ANTIBACTERIAL EFFCT OF GUANIDINE DERIVATIVE NLO SINGLE CRYSTALS GROWN BY TEMPERATURE GRADIENT METHOD

D. SATHYA[1], S. BALASUBRAMANIAN[2], S. ANBU RADHIKA[3*]

[1]Dept of Physics, Sadakathullah Appa College, Rahmath Nagar, Tirunelveli-627011, Tamilnadu, India

[2]Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam- 629502, Tamilnadu, India.

[3*]Dept of Zoology, PMT College, Melaneelithanallur- 627953, Sankarankovil, Tamilnadu, India

*Address for correspondence: Email - sanburadhika90@gmail.com

Abstract- A solitary precious stone of Guanidine subordinates have developed by sluggish vanishing arrangement development procedure. The limiting of Guanidinium with amino acids was examined. The Guanidine subsidiaries were identified through powder XRD crystallography and evaluated for its antimicrobial action. In the current work Organic and metal organic Guanidine derivative single crystals are taken into Powder XRD, Single crytstal XRD and antimicrobial movement. Guanidine family single gems (Guanidine tartarate, Guanidine acetic acid derivation, Guanidine maleate and Guanidine acrylate single crystals) are thought about for this work. The Guanidine subordinates displayed inhibitory outcomes against Escherichia coli, Enterobacter aerogens, Pseudomonas aeruginosa and Proteus vulgaris the obvious upgrade in the strength as antibacterial specialist. The examples are taken into well dispersion strategy with various antimicrobes the resultant better obstruction from the microorganisms are noticed. The presence of unequivocal zone of hindrance of any size around the very much showed antibacterial action and the zone of restraint was estimated. The compound was viewed as dynamic against a bacterium on the off chance that the hindrance zone was 6mm or more. The tests showed superb bactericidal impact of acidic corrosive and acrylic corrosive, especially with P. aeruginosa. The less inhibitory action displayed against the germicide Guanidine subsidiaries by P. vulgaris. In contrast with our as of now involved kanamycin as germicide arrangement, it showed comparative - in certain microscopic organisms, far better - bactericidal properties. It tends to be finished up Guanidine acetic acid derivation and Guanidine acrylate make incredible bactericidal difference and, in this manner, is by all accounts reasonable as a neighborhood sterile specialist, yet further clinical investigations are important.

Key Words- Guanidine subsidiaries, Escherichia coli, Enterobacter aerogens, Pseudomonas aeruginosa and Proteus vulgaris

I. INTRODUCTION

The expanded obstruction of microorganisms to antimicrobial specialists forces the quest for option and more compelling specialists. By and large, antibacterial specialist compound blend is separated into two classes: natural and inorganic antibacterial specialist [1]. Generally, natural mixtures have found boundless purposes in medication and veterinary practice [2]. As of late, natural guanidine compounds have drawn in emphatically consideration because of their adaptable organic action, for example, antifungal and antibacterial [3-5]. Guanidine is a sort of straightforward and solid base in a natural science and has broad application in many fields [6-8]. Guanidine bases and their naturally dynamic buildings have been frequently utilized as denaturant of proteins in the natural science of change components as radiopharmaceuticals for absorption, as model frameworks for organic macromolecules, as impetuses and as protein denaturant [9]. Guanidine has been generally utilized for a long time as a clean in medication and the food business as a mouth wash, as a sanitizer for an assortment of strong surfaces and furthermore in water medicines [10]. Vivek et al. explained about Development of organic crystalline nature guanidinium nitrate (GuN): structural, frontier molecular orbital, optical, thermal, mechanical, theoretical and experimental ways [11]. Various types of Guanidine salts have been presented as microbicides [12]. Atomic guanidine, similarly as the anionic and cationic salts (i.e., NaCN3H4 and CN3H6Cl) of the key biomolecule, is an ideal model substance to concentrate on hydrogen-reinforced networks and the impact of charges and counterions on these organizations [13]. While cationic salts of guanidine have been known for a long time and are industrially accessible, the anionic mixtures were incorporated as of late and offer intriguing new designs [14]. Fluid translucent material gives new chances to plan material connection points that can report designated natural collaborations Liquid glasslike materials [15]. A good quality biologically active single crystal of L-tryptophan L-tryptophanium bromide (LTTB) with molecular formula C22H27BrN4O5 was successfully synthesized and grown from aqueous solution using slow evaporation solution growth technique was proposed by Darling et al. [16]. The single gems arranged from Guanidine admixtured aminoacids. In natural single gems, Guanidine subordinates have been broadly utilized as great stage move impetuses in alkylation and epoxidation particular base impetuses in lopsided responses [17]. The LDT value of MCAM crystal was found by using Nd: YAG laser. Also Hirshfeld surface and antibacterial activity analyses for the sample have been carried out by Vasumathi et al. [18]. Coincidentally, the Guanidine acetic acid derivation, Guanidine tartarate, Guanidine maelate has been accounted for to have intriguing germicide specialist and natural action [19]. So that, to investigate the impact of the microstructure and gem on the

