $BS^{+\bullet}$., then $PS^{-\bullet}$ donates its extra electron to oxygen to form superoxide anion $O_2^{-\bullet}$. Other radicals produced during the propagation stage are hydroxyl radicals (HO$^\bullet$) and hydroperoxyl radicals ($HOO^\bullet$) and all these species could kill an organism (Benov, 2015; Kwiatkowski *et al*., 2018; Niculescu and Grumezescu, 2021). The second route (Type II) involves energy transferred from ^{3}PS to molecular oxygen. This changes the state of molecular oxygen from its triplet ground state ($^3O_2$) to an excited singlet state ($^1O_2$). The excited singlet state of oxygen is highly reactive

and interacting with the proteins, nucleic acid, or lipids within the cells results in cell death. The singlet oxygen where the two electrons in the antibonding orbitals are paired, being more highly energetic, quickly relaxes to the singlet state where the electrons fill in singly (Benov, 2015; Kwiatkowski *et al*., 2018; Niculescu and Grumezescu, 2021). The skin which is capable of the natural process to self-heal when there is tear/damage could provide slight opportunity for the normal flora on the skin to infect the wound, support bacterial growth, resulting in increased inflammation and a lengthened wound healing process. These microorganisms could also develop a biofilm on the wound that serves as a defense mechanism against antimicrobial treatment and prevent immune response, leading to chronic wound infection. Chronic wounds are wounds that do not heal after 12 weeks and often occur in elderly and diabetic patients (Wilkinson and Hardman, 2020). The incessant growth of bacteria could be a result of underlying health conditions decreasing the body's natural ability to fight infections. In some cases, it might be due to cellular senescence (the cells aged, stops dividing but do not die) (Wilkinson and Hardman, 2020). These cells release cytotoxic substances that damage the nearby healthy cells further preventing the natural healing process. In this era where there are several multidrug resistance pathogens, it becomes more difficult to manage these conditions and sometimes amputation is required when all other means of treatment of wound infections have failed (Järbrink *et al*, 2017). Oftentimes, it takes decades for some chronic wounds to heal up, subjecting patients to serious and constant pain with increasing morbidity cases. Such wounds often produce bad odours especially when poorly managed, leading to isolation and as result have a negative impact on their mental health (Järbrink *et al*, 2017; Sen, 2019).

The advantage aPDT has over other antibacterial therapy is, it is non-toxic, non-invasive and apart from the photosensitizer, all other components of this therapy are eco-friendly and free. Secondly unlike other treatments, it does not target a single site within the pathogen; rather it targets various structures, which in turn alters different metabolic pathways of the organism. Thirdly, the photodynamic process occurs so rapidly that the organism does not have "enough time and resources" to recover and engage in adaptive survival and to confer cross-generation adaptivity mechanism against the therapy (Feese *et al*., 2011; Ranjbar and Ashrafzadeh, 2016).

The conversation on the application of porphyrin as photosensitizers in aPDT is ongoing (Milanesio *et al.,* 2001; Lui *et al.,* 2015; Amos-Tautua *et al.,* 2019; Ion 2021; Oyim *et al.,* 2021) and several studies into the antibacterial efficacy of porphyrins have been done, many of which utilized the free-base molecules and mostly against *Staphylococcus aureus* and *Escherichia coli;* some of which have been listed in Table 1.

Table 1. Some research reports on the investigation into the use of porphyrins as an antibacterial agent or in aPDT.

	Porphyrins		**Organisms**	**Activity**	**Reference**
Formula	Functional group (R)	Metal			
TMePP	$-OCH_3$	Nil	*S. aureus, K. pneumoniae, E. coli* & *C. albicans.*	Antibacterial	Jagessar (2018)
TCPP	-COOH	Nil			
TMePP-TCPP					
TPP	-H	Nil	*E. coli* & *S. aureus*	aPDT	Wang *et al*. (2018)
TPPOH	-OH	Nil			
$TPPNO_2$	$-NO_2$	Nil			
$TPPNH_2$	$- NH_2$	Nil			
$TPPS_4$	$-SO_3^-$	Ni & Zn	*E. coli*	aPDT	Zoltan *et al.* (2015)
$TNPS_4$	$-NAPH-SO_3^-$	Zn			
	$-N^+CH_2COOC_2H_5$	Ni & Zn			
TallPyP	4-N-allylpyridyl	Nil	S. *aureus*, *E. coli*, *P. aeruginosa* & *C. albicans*.	aPDT	Yu *et al.* (2009)
Deutero-porphyrin	4 β-substituted-CH_3 groups 2 β-substituted carboxylic acid groups	Nil	*S. aureus*, *E. coli*, & *P. aeruginosa*	aPDT	Orenstein *et al.* (1997)
TOE_4PyP	$4\text{-}N^+\text{-}C_4H_7$	Zn & Ag	*E. coli* & *S. aureus*.	aPDT	Gyulkhandanyan *et al.* (2016)
$TBut_4PyP$	$4\text{-}N^+\text{-}C_2H_4OH$				
$Tall_4PyP$	$4\text{-}N^+\text{-}C_3H_5$				
$TMetAll_4PyP$	$4\text{-}N^+\text{-}C_4H_7$				
TAPP	$-NH_2$	Zn	*S. aureus, E. coli, & P. aeruginosa*.	aPDT	Fayyaz *et al.* (2016)
TMAP	$-N^+(CH_3)_3$	Nil	*E. coli*	aPDT	Caminos *et al*. (2008)
TMPyP	$-N^+CH_3$	Nil	*P. aeruginosa*	aPDT	Collins *et al.* (2010)
TMPyP	$-N^+CH_3$	Nil	*E. coli* & *S. aureus*.	aPDT	Muehler *et al.* (2022)
T_4MPyP	$R_{1\text{-}3} = -N^+CH_3$ $R_4 = -N^+C_nH_{2n+1}$ (n = 1, 6, 10, 14, 18, 22)	Nil	*E. coli* & *S. aureus*.	aPDT	Reddi *et al.* (2002)

