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Antibacterial Activities of Guanidine Family Single Crystals against *Bacillus Subtilis* and *Staphylococcus Aerus*

D. Sathya[1], S. Anbu Radhika[2], V.Sivashankar[1], D. PremAnand*[1]

[1]Dept of Physics, St. Xavier's College, Palayamkottai, Tirunelveli-627002, Tamilnadu, India
[2]Dept of Zoology, PMT College, Melaneelithanallur, Sankarankoil, Tamilnadu, India

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Abstract: A Guanidine derivative single crystal has been grown by simple ambient temperature gradient solution growth technique. The as grown single crystals tested against two bacterial pathogens like *Bacillus subtilis, Staphylococcus aerus.* The result shows that the single crystals have better resistance against the microbes. The standard antiseptic solution like kanamycin, it showed less or equal activity in pathogenic bacteria. In comparison to our currently used Guanidine derivatives single crystals, Guanidine Acetate shows better zone of inhibition against both the pathogens. But in contrast the Guanidine maleate and Guanidine Tetrafluroborate reveals lowest zone of inhibition against *Bacillus subtilis* and *Staphylococcus aerus* respectively. It can be concluded that Guanidine acetate exhibit excellent antibacterial effect and therefore, seems to be suitable as a local antiseptic agent, but further clinical studies are necessary.

Keywords: *Bacillus subtilis*, *Staphylococcus aerus*, kanamycin, Guanidine acetate, antibacterial effect.

INTRODUCTION

Guanidine single crystals have vast applications in telecommunication and Photonic devices and also in other optical devices. Recently Guanidine crystals found application in biological field, for example, guanidinium base is present in most of the drugs clinically used such as guanabenz, an α2-adrenoceptor agonist used as an antihypertensive, the anticancer drug imatinib[1]. Pharmacological investigation of new guanidine compounds has led to the development of medications with interesting activities for the treatment of infectious diseases. From the chemical point of view, guanidine is a strong Lewis base and the guanidinium cation may be easily anchored onto numerous inorganic and organic anions and poly-anions through hydrogen-bonding networks[2]. Guanidinium ion is relatively simple chemical species, whose structure is related to those of amides and proteins in which there is considerable interest. The guanidinium ion can form a broad family of hydrogen bonded crystals[3]. The physiological aspect of single crystals has received considerable attention, and much effort has been devoted to the synthesis of different kinds of guanidine compounds are arranged to get better antibacterial activities[4]. In the present study, we have developed guanidine-based organic single crystals and assessed their antibacterial activities against standard strains of pathogenic bacteria's like *Bacillus subtilis* and *Staphylococcus aerus* in order to address the antiseptic stability of guanidine derivatives. In continuation of our previous studies[5-8] in Guanidine compounds, we report here the synthesis and antibacterial activity of Guanidine derivatives which was prepared as a single crystal in the reaction of Guanidine carbonate admixture aminoacids like tartaric acid, acetic acid, maleic acid, acrylic acid and tetrafluroboric acid in absolute solvent upon low temperature solution growth technique. From that, the Guanidine tartarate (Guhtt), Guanidine acetate (GuAce), Guanidine maleate (Gumlt), Guanidine acrylate (GuAcr) and Guanidine tetrafluroborate (Gutfb) single crystals are harvested.

MATERIALS AND METHODS

Crystallization method: Single crystals of Guanidine maleate are grown by slow evaporation method. The maleic acid and guanidinium carbonate taken in the equimolar ratio and dissolved in double distilled water. The solution is stirred continuously using magnetic stirrer for 5 hours. The reactor is filtered, kept undisturbed in room temperature for slow evaporation. The acidic portion of maleic acid is the carboxyl group; it reacts with the Guanidine cation to form Gumlt single crystals. The final product of synthesis is purified by repeated crystallizations from water solution. The transparent single crystals are obtained after 20 days of the growth. Similarly, Guanidine tartarate, Guanidine acetate, Guanidine acrylate, Guanidine tetrafluroborate single crystals are also grown.

Antimicrobial test: The antibacterial assay was done according to the Bayer method[9]. The bacterial test pathogens were spread on fresh Muller Hinton Agar (MHA) plates with the help of cotton swabs to form an even lawn of the test bacteria. The Whatmann filter paper disc impregnated with the test compounds were placed on the surface of the MHA plates seeded with test bacteria and the plates were incubated in a BOD Incubator (Caltan-152, Narang Scientific Works, New Delhi, India) for 24 hours at 37 ± 2ºC. The inhibition zones around each disc (Plate 1) were measured after 24 hours of incubation with the help of zone reader.

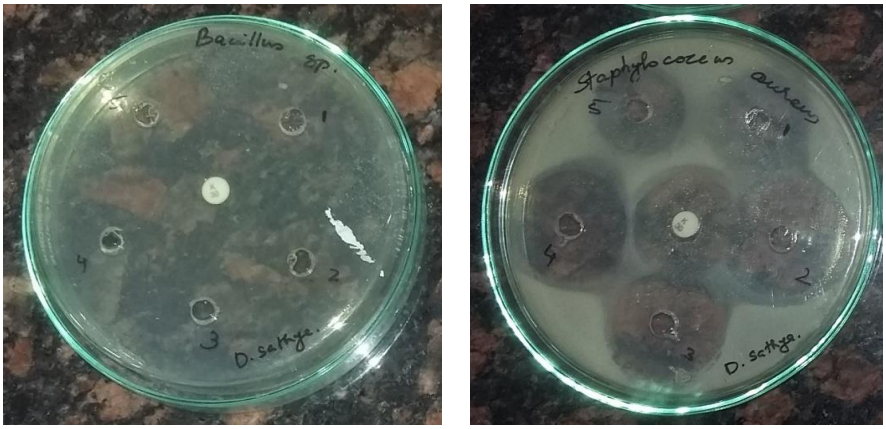

Plate 1. The antibacterial activity of the Guanidine derivatives.