antibacterial properties of Guanidine family precious stones (Guanidine tartarate, Guanidine acetic acid derivation, Guanidine maleate, Guanidine acrylate and Guanidine tetrafluroborate), the single precious stones were blended by sluggish dissipation strategy from various aminoacids and the antibacterial exercises against Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, and Enterobacter aerogens were assessed. Additionally, the antibacterial exercises of Guanidine subordinates were concluded.

II. MATERIALS AND METHODOLOGY

2.1. CRYSTALLIZATION METHOD

Single crystals of Guanidine acetate were obtained by slow evaporation of an aqueous solution containing Guanidine carbonate (AR grade) and glacial acetic acid (AR grade) in the 1:1 stoichiometric ratio. The solution was stirred continuously using a magnetic stirrer. The prepared solution was kept undisturbed in room temperature. The product was then purified by repeated crystallization until optically clear tiny crystals were obtained. Small (seed) crystals were grown from saturated aqueous solution by the free evaporation technique at constant temperature (30 oC). Good quality seed crystals were selected for the growth of large single crystals. Large size single crystals were grown by the slow cooling method using an optically heated constant temperature bath of control accuracy ± 0.01 oC set at 45 ◦C. A cooling rate of 0.5 oC per day was employed throughout the growth period. The natural NLO Guanidine acetic acid derivation single gems of aspects 12 × 8 × 5 mm3were gathered within three to four weeks (Fig. 1). Additionally, Guanidine maleate (Gumal), Guanidine tartarate (Gutt) and Guanidine acryalte (GuAcr) single precious stones likewise developed.

2.2. STRUCTURAL ANALYSIS

2.2 A) POWDER X- RAY DIFFRACTION ANALYSIS

The powder X-ray diffraction technique has been employed to identify the crystalline phases of the samples using monochromatized Cu- Kα: Aλ = 1.5056 Å) on X-ray diffractometer (Model: PW3040/60 X' pert PRO) available at CECRI, Karaikudi. The data collection was over the 2-theta range of 3º-80º in steps of 0.1º /sec. The particle size was measured from X-ray broadening by employing the well-known Scherrer equation,

$$D = 0.96\lambda/\beta \cos\theta$$

Where, D - particle size of the sample (nm), λ - wavelength of the X-ray (1.5056 Å), β - width of the XRD pattern line at half peak-height (Rad), θ - angle between the incident and diffraction beam (0).

2.2 B) SINGLE CRYSTAL X- RAY DIFFRACTION ANALYSIS

The as grown Guanidine derivative single crystals (Guanidine Acetate (GuAce) Guanidine maleate (Gumal), Guanidine tartarate (Gutt) and Guanidine acryalte (GuAcr)) were subjected to characterize single crystal X-ray diffraction. XRD data were collected using an ENRAF NONNIUS- CAD 4 single crystal X-ray diffractometer with MoKα (λ= 0.71073 Å) radiation at room temperature to estimate lattice parameter values.

2.3 SHG EFFICIENCY

The Second Harmonic generations of the as grown single crystals were perfomed using a Q-switched Nd: YAG laser by employing Kurtz powder test. The crystalline powder samples were filled with microcapillary tubes of about 1.5 mm in diameter. Nd:YAG laser λ=1064 nm with input beam energy 3.8 mJ/pulse and pulse width 10 ns with a repetition rate of 10 Hz were used. The crystalline powdered samples with a uniform particle in the microcapillary tube were exposed to the laser radiation. The second harmonic signal generated from crystalline powder sample confirmed by the bright green light emission (k = 532 nm) is observed, which indicates the second harmonic generation behavior of the particular single crystal. It was detected by a Photomultiplier Tube (PMT) and viewed on the oscilloscope. The optical signal fed into the PMT was converted into voltage output at the oscilloscope. The value obtained in the oscilloscope was compared with the voltage output for a standard KDP sample. The ratio of the two values gives the relative Second harmonic generation efficiency of the tested sample material.