We propose that this treatment can be administered on the surface of the wounds, activating the compound by exposing to sunlight rays for few hours thus, initiating the killing of the colonizing bacterial isolates.

Therefore, the aim of the study was to conduct a screening investigation of several porphyrins - neutral, positively and negatively charged porphyrins and each metallated with zinc, tin, and silver. The neutral porphyrins were meso-tetra(4-phenyl) porphyrin, (TPP), meso-tetra(4-methoxyphenyl) porphyrin, (TMPP), meso-tetra(4-hydroxyphenyl) porphyrin, (THPP). The negatively-charged porphyrins were meso-tetra(4-carboxyphenyl) porphyrin, (TCPP), and meso-tetra(4-sulphonatophenyl) porphyrin, (TPPS), and the positively-charged porphyrin was meso-tetra(N-methyl-4-pyridyl) porphyrin, (TMPyP). The antibacterial photodynamic properties of these compounds were tested against four bacterial isolates obtained from chronic wounds; *Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli* (Gram-negative), and *Staphylococcus aureus* (Gram-positive). We have reported the aPDT activities of TPP, TPPS and their complexes (Zn, Sn and Ag) elsewhere (Daramola *et al.,* 2021) and would be comparing their activities with these compounds.

MATERIALS AND METHODS

All reagents and solvents were purchased from Merck and Sigma Aldrich and used without further purification. Thin-layer chromatography (TLC) was performed with Merck aluminium plates coated with silica gel 40 mesh to test the purity of porphyrin molecules and to explore the chromatographic conditions necessary to purify them and column chromatography was performed on silica gel 60 mesh (0.040 – 0.063 mm) manufactured by Merck. Ultraviolet/Visible (UV/Vis) spectra were recorded on a Shimadzu UV–200 spectrophotometer using a path length of 1 cm, quartz cells and spectra were plotted using OriginPro 7.0 software (USA) installed in a personal computer. Fourier Transform Infrared (FT-IR) (4000–400 cm^{-1}) was recorded in KBr disk on Shimadzu FT–IR 8400. Proton Nuclear Magnetic Resonance (^{1}H–NMR) was recorded using a Bruker EMX 400 MHz NMR spectrometer.

Synthesis of porphyrin

The Alder-Longo technique, which is a condensation reaction between pyrrole and an aldehyde with a catalyst usually an acidic solvent and an oxidant, is the standard procedure for the synthesis of most porphyrins. The catalyst and oxidant used in the work were propanoic acid and nitrobenzene respectively (Adler *et al.,* 1967; Nascimento *et al*., 2007). To obtain each porphyrin equal moles of freshly distilled pyrrole and the corresponding aldehydes were used. All the compounds

synthesized were characterized using UV–Vis, Infra-Red and ^{1}H–NMR, spectroscopic techniques. Detail description of the synthetic procedure, characterization and spectra for each porphyrin are in the Supplementary Information (SI). The metalation of the free-base porphyrins H_2P (=TCPP, THPP, TMPP, TPyP) were carried out following Alder *et al*. (1970) methodology (See SI for details). MTPyP was quaternized using methyltosylate yielding a positively charge molecule and making it water-soluble. This was carried out following the procedure of Durmus and Nyokong (2007).

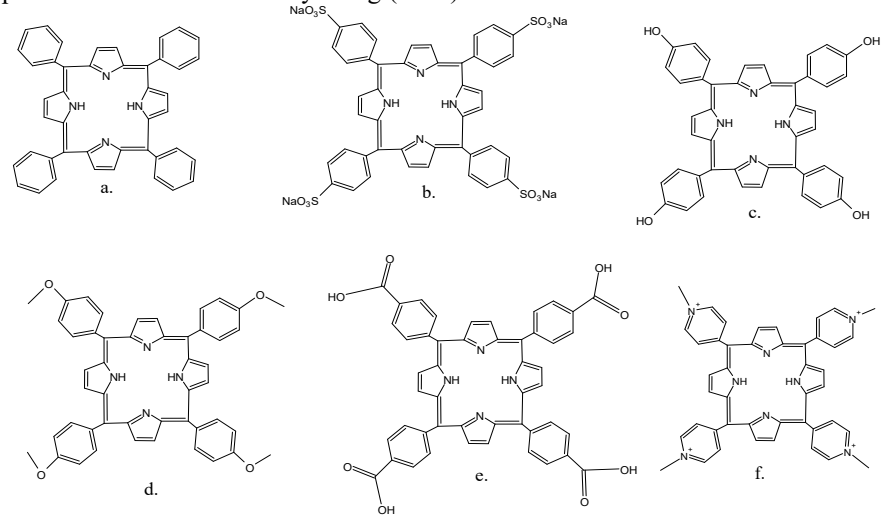


Figure 3. Structure of the various porphyrins (a) TPP (b) TPPS (c) THPP (d) TMPP (e) TCPP (f) TMPyP.

