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Mesoporphyrin (ix) Dihydrochloride as a Non-Selective Antimicrobial Agent

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Antimicrobial activity of mesoporphyrin ix dihydrochloride (1) was investigated using the Agar diffusion and Poison plate methods. Compound (1) was shown to be both antibacterial and antifungal with a higher degree of selectivity for *S. aureus* and *C. albicans* versus *E. coli.*

Key words: Antimicrobial activity, Mesoporphyrin (ix) dihydrochloride , Agar well diffusion, Poison plate methods.

There has been considerable interest in antimicrobial activity in recent years because of their potential applications. For example, recently, the search for novel antifungal and antibacterial compounds has received special attention as a result of enhanced microbial resistance to current pesticides[1]. Initially, plant extracts[2-9] and isolated pure natural products[2,3] have been used as initial antimicrobial agents. Following this, the isolation and structural elucidation of bioactive compounds from fractionated extracts have led chemists to mimic and synthesized similar compounds as antimicrobial agents[11-12]. Via a modulation of the structure of drugs, variation in the potency of their antimicrobial activity have been achieved[12-15]. Even though there are several reports of synthesized compounds such as porphyrins and other porphyinoid as antimicrobial agents[13-16], there is no documentation of mesoporphyrin(ix) dihydrochloride as an antimicrobial agent. In our continuing search for novel, potent and selective antimicrobial agents, we report here an investigation of the antimicrobial activities of (1) against *Candida albicans*, *S. aureus* and *E. coli* via Agar diffusion and Poison plate techniques[17,23]. We have recently communicated the antimicrobial activity of α,β, unsaturated carbonyl compounds[34,35]. *Candida albicans* is a diploid fungus (i.e a form of yeast) and is a casual agent of opportunistic oral and genital infection in humans[18]. *S. aureus* can cause furuncles (i.e. boils) and carbuncles[19]. *E. coli* can cause several intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonea, mastitis, septicemia and gram negative pneumonia[20].

Porphyrins are a versatile class of chemical compounds, having found applications in the field of supramolecular chemistry[24-30], catalysis [27-28], oxygen haemoglobin mimics[29-31] and in nanotechnology[32-33]. etc. Compound (1), mesoporphyrin (ix) dihydrochloride (7,12-

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diethyl-3,8, 13, 17 – tetramethyl – 21H, 23H-porphine-2,18-dipropionic acid dihydrochloride, $C_{34}H_{38}N_4O_4$ was purchased from Aldrich chemical company in the USA and its structure is shown below.

MATERIAL AND METHODS

Reagents and materials

Mesoporphyrin (ix) dihydrochloride (1) and solvents were purchased from Aldrich. Antibiotics, Ampicillin and nystatin, Mueller Hinton agar, agar plates and microbial discs were purchased from the International Pharmacy Association in Guyana. Bacterial and fungal cultures were obtained at John's Science Centre Berbice Campus, University of Guyana.

Preparation of Mesoporphyrin (ix) dihydrochloride

Compound (1) was made up to the appropriate concentration of 10 mg in 10ml (1mg in 1 ml) of dichloromethane in a 25 ml round bottom flask and was stored under aseptic conditions.

Source of microorganisms

For the bacterial organisms, gram negative bacteria used was *Staphylococcus aureus* (ATCC 25923). For the fungi, yeast of the *Candida albicans* (ATCC 1023) species was investigated. These microorganisms were stored in a refrigerator at the microbiology laboratory at John's Science Centre Berbice Campus.

Reference and Control

The references were antibiotic in nature. Ampicillin and Nystatin, Ampicillin was choosen as the reference for all bacterial species used: *E. coli* and *S. aureus*. Nystatin was used as the reference for the fungus, *Candida albicans.* The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion [20].

Antimicrobial tests

Compound (1) was investigated for their antimicrobial activity using the Agar diffusion[17,23] and Poison Plate techniques[17,23] under aseptic conditions.

Aseptic conditions

The aseptic chamber consists of a wooden box (1m x 1m x 0.5m) with a door which was cleaned with 70% ethanol and irradiated with short wave UV light for 1 hour.

Mother plates

These were made by culturing *C. albicans* on PDA (Potato Dextrose Agar). A sterilized 9 cm cork borer was used to cut agar discs in the plate.