RESULTS AND DISCUSSION

The potency of the organic guanidine single crystals exposed to antibacterial drug was evaluated against two pathogenic bacterial strains. The biological activities of the Guanidine derivatives have been studied for its antibacterial activities using disk diffusion method[10] in vitro against two pathogenic bacteria *Staphylococcus aureas* and *Bacillus subtilis* at concentration of 10g/ml. Kanamycin was used as standard drug for the comparison of bacterial results. The newly synthesized Guanidine derivatives have exerted significant inhibitory activity against the growth of the tested bacterial strains and data reveal that Guanidine derivatives have significant influence on the antibacterial profile of *S. aureus* and *B. subtilis* as reported in the **table 1**. From that, the Guanidine acetate exhibits the highest activity against both *Bacillus subtilis* and *Staphylococcus aerus* pathogens. The figures, shows the GuAce act as the best antibacterial agent when compared to the standard kanamycin.[11]. Because of the acetic acid has been commonly used as disinfectant of wounds and, especially, as an antiseptic agent in the treatment and prophylaxis of the plague [12].

Table 1. The antibacterial activity of the Guanidine derivatives.

Sample ID	Zone of inhibition (mm)	
	Bacillus subtilis	*Staphylococcus aerus*
1-Guhtt	26	26
2- GuAce	35	32
3-Gumal	26	27
4-GuAcr	30	28
5 – Gutfb	27	21
Standard Kanamycin (30 µg)	27	25

The Guanidine maleate and Guanidine tartarate shows a minimum activity against *Bacillus subtilis* bacteria and Guanidine tetrafluroborate shows the low activity against *Staphylococcus aerus* pathogen. The Guanidine derivatives with malaete and tartarate have less antibacterial activity against *B. subtilis*; and Guanidine derivatives with tetrafluroborate have less activity against *S. aerus*. Gautam et al. 2015 [13] also confirmed the antibacterial activity of *S. aerus* against 2-Chloro-5-Ethoxy-3,6- Bis(Methylamino)-1,4-Benzoquinone single crystals.

The activity of the Guanidine compounds towards *Bacillus subtilis* decreases in the order GuAce > GuAcr > Gutfb = Kanamycin > *Guhtt= Gumal*. The same activity of *Staphylococcus aerus* decreases in the order GuAce > GuAcr > Gumal> Guhtt > kanamycin > Gutfb **(Figure 1).**

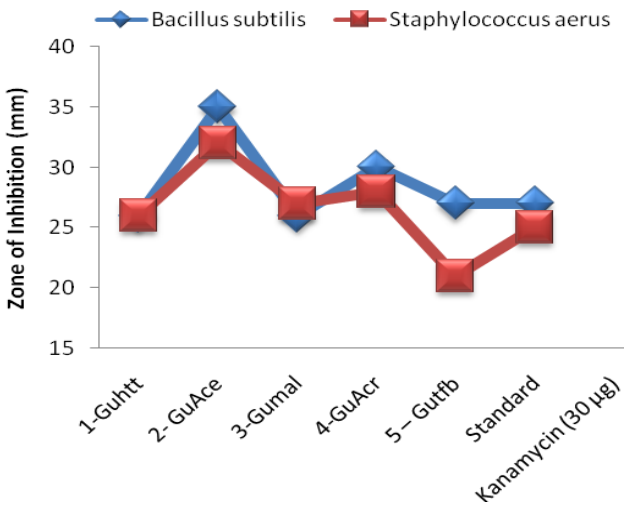

Figure 1. The antibacterial activity of the Guanidine derivatives.

In this study, the Guanidine derivates are demonstrated and exhibits excellent elimination of all tested bacterial pathogens. Although there have been earlier studies[14] investigating the bactericidal effect of acetic acid, this study is the first to compare the bactericidal effect of commonly used topical antiseptics with the effect of acetic acid in a wide spectrum of pathological bacteria.

CONCLUSIONS

The present work summarizes a range of approaches that lead to Guanidine derivative single crystals that are potentially suitable for biological applications. The Guanidine derivatives were grown by the simple slow evaporation technique. The results of the preliminary antimicrobial screening against bacterial species indicate that the Guanidinium derivatives are moderately active and could be further screened *in vitro* against a wide range of pathogens. From the observation, the Guanidine acetate is quite specific in controlling the growth of both pathogens *Bacillus subtilis* and *Staphylococcus aerus*. Hence, this result provides a strong platform for further researchers to probe and develop organism specific antibiotic and

Guanidine acetate exhibit excellent antibacterial effect. It seems to be suitable as a local antiseptic agent, but further clinical studies are necessary.

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*** Corresponding author: D. PremAnand;**

[1]Dept of Physics, St. Xavier's College, Palayamkottai, Tirunelveli-627002, Tamilnadu, India

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