Antibacterial photodynamic screening

Collection and evaluation of bacterial test isolates

For this study, bacterial isolates source and antibiotic susceptibility profiles were reported in our previous study (Hudzicki, 2009; CLSI, 2018; Daramola *et al.,* 2021). They include *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis*.

Bacterial inhibition activities of porphyrin derivatives

To evaluate the photodynamic activities of the porphyrin derivatives on the test isolates, the agar well diffusion method described by Gyulkhandanyan *et al.* (2012) was used with slight modifications reported in our previous study on TPP

and TPPS (Daramola *et al.,* 2021). The test isolates grown in test tubes containing Tryptic soy broth were incubated for 18-24 hours. The cultured broth was centrifuged at 4,000 rpm for 10 minutes. The cultured broth was decanted and the cell pellets were washed by resuspension in sterile normal saline, vortexed, and centrifuged at 4,000 rpm for 5 minutes. After washing, the cell pellets were vortexed and adjusted to 0.5 McFarland standard at 540 nm wavelength. Sterile swab sticks soaked with the test isolates were seeded on sterile Mueller-Hinton agar (MHA) plates after which 7 mm wells were drilled on the seeded plates using a sterile cork-borer. About 30 µL of the porphyrin derivatives of the working concentration as described in our previous studies were dispensed in the agar wells. Streptomycin sulfate (84 mg/mL) and sterile distilled water were used as positive and negative control, respectively. The experimental parameters were prepared in two sets to assess the toxic and photo-toxic activities of the porphyrins used.

For the toxicity activity, the first set was evaluated by incubating in the dark at 37 ^{o}C for 18 – 24 hours. The photo-toxic profiles of the porphyrin derivatives were observed under a controlled condition (Fayyaz *et al.*, 2015). A Tungsten lamp (100 Watt) was used as light source and placed at a distance of 35 cm above the seeded MHA plates. A glass tray containing about 5 L of water was positioned between the tungsten lamp and the MHA plates to absorb heat in the incubating chamber. Data obtained in this study were expressed as mean ± standard deviation using Microsoft excel and Graph Pad Prism version 6.01.

RESULTS AND DISCUSSION

Molecular characterization

The electronic spectrum of a free-base porphyrin has an intense soret band occurring around 400 – 420 nm and four weak Q-bands between 500 – 750 nm. Upon complexation, the symmetry of the molecule changes from D_{2h} to D_{4h}. The consequence of this is a decrease in the number of Q-bands from four to two or one, accompanied by a shift in λ_{max} of the soret-band (Gouterman, 1961). Most of the Ag-porphyrins had a single Q-band due to the 'sitting–atop' position of silver as a result of its large ionic radius (1.29 nm) relative to the distance between opposite nitrogen atoms (0.4 nm) (Senge, 1999; Punnagia *et al.,* 2004; Smith, 2006). Several researchers have studied the IR spectra of various porphyrins and have assigned certain bands (Thomas and Martell, 1956, 1959; Kincaid and Nakamoto, 1975; Zhang *et al*., 2003). The N – H stretching frequency of pyrrole occurs around 3500 cm^{-1} but in porphyrins, it occurs at a lower frequency due to strong hydrogen bond (N – H^{IIIII}N). The C–H (sp^2) stretching vibrational

frequency of the phenyl and pyrrole rings occur in range 3090 - 2920 cm^{-1}. The C=C bands of the conjugated phenyl are often regarded as the skeletal in-plane stretching vibrations and occur around 1610 - 1590 cm^{-1}. For the methine and pyrrolic groups, the C=C stretching vibrations are found around 1575 - 1520 cm^{-1}. The phenyl ring mode which is the expansion and contraction of the ring have been assigned values 1495 – 1460 cm^{-1} (Smith, 2016). The C – H bending vibrations of pyrrolic ring are observed around 1445 – 1410 cm^{-1} while the C – N stretching bands are observed between 1355 - 1335 cm^{-1}. The C – H rocking frequency often noticed as 3 bands occur around 1080 - 960 cm^{-1}. The peaks around 760 – 700 are assigned to C – H deformation of mono-substituted phenyl groups.