Potato dextrose agar (PDA)

This is an agar medium on which the fungi was cultured. The potato was peeled and 200g weighed, finely chopped and boiled to a mash in distilled water. The dextrose was weighed (12.5g) and placed in a 1L measuring cylinder. Agar was weighed (12.5g) and added to the measuring cylinder (with the dextrose). The potato mash was stirred and strained into the cylinder. Distilled water was added to make up the solution to 500mL.The contents was continuously stirred until consistency was achieved and was then poured into a conical flask, plugged with cotton wool, over which aluminium foil was tightly wrapped. The flask was then autoclaved at 121^0C, 15psi for fifteen minutes.

Agar diffusion Technique

The spore suspension of pathogens was seeded into a molten PDA medium or poured into petri plates. When the medium solidified, a 9 cm well was made at the centre of the plate with the help of a sterile 9 cm cork borer. A solution of the test compound (1), at a concentration of 1mg in 1ml was transferred into the well and incubated for three days. The zone of inhibition in mm^2 was measured for the test compound and recorded. From these values, the area of inhibition was calculated.

Poison Plate technique

Under asceptic conditions, the test compound was seeded into molten PDA medium and poured into Petri plates. The plates were covered and allowed to cool. As soon as the agar was solidified, a 9 cm sterile cork borer was used to make a disc on the pathogen plate (Mother plate). A pathogen disc was taken from pathogen plate (mother plate) and kept at the centre of test compound seeded plate with help of a sterile innoculum needle and was incubated for 3 to 4 days. The innoculum needle was sterilized with alcohol and flame before each application.

The zone of inhibition was measured for pathogenecity of test compound. The experiment was repeated thrice. Triplicates were maintained for each test compound at every 24 hours interval. The diameter of mycelium growth was measured and the average value taken.

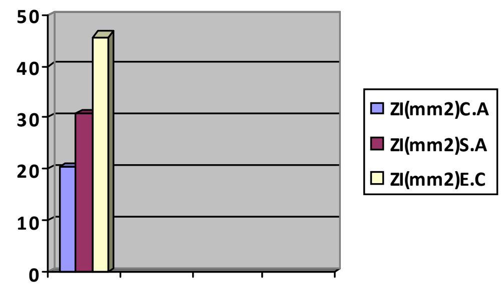
RESULTS AND DISCUSSION

This paper focuses on the investigation of the antimicrobial activity of mesoporphyrin (ix) dihydrochloride (1) against pathogenic microrganisms: *Candida albicans*, *E.coli* (gram negative bacteria) and *Staphylococcus aureus* (gram positive) using the Agar diffusion and Poison plate methods[17-23]. For each microbial experiments, triplicates were done and the average value taken to calculate the area of inhibition.

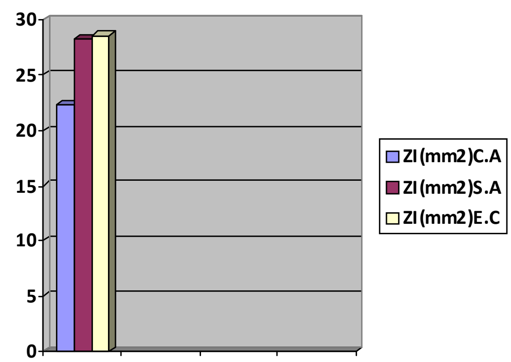
The zone of inhibition (mm^2) is quoted at the ED_{50} value and as the area of inhibition (mm^2). ED_{50} is the effective dose concentration of the sample required to kill 50% of the pathogen growth. The zone of inhibition in mm at ED_{50} was calculated and converted into area of inhibition, mm^2. First, a random check of the compound antimicrobial activity was investigated using both methods, Table 1. These served as the reference experiments. Ampicillin was used as the reference for bacterial species and Nystatin as the reference for the fungal species. For fungus *Candida albicans* with Nystatin and bacteria, *S. aureus*, the area of inhibition (mm^2) was 29.59 mm^2 (ED_{50} = 3.07 cm) and 28.26 mm^2 (ED_{50} = 28.26 cm) respectively. A controlled experiment was also investigated using the pure solvent, CH_2Cl_2 with the microorganism as the innoculant[23]. It was found that the pure solvent induced negligible zone of inhibition (< 5 mm) on the agar medium, Table 2. Thus, the zone of inhibition are indeed due to compound (1) rather than to the pure solvents.

