Research Writing

**Abstract**Traditional antibacterial treatments always rely on antibiotics, which has led to the attrition of antibiotics and resulted in the unfolding of multidrug-resistant (MDR) bacteria in the last few years. This study analyses the antibacterial nature of TCPP –Tetrakis(4-carboxyphenyl)porphyrin with E. Coli.

This study encapsulates the antibacterial nature of TCPP with the Kirby-Bauer method ( Disc Diffusion), Minimum Inhibitory Concentration, and Colony Counting. The result manifests that TCPP is an antimicrobial compound. The result provides a scientific basis for using TCPP in upcoming antibiotics.

**Keywords**Antibacterial activity; Antibiotics ; Bactericidal activity ; Minimum Inhibitory Concentration ; TCPP –Tetrakis(4-carboxyphenyl)porphyrin

**Introduction**

The expeditious advent of resistant bacteria is a reality today; this endangers the efficacy of antibiotics, thus becoming a taxing situation where millions can be in danger. Since the first patients were treated with antibiotics, bacterial infections have become a looming threat. The antibiotic resistance quandary has been accredited to the overuse and misuse of antibiotics, genetic evolution in bacteria cultures, and lack of new drug research by the pharmaceutical industry due to unviable economic incentives. Thus, this study aids in taking a small step in the right direction.

To expand the spectrum of antimicrobial agents, we have conducted tests on TCPP to confirm its antibacterial nature.

**Note:** Antimicrobial and antiviral activities of porphyrins are based on their ability to catalyze and oxidase reactions, absorb photons, generate reactive oxygen species (ROS), and partition into lipids of bacterial membranes.

**Experimental**

Preparation of TCPP

TCPP is a water-soluble compound taken in the test tube in required quantities, mixed with water, and vortexed to make the desired concentrations.

**Bacteria Cultures**

All experimentation was conducted on E. Coli, where nutrient broth and nutrient agar were taken. E. Coli culture was obtained from Microbial Type Culture Collection and Gene Bank (MTCC).

All apparatus was autoclaved to prevent contamination.

**ZONE OF INHIBITION- is qualitative**

**Antibacterial Experiment**

**1. Agar solution-** In a conical flask, 4.5g of nutrient agar powder was

dissolved in 200mL of autoclaved distilled water and agar solution was

autoclaved for 15mins at 120C.

**2. Broth solution-** In a conical flask, 2.6g of nutrient broth powder was

dissolved in 200mL of autoclaved distilled water, and the broth solution was autoclaved for 15mins at 120C.

**3. Cell preparation-** 50µl of E. coli was spread over a nutrient agar plate for growing at 37C for 16 hours. A single colony was inoculated from the plate in nutrient broth at 37C for 16-20 hours to obtain the bacterial culture. The culture was transferred to a centrifuge tube and centrifuge for 10mins, the supernatant was decanted, and cells were re-suspended into the fresh nutrient broth. Bacterial culture was diluted to obtain 10 6 to 10 7 CFU

mL -1 .

**4. Antibacterial activity-** Well method was used to evaluate the inhibitory

effect of the sample against E. coli. The agar plate was prepared using nutrient agar, and diluted E. coli cells were spread evenly using a cotton plug; holes were made in the agar plate using a cork borer, and 100µL of different samples were poured into holes, followed by incubating the plate at 37C for 16 hours. After that, the antibacterial activities of the prepared composites were evaluated by measuring the zone of inhibition (ZOI), which is the transparent region around the disc saturated with an antimicrobial agent on the agar surface.

**Colony Counting- is quantitative**

Note: CFU- Colony Forming Unit is the unit used in Colony Counting

First, we find the Optical Density of the compound with the U V spectrometer.

1 OD is 8\*10 8 CFU

2 OD is 16\*10 8 CFU

We know m1v1=m2v2

16\*10 8 CFU \*x=10 8 \*1 ml

x=1/16=0.0625ml

= 62.5 microliters (bacterial stock) + 937.5 microliters (nutrient broth)

to make a 1 ml solution in appendove.

We must also dilute to check concentrations of 10 7 and 10 6.

As a precaution, we should prevent lawn formation.

For our compound OD=0.120 Angstrom\* 10=1.2 A

1.2=1.2\*8\*10 8 CFU

=9.6\*10 8 CFU

9.6\*X\*10 8=10 8

x=1/9.6= 0.104ml

=104 microliters (bacterial stock)

broth (1000-104=896 microliters) per 10 8 CFU per ml.

Need to take 5mg TCPP.

Need to sonicate 5mg TCPP solution if needed. (Here not needed as TCPP is water soluble). (doubt)

Need to take 2ml broth in cubet and auto zero.

200 microliters of broth were removed from the cubet, add 200 microliters of bacteria were, and then use the UV spectrometer.

Adsorbance for 10 times diluted solution=0.145 A

no dilution=1.45 A

1.45=1.45\*8\*10 8 CFU=11.6 CFU

11.6\*10 8 \* x= 10 8x=1/11.6=0.08620ml

almost 85 microliters and thus broth (1000-85=915microliters)

For colony counting, we take 5 test tubes to make the following concentrations:

500microliters

250microliters

125microliters

62.5microliters

31.25microliters

at 10 7 CFU

Take a 2 mg/ml sample, add 0.5 ml in the following solvent, and repeat to dilute.

Add 500 microliters of bacteria solution.

In the end, incubate in the oven at 37C for 20hr (16-20hr)

Next morning, put all test tubes in the oven, and in the evening, take 50 microliters of sample and spread them out in 5 agar plates using a glass spreader.

The next day count the number of colonies on the plate using a digital colony counter.

**MINIMUM INHIBITORY CONCENTRATION (MIC)**

For MIC, we use 96 well plate and an ELISA reader

1 well volume= 300 microliters

we have a solution of 5mg/ml

5\*V=1\*150

v=30micro liter

and add 120 microliters of water

then dilute

Note-

1. Use a multichannel pipette

2. Reactions are done in duple or triple concentrations

3. Always take two columns as control, i.e., with no bacteria to make a baseline

After this, add 10 7CFU 10 microliters bacteria and mix, then put in the oven.

The next day put 96 well plate in the ELISA reader to check the optical density (OD) of wells and make a graph where the baseline is in control.