According to the report of Kancaid and Nakamoto (1975), it is difficult to assign vibrational modes to the metalloporphyrin, due to a high degree of coupling of these modes. Furthermore, there are no pure M – N vibrational modes because these occurring at the low-frequency region are mixed with the phenyl deformation modes. However, they reported that certain bands are metal sensitive and these peaks occur around 1491 ($\nu_{\text{phenyl ring}}$), 1350 cm^{-1} (ν_{C-N}) 1000, and 967 cm^{-1} ($\nu_{C-H\ \text{rocking}}$) for TPP. These are referred to as the porphyrin skeletal modes. These metal sensitive bands were used in the characterization of the metal complexes in this work. A shift in wavenumbers of these metal sensitive bands were observed and some of these bands were also absent in a few of the metalloporphyrins. The NH band disappears due to the complexation, therefore any broadness in this region is likely due to the presence of water as an axial ligand or the porphyrin is hydrated.
As regards to proton-NMR, the aromatic ring of a porphyrin experiences anisotropy. Johnson and Bovey model described the flow of the delocalized electron in the magnetic field as occurring in loops above and below the ring and this ring currents result in a change in the position of the chemical shift when compared to regular non-aromatic compound (Johnson and Bovey, 1958; Abraham, 1961). The N–H protons in the center cavity are intensely shielded resulting in their chemical shift to positions sometimes less than 0.0 ppm (Mamardashvili and Golubchikov, 2001). While protons outside the ring are desheilded, these are the β- and meso protons and they occur more downfield than usual. The meso protons are more downfield than the β-protons, which is a result of the electron deficiency at the meso carbon because the pyrroline unit has five π-electrons versus the six in pyrrole. Porphyrin molecules exhibit molecular flexibility (Cheng *et al*., 1997) and adopt various conformations (Kingsbury and Senge, 2021). The interactions between the four $^{+}N–CH_3$ groups and the ring current affects the out-of-plane distortion of the compound which results in the protons of the $^{+}N–CH_3$ groups being in different chemical environments, creating the two peaks, $\delta = 1.08$ and $\delta = 1.20$ in the TMPyP (Gjuroski *et al*., 2021).

Screening study

The zone of inhibition shown in Figure 4 is the clear region that indicates the absence or inhibition of the growth of organism by the porphyrin molecules (See SI for data). In general, most of the porphyrins showed antibacterial activity in the dark with an increased zone of inhibition when exposed to light (Figure 5). An exception to this was observed for TPPS against *E. coli,* displaying a larger zone of inhibition in the dark than in the presence of light (Figure 5a). *S. aureus* was sensitive to nineteen of the porphyrin except for TPP, TMPP, AgTMPP, TCPP, and TMPyP (Figure 5b). *P. mirabilis* showed the highest resistivity amongst the four tested isolates, exhibiting sensitivity only towards ZnTHPP, SnTHPP, ZnTPPS, ZnTCPP, SnTCPP, and ZnTMPyP (Figure 5c).

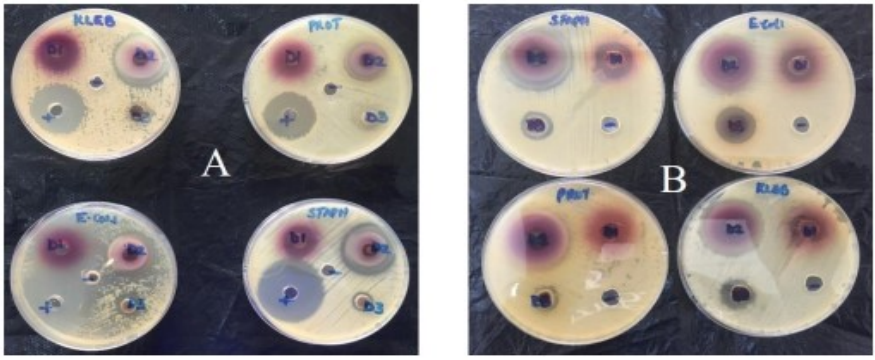
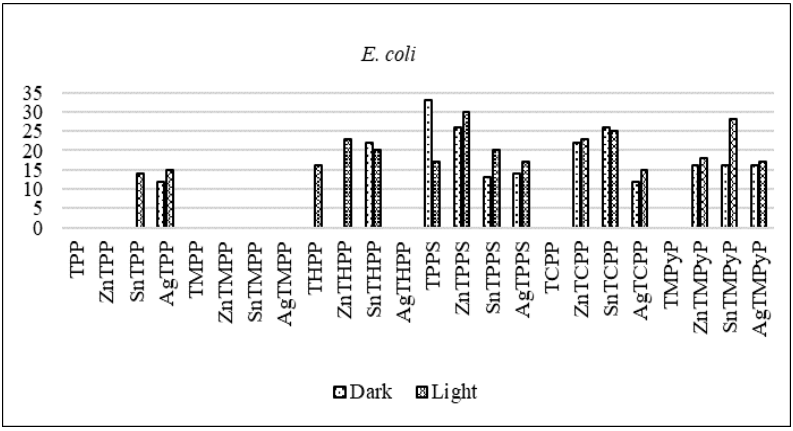


Figure 4. A picture showing the zone of inhibitions TPPS series A was in the dark and B under light) (Daramola *et al*., 2021)

