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RESEARCH ARTICLE

BIO TECHNOLOGY

ANTIBACTERIAL PROPERTIES OF ORGANIC GERMANIUM AGAINST SOME HUMAN PATHOGENS

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ABSTRACT

The antibacterial activity of Ge-132 (bis-betacarboxy-ethylgermanium sesquioxide/organic germanium) was investigated against gram positive (*Bacillus subtilis, Streptococcus pyogenes* and *Methicillin-resistant Staphylococcus aureus*) and gram negative bacteria (*Aeromonas veronii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Vibrio fischeri*) using well diffusion and broth micro dilution technique test. Ge-132 showed inhibition activity towards both gram positive and gram negative bacterial species except *E. coli*. The results demonstrate that the test compound showed greater high inhibitory activity against gram positive strains than gram negative strains. Treatment for 250µg/ml concentration within 8-12h incubation results a maximum inhibition. Our findings suggest that organic germanium compound have potential to be developed as an antimicrobial agent against some bacterial infections.

KEYWORDS

Organic germanium, Antibacterial activity and well diffusion micro dilution test.

INTRODUCTION

Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health and its impact is particularly large in developing countries due to the emergence of widespread drug resistance[1]. Micro organisms are the causative agents of almost all kinds of acute and chronic diseases[2]. Metals are present in trace amounts as essential elements for biological systems which plays an important role in bioinorganic chemistry. In order to understand the role of metal ions in biological systems, structural studies of the biological compounds and their metal complexes are extremely important[3]. The antibacterial properties of metals have been recognised for centuries and have represented the most fundamental breakthroughs in medicinal history. The first laboratory antibacterial experiments involved a metal compound, investigated and confirmed the activity of mercuric chloride on anthrax spores[4].

Germanium and its compounds were used as dietary supplements since 1970s. It also induce erythropoietic, antimicrobial or immunomodulating activities, anti-viral activity, immunity enhancement, oxygen enrichment, free radical scavenging, heavy metal detoxification, induction of interferon, macrophages and T-suppressor cells and augmentation of natural killer cell activity, healthy people and patients undergoing therapeutic treatments use germanium compounds daily[5]. Other organic germanium such as germatranes, spirogermanium, 2-carboxy germanium sesquioxide, carboxy germanium sesquisulfide and germa-γ-lactones have been reported for a variety of activities including their possible use in suppression of the growth of certain tumours, proliferation of the normal marrow cells in the tumour bearing animals, in pain relief, hepatic cirrhosis, cardiovascular function, motor activity and stimulation of red blood cells [6]. Germanium rapidly removed from plasma and does not accumulate in tissues [7]. The series of trialkyl, ethyl, propyl germanium acetates have antifungal and antibacterial activity [8]. Reports on the antibacterial activity of Ge-132 are not sufficient to confirm the antibacterial activity of this organic metallic compound. Our study tried to explore the *In vitro* antibacterial property of bis-betacarboxy-ethylgermanium sesquioxide against some gram positive and gram negative pathogens that causes the most common infectious diseases.

MATERIALS AND METHODS

(i) Bacterial stains and inoculums preparation:

All the microbial strains of human pathogens used in the antimicrobial bioassay were *Aeromonas veronii* (MTCC 3249), *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 424), *Streptococcus pyogenes* (MTCC 1924), *Methicillin-resistant Staphylococcus aureus* (MRSA)(MTCC 84), *Vibrio fischeri* (MTCC 1738) procured from MTCC Chandigarh, India. The stock cultures of these micro organisms were maintained at -20^{o}C. The inoculums were prepared from stock cultures by streaking on Mueller-Hinton agar (Hi- Media, India) incubated at 37^{o}C for overnight. The 5ml broths were inoculated with respective cultures and incubated on an orbital shaker (150 rpm) overnight at 37^{o}C [9].

(ii) Drug:

Bis-betacarboxy-ethylgermanium sesquioxide (Alfa Aesar India), Ciprofloxacin (Sigma Aldrich, USA) was dissolved and further diluted in Dimethyl sulphoxide (Qualigens, India).

(iii)Agar well diffusion method:

Well diffusion method was previously described [10]. The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile Petri dish Mueller-Hinton agar. Four wells (6mm diameter) were made in each of these plates using sterile cork. About 5µL, 10µL, 20µL of drug were added to each of the wells and allowed to diffuse at room temperature for 24h under aerobic condition. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm. The effect of test compound was compared with the standard antibiotic Ciprofloxacin. Triplicates were maintained and the mean values were recorded.