Results indicate that with both methods of investigations, significant area of inhibition from 28.28 m^2 to 22.38 m^2 were noted. The largest zone of inhibition was obtained for the bacterial species over the fungal species, *Candida albicans*. Here again, as in our previous work, a variation in the structure of the porphyrin skeleton can lead to a variation in its structure activity relationship[33-34].

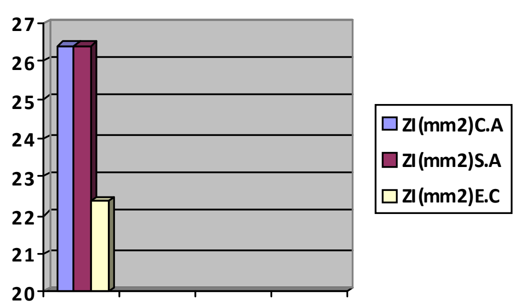
The largest area of inhibition was observed for bacteria, *E. coli* using the diffusion



a) A plot of area of inhibition (mm^2) versus organism type for Random check experiment



(b) A plot of area of inhibition (mm^2) versus microbial organisms type for compound (1) using the diffusion plate technique



(c) A plot of zone of inhibition (mm^2) versus microbial organisms type for compound (1) using the poison plate technique

Key to Graphs:
C. A = *Candida albicans*
S.A = *Staphylococcus aureus*
E.C = *Eschericia coli*

plate technique. However, with poison plate, this value was lowered. For example, with bacteria, *E. coli*, area of inhibition of 28.62 mm^2 (ED_{50} = 3.0 mm) was observed using the diffusion plate. However, with the poison plate, a value of 22.38mm^2 (ED_{50} = 2.67 mm) was observed for the same bacterial species.

It is interesting to note that the zone of inhibition induce by sample (1) against bacterial and fungal species are close to the value induced by the reference compounds, Nystatin for fungal species and Ampicillin for bacterial species, staphylococcus *S. aureus* and *E. coli*. For example, values of 22.38 mm^2, 28.26 mm^2 and 28.62mm^2 were obtained against *Candida albicans*, *S. aureus* and *E. coli* respectively. Comparatively for the diffusion plate, values of 22.38 m^2, 28.26 m^2 and 28.62 mm^2 were observed for *Candida albicans*,

Table 1. Random check (1 mg in 1 ml) (Reference experiments)

	Sample Number	Fungus, (*Candida albicans*) with Nystatin)	Bacteria, (*Staphylococcus aureus* with Ampicillin	Bacteria, (*E .coli)* with Ampicillin
(Average of Triplicate) $ED_{50\ (mm)}$	(1)	3.07	3.0	3.07
Area of inhibition (mm^2)	(1)	29.59	28.26	29.59

Table 2. Controlled experiment (1 mg in 1 ml)

	Sample Number	Fungus, (*Candida albicans*) with Nystatin)	Bacteria, (*Staphylococcus aureus* with Ampicillin	Bacteria, (*E .coli)* with Ampicillin
(Average of Triplicate) $ED_{50\ (mm)}$	(1)	< 5	< 5	< 5
Area of inhibition (mm2)	(1)	< 5	< 5	< 5

Table 3. Diffusion Plate (1 mg in 1 ml)

	Sample Number	Fungus, (*Candida albicans*) with Nystatin)	Bacteria, (*Staphylococcus aureus* with Ampicillin	Bacteria, (*E .coli)* with Ampicillin
(Average of Triplicate) $ED_{50\ (mm)}$	(1)	2.67	3.00	3.00
Area of inhibition (mm2)	(1)	22.38	28.26	28.62

Table 4. Poison plate (1 mg in 1ml)

	Sample Number	Fungus, (*Candida albicans*) with Nystatin)	Bacteria, (*Staphylococcus aureus* with Ampicillin	Bacteria, (*E .coli)* with Ampicillin
(Average of Triplicate) $ED_{50\ (mm)}$	(1)	2.90	2.90	2.67
Area of inhibition (mm2)	(1)	2.90	2.90	2.67

S. aureus and *E. coli*. Compound (1) has potent antimicrobial properties against *S. aureus* and *E. coli* compared with the synthetic antibiotic, Ampicillin.

Graphs 1(a), (b) and (c) depicted, is a plot of the area of inhibition versus pathogenic organisms for the random check, diffusion plate and poison plate respectively. These graphical results mirror those in Tables 1-4.

Thus, sample 1 can be described as a potent antimicrobial agent that is non selective since there seems to be not much of a difference in its potency against the three pathogenic strains.

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