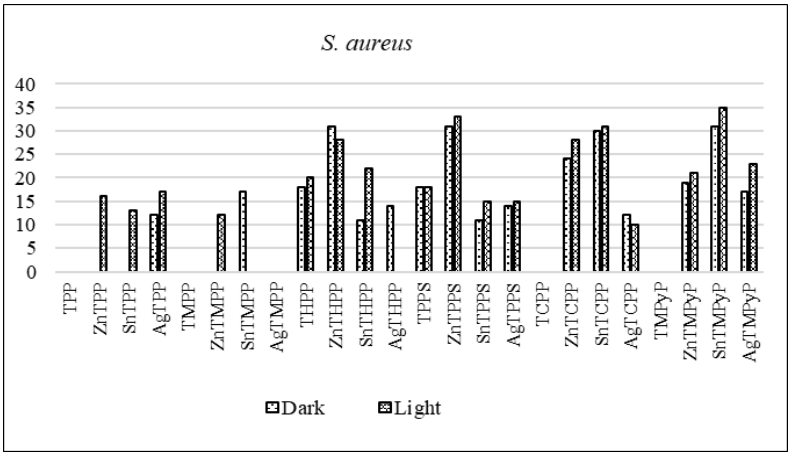
Some porphyrins exhibited antibacterial properties in the dark only. For example, SnTMPP and AgTHPP were found effective against *S. aureus* and AgTMPP and AgTCPP against *K. pneumoniae* (Figure 5b and d, respectively). In some instances, the photodynamic antibacterial activities of the porphyrins were activated as they showed no activity in the dark. This was observed for ZnTPP, SnTPP and ZnTMPP against *S. aureus;* THPP, SnTPPS and TCPP against *K. pneumoniae*; ZnTHPP and ZnTMPyP against *P. mirabilis* as well as SnTPP and THPP against *E. coli* (Figure 5).

From the TPP series, only AgTPP showed activity in the dark and it was against *S. aureus, E. coli* and *K. pneumonia.* The metal complexes of TPP exhibited photodynamic inactivation against at least one pathogen (Figure 6a). Amongst the TCPP series, SnTCPP and ZnTCPP showed antibacterial activities against all the test isolates under both experimental conditions (light and dark), with SnTCPP having the highest stack mean values of the zone of inhibition (Figure 6b). For the

THPP series, similar findings are reported for SnTHPP, while antibacterial photodynamic activity was evident with THPP and ZnTHPP as mentioned earlier (Figure 6c). All the metal complexes of TPPS showed antibacterial activity against at least two of the bacterial isolates while ZnTPPS had an increased zone of inhibition under light conditions against all the bacterial isolates (Figure 6d). Metal complexes of TMPyP had antibacterial activity against the bacterial isolates with an increased zone of inhibition in light except against *P. mirabilis* (Figure 6e). The TMPP series had the weakest antibacterial activity both in the dark and in light. SnTMPP showed activity against *S. aureus* and *K. pneumoniae* in the dark while ZnTMPP exhibited phototoxity but towards *S. aureus* only (Figure 6f).



(a)



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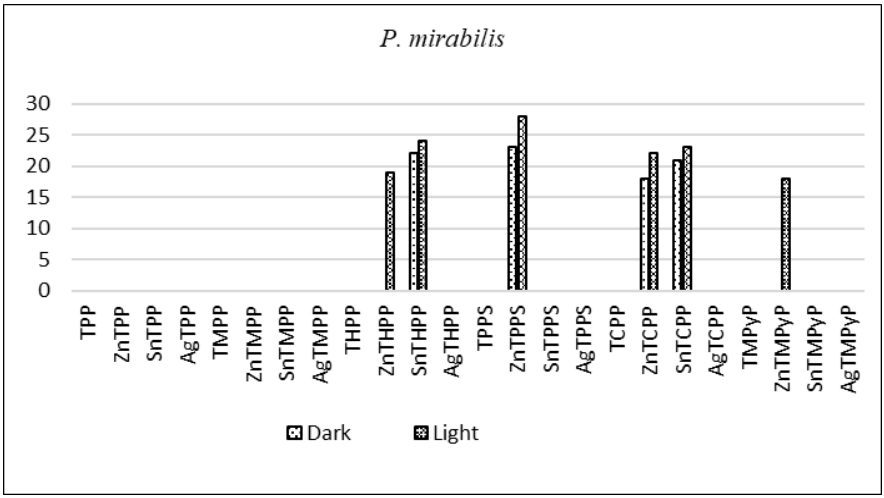
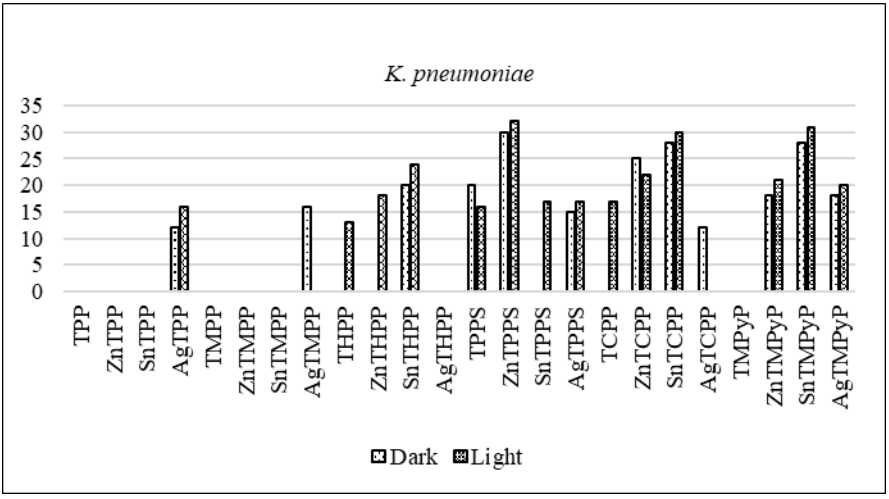
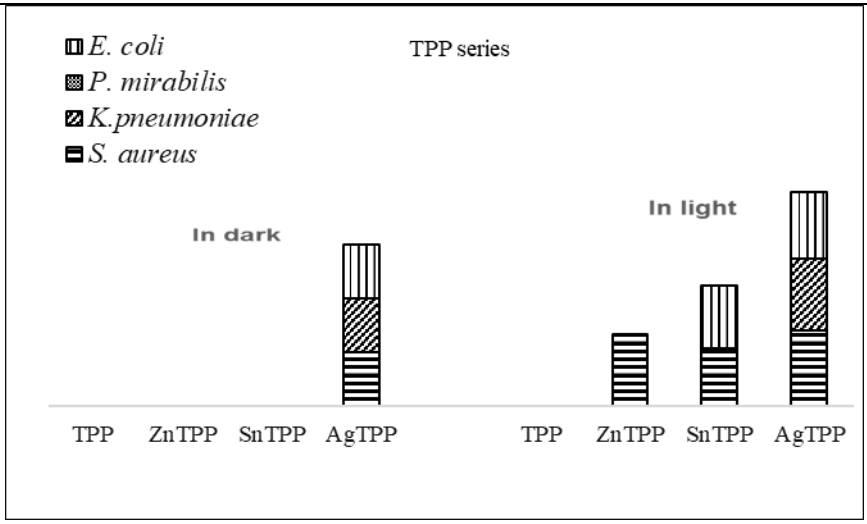