(iv)Broth micro dilution test

The strains were tested using the micro dilution method [11], using two different culture media: Mueller-Hinton broth and Luria Bertania (Hi- Media Laboratories, India). A total of 10 µL of each of the drug dilution was inoculated with 5 µL of a bacterial suspension (10^8 CFU/mL or 10^5 CFU/well), and the mixture was incubated at 36ºC for 18 h for growth. Further 1:2 serial dilutions were performed by addition of culture broth to reach concentrations ranging from 2 to 0.0156 mg/mL; 100 µl/L of each dilution were distributed in 96-well plates, as well as a sterility control and a growth control (containing culture broth plus DMSO, without antimicrobial substance). Minimum inhibitory concentration (MIC) values were defined as the lowest concentration at 99.9% of the inoculum was killed. The results were expressed in milligrams per millilitres.

RESULT AND DISCUSSION

The purpose of this study was to investigate the effects and potency of ge-132 on human pathogenic micro organism. The compound was tested for the antibacterial activity against strains of gram positive and gram negative bacteria using Well diffusion and Micro dilution method has been listed in Table1 and 2.

Table - 1

Antibacterial activity of the Organic germanium (Ge-132) selected human pathogens

Micro organism	Zone of inhibition (in mm)			
	Ciprofloxacin (µg/ml)	Ge-132(µg/ml)		
	30	250	500	1000
Gram positive bacterial strains				
Bacillus subtilus	21	21	25	32
Streptococcus pyogenes	26	18	21	24
Methicillin-resistant Staphylococcus aureus	31	16	21	23
Gram negative bacterial strains				
Aeromonas veronii	35	9	13	22
Escherichia coli	25	-	10	8
Klebsiella pneumoniae	29	-	18	9
Pseudomonas aeruginosa	41	10	20	25
Vibrio fischeri	21	12	13	19

- *indicates no activity*

The Ge-132 possesses more antibacterial activity against gram positive bacteria than gram negative bacteria. The compound showed the highest and strongest inhibitory property against *Bacillus subtilis* (21mm at 250µg/ml, 25mm at 500 µg/ml, and 32mm at 1000 µg/ml) compare to standard, also showed moderate activity against gram negative bacteria except *E. coli.* In present study the highest antimicrobial activity of the compound was observed to be in minimum concentration. The inhibitory action decreases as the compound concentration increases.

The organo selenium compounds showed moderate to good inhibition activity against the bacterial species, showed significant inhibitory activity at lower concentrations against both gram positive and gram negative bacteria except expect *S. typhimurium*[12]. The inhibition effect of the compound was determined using well diffusion method, confirmed by the MIC. The MIC of Ge-132 resistant to bacteria range from 15.8 to 500 µg/ml and showed good inhibitory activity against both gram positive and gram negative bacteria in lowest concentration. Ge-L (Germa-c-lactones) has antibacterial activity against a wide range of gram-negative *Bacilli* (such as *Klebsilla pneumonae*, *P. mirabilis* and *P. vulgaris*), very significant on *P. mirabilis*, whereas a weak effect was found on other *Bacilli* [13]. Ge-132 has antibacterial activity against a wide range of gram-positive strains in which *Bacillus subtilis* showed inhibitory property at lower concentration than other two gram positive bacteria.

Table - 2
Antimicrobial activity of Organic Germanium (Ge-132) eight selected species of bacteria assayed by the agar dilution method

Microorganisms	Ciprofloxacin (µg/ml)	Ge-132 (µg/ml)
Aeromonas veronii	7.9	62.5
Bacillus subtilis	2.5	15.8
Escherichia coli	2.5	>500
Klebsiella pneumoniae	15.8	500
Methicillin-resistant Staphylococcus aureus	5	125
Pseudomonas aeruginosa	7.9	62.5
Streptococcus pyogenes	31.8	<500
Vibrio fischeri	1.25	250

Gram-negative organisms have an outer membrane, which provides an effective control over the uptake of toxic substances, and as a consequence are generally more resistant to antibacterial agents [14]. Table 1 confirms that among gram negative bacteria *Pseudomonas aeruginosa* and *Vibrio fischeri* has moderate inhibitory property than *Klebsilla* and *Aeromonas* in minimum concentration. The efficiency of Ge-132 on gram positive bacteria is slightly greater than gram negative bacteria. This specificity of activity may be due to the sensitivity of the test compounds is associated with the different cell wall structure. Similarly the metal Cobalt, Nickel, and Zinc from metal complex 2-(2'hydroxynaphthyl) benzoxazoles showed good antibacterial activity against *Bacillus subtilis* and Staphylococcus aureus[15]. When we compare the results of well diffusion method and MIC, *Bacillus subtilis* showed more susceptible to the compound at lower concentration within 8-12h incubation at 37^{o}C and delay or no growth in micro organism after a period of 2 days dependent on the concentration tested. Ge-132 had less or no activity against *E. coli*.

CONCLUSION

In conclusion, we have shown that a systematic significant antimicrobial activities against some selected gram positive and gram negatives bacterial strains. *Bacillus subtilis* is more sensitive to Ge-132, but *E. coli* showed less sensitivity. It had a potential antibacterial effect on other strains of bacteria which indicates that germanium 132 and its organic derivative may have significant clinical efficacy.

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