(c)

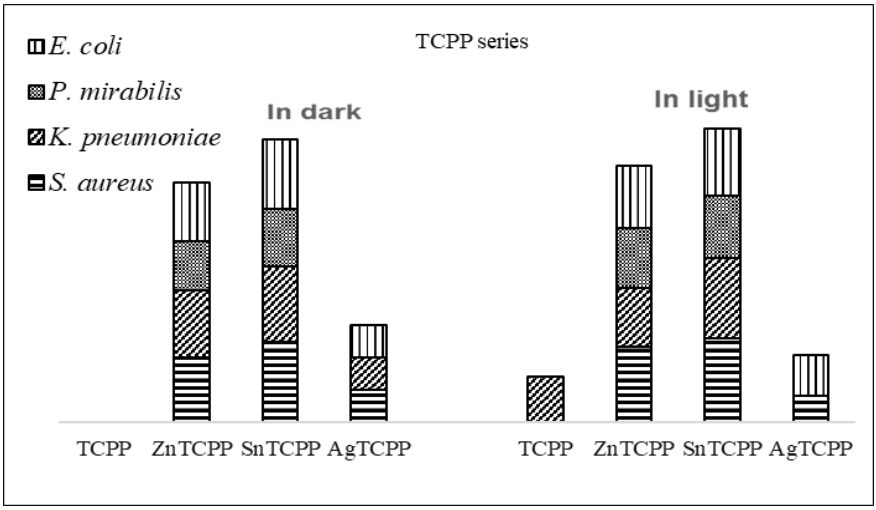


(d)

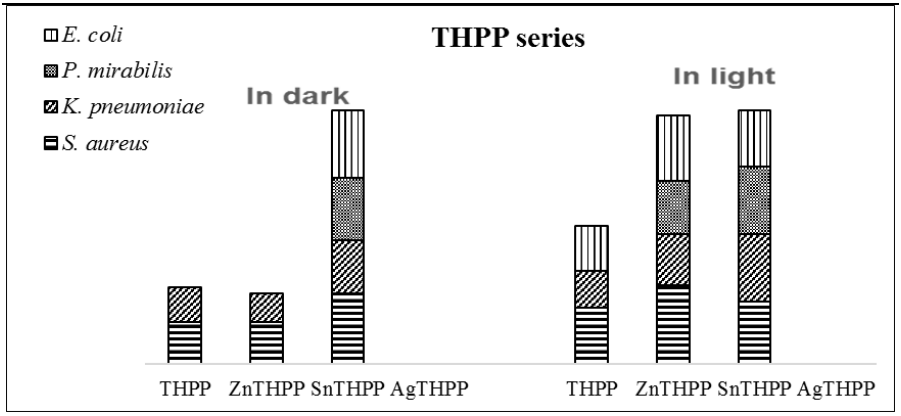
Figure 5. Bar charts of the zone of inhibition (mm) of each bacterial isolates against all the porphyrin 0.01 g/10 ml.


E. coli
P. mirabilis
K.pneumoniae
S. aureus
TPP series
In dark
In light
TPP
ZnTPP
SnTPP
AgTPP
TPP
ZnTPP
SnTPP
AgTPP

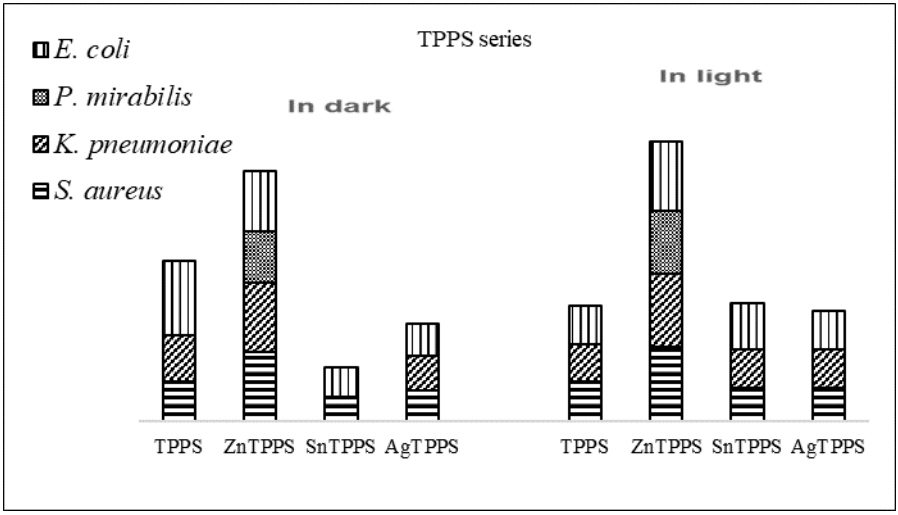
(a)


E. coli
P. mirabilis
K. pneumoniae
S. aureus
TCPP series
In dark
In light
TCPP
ZnTCPP
SnTCPP
AgTCPP
TCPP
ZnTCPP
SnTCPP
AgTCPP

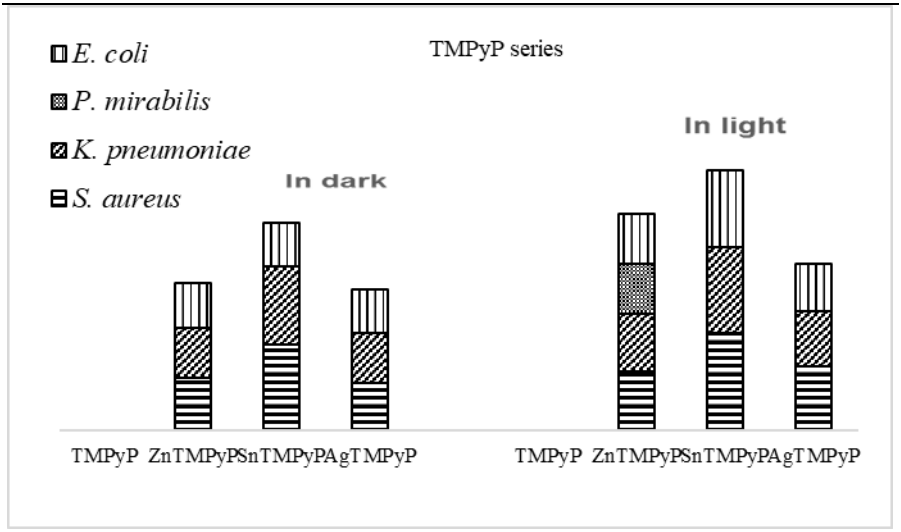
(b)


E. coli
P. mirabilis
K. pneumoniae
S. aureus
THPP series
In dark
In light
THPP
ZnTHPP
SnTHPP
AgTHPP
THPP
ZnTHPP
SnTHPP
AgTHPP

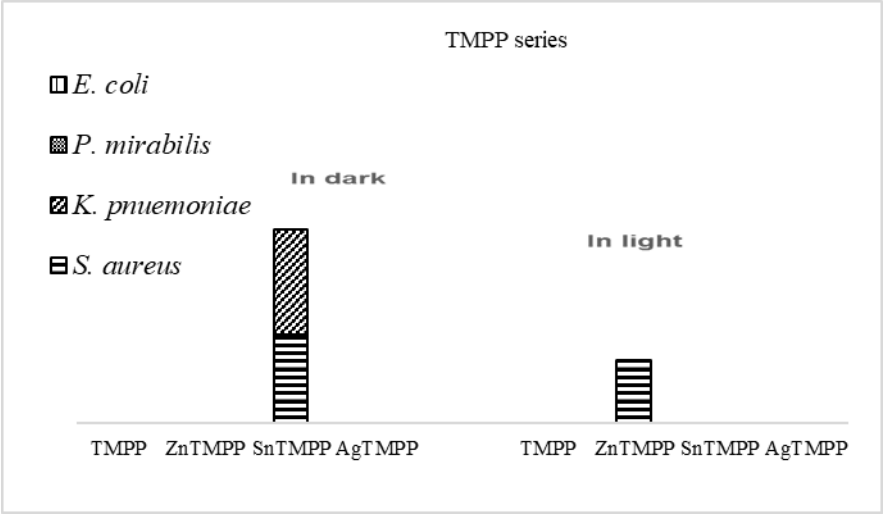
(c)


E. coli
P. mirabilis
K. pneumoniae
S. aureus
TPPS series
In dark
In light
TPPS
ZnTPPS
SnTPPS
AgTPPS
TPPS
ZnTPPS
SnTPPS
AgTPPS

(d)



(e)



(f)

Figure 6. Bar charts of the zone of inhibition (mm) of each porphyrin (0.01 g/10 ml) series against the bacterial isolates.

Comparing the antibacterial activity of the standard streptomycin sulphate (84 mg/ml), to that of the porphyrin compounds (30 mg/ml), streptomycin had biocidal activity against all the pathogens with higher mean values of zone of inhibition with the exception of SnTCPP, ZnTPPS and SnTMPyP against *K. pneumonia*, ZnTPPS against the *P. mirabilis* and SnTMPyP against *S. aureus*.

The outermost layer of all bacteria has an overall negative charge on their surfaces due to the presence of numerous phosphate (PO_4^{2-}) and carboxylate (CO_3^{2-}) groups and having Ca^{2+} and Mg^{2+} as counter ions (Brown *et al*, 2013; Liu *et al.,* 2015). It is therefore assumed that a positively charged PS will interact or bind more strongly to the cell wall resulting in a more effective photosensitization (Hamblin *et al*., 2002). The results obtained in the work show the contrary. The cumulative mean of the anionic compound (TPPS and TCPP series) was higher than those obtained in the cationic compounds (TMPyP series). According to literature report which explained that although the presence of the phosphate and carboxylate groups present a challenge for negatively charged PS to pass through this lipophilic barrier but once the Ca^{2+} and Mg^{2+} ions are displaced or replaced, the structural integrity of the bacterial membrane will be lost (Demidova and Hamblin, 2005; Silhavy *et al*., 2010). This has been reported that the replacement of these counter ions in *E. coli* was responsible for its susceptibility to porphyrins (George *et al.,* 2009). Therefore, it is probably that the reason for the results obtained in the study was due to the fact the negatively charged porphyrins were able to deionize this layer (TCPP series) and/or replace Ca^{2+} and Mg^{2+} with Na^{+} as the TPPS series are a sodium salt. The cumulative means of the zones of inhibition of the TPPS series were greater than those of the TCPP series. This might be due to a stronger binding or affinity by the SO_3^{2-} group because it is the conjugate base of a strong acid compared to -COOH group which is a weaker acid.

According to Wang *et al.* (2018), THPP has a high lipophilicity because of its electron donating –OH groups which increases the electron density on the pyrrolic ring making it more basic and readily susceptible to protonation; therefore, in aqueous solution it has more positive charges hence would readily bind on the surface of bacteria. Methoxy groups ($–OCH_3$) are also electron donating but TMPP exhibited very poor biocidal activity. It therefore shows that the electron donating properties might not be responsible for the higher antibacterial activity of THPP but simply that the –OH groups have better binding for the bacteria cell walls. Based on the output of this study it can be stated that for the neutral porphyrins the binding ability increases in the order: $OCH_3 < H < OH$.

The antibacterial activity of the synthesized complexes was greater than the corresponding ligands, indicating that the metal ions play an important role in the antibacterial activity. First this could be due to an increase in the triplet state

quantum yield of the PS due to the presence of the closed shell metal ions, which in turns resulted in an increase in the concentration of the ROS (Rodriguez *et al.,* 1989). The increase in antibacterial activity has also been attributed to an increase in lipophilicity of the metal complexes compared to its corresponding free-base porphyrin (Belete *et al.,* 2020). The outermost layer of the all bacterial contains acidic lipopolysaccharides and according to Overtone's theory on cell permeability, only lipophilic substances can pass through the cell membrane (Ličina *et al*., 2016). Porphyrin is a tetradentate chelating ligand and according to the chelating theory which states that the effect of chelation increases the lipophilicity of a metal complex. This arises from the decrease in the polarity of metal ions as a result of overlapping of the ligand orbitals with the d-orbitals of the metal ion as well as the partial sharing of the positive charge of the metal ion with the donor groups of the ligand (Tweedy *et al.*, 1964). The penetration of these metal complexes into the bacteria are likely to block the metal binding sites of enzymes in the microorganisms thereby inhibiting the respiratory process and cell growth/replication (Belete *et al*., 2020).

Furthermore, the peptidoglycan layer is very porous in both Gram species however, it is the outermost layer of the Gram-positive bacteria such that even anionic and neutral PSs will diffuse through this thick layer (Maisch *et al.*, 2011). This explains why *S. aureus* has shown sensitivity to most of the porphyrins. Another factor to consider is the hydrophobic and hydrophilic interaction of the PS with the cell wall, facilitating the penetration of the PS into the bacterial cellular barriers (Shargel *et al.,* 2012). This is attributed to presence of the –OH groups in THPP and TCPP accounting for their activity and further suggesting why TPP and TMPP showed poorer activity.

CONCLUSION

No doubt people suffering from chronic wounds are faced also with a major physical health challenge, and can develop mental health challenges as well. Antibacterial photodynamic therapy provides potential solution for the management of these chronic sores. A study was carried out to idetermine the potency of porphyrins as photosensitizers in antibacterial photodynamic therapy to inactivate *Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis,* and *Escherichia coli.* The free-base porphyrins showed poor activity against the pathogens except for TPPS and THPP but activity improved when they were metallated. It is obvious that the effect of functional groups on porphyrins and metal ions in complexes are additive. However, irrespective of the specific nature of the metal, it appears that the effect of the functional groups on the activity of the compounds is in an increasing order: TMPP < TPP < THPP < TCPP < TMPyP <

TPPS. As an antibacterial, Zn-porphyrins inactivated them best and Ag-porphyrins least. Most of the porphyrins showed biocidal activities against the pathogens except the recalcitrant *Proteus mirabilis.* This study also showed that the aPDT potential of porphyrin is not limited to positively charged porphyrins. ZnTMPyP and ZnTHPP showed strong photo-toxic activities against all four bacterial isolates. SnTHPP, ZnTPPS, ZnTCPP, and SnTCPP exhibited inactivation activity against all four bacterial isolates under both experimental condition and therefore might be the most suitable for aDPT for chronic wounds.

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