2.3. ANTIBACTERIAL TESTS

The antibacterial tests were completed in gem research focus, St. Xavier's College, Palayamkottai. Tirunelveli Dist. The tests were done on four pathogenic microorganisms, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris and Enterobacter aerogens acquired from Expression Biotech, Nagercoil. KK Dist. Reference antibacterial medication Kanamycin drug was assessed for their antibacterial movement and their outcomes were contrasted and guanidine subsidiaries.

The well dispersion technique, utilizing Muller Hinton Agar (MHA), from the convention depicted by the National Committee for Clinical Laboratory Standard (NCCLS, 2004) was utilized for starter screening. Mueller-Hinton agar was ready from a monetarily accessible got dried out base as per the maker's directions. A few provinces of every microorganism were gathered and suspended in saline (0.9% NaCl). Then, the turbidity of the test suspension was normalized to match that of a 0.5 McFarland standard (relates to around 1.5 × 108 CFU/ml for microscopic organisms or 1 × 106 to 5 × 106 cells/ml for yeast). Each compound or reference was precisely gauged and disintegrated in the proper diluents (DMSO at 10%, methanol at 10%, or refined water) to yield the expected fixation (2mg/mL for compound or 1mg/mL for reference drug), utilizing sterile dish sets.

Whatmann channel paper number 1 was utilized to get ready plates around 6mm in measurement, which were gotten together with aluminum paper and cleaned via autoclaving. Then, at that point, 25 μL of stock arrangements of compound were conveyed to each circle, prompting 50 μg of compound or 25 μg of standard kanamycin drug. The dried surface of a MHA plate was vaccinated by flooding over the whole sterile agar surface with 500 μL of inoculum suspensions. The top was passed on a container for 3 to 5 minutes to consider any abundance surface dampness to be retained prior to applying the medication

impregnated circles. Circles containing the mixtures or antimicrobial specialists were applied in the span of 15 minutes of immunizing the MHA plate. Six circles for each petri dish were plated. The plates were upset and set in a hatchery set to 35oC. Following 18 hours (for microscopic organisms) and of hatching, each plate was analyzed. The distances across of the zones of complete hindrance (as decided by the independent eye) were estimated, including the width of the plate. The antibacterial action is communicated as the zone of hindrance in millimeters, which is estimated with a zone peruser [17]. The presence of unequivocal zone of restraint of any size around the very much demonstrated antibacterial movement and the zone of hindrance was estimated. All analyses were done in copy. The compound was viewed as dynamic against a bacterium assuming the hindrance zone was 6mm or more.

III. RESULT AND DISCUSSIONS

3.1. As grown single crystals

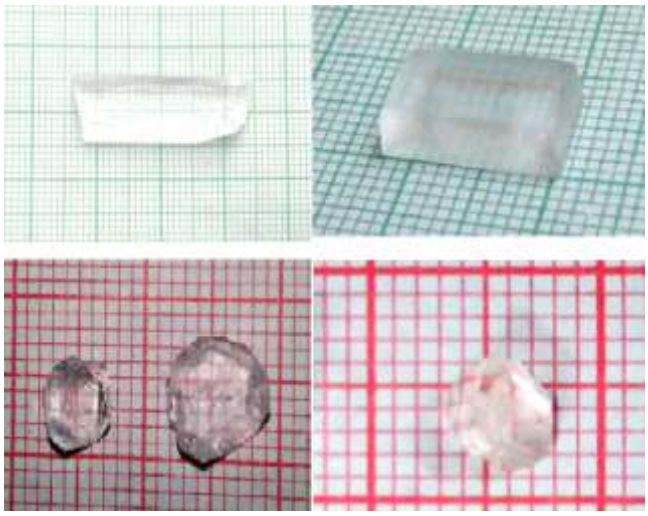

Figure 1: As grown single crystals of a) Gutt b) GuAce c) Gumal d) GuAcr

3.1. STRUCTURAL ANALYSIS

The grown Gutt single crystal belongs to orthorhombic system with the space group P212121. The lattice parameters are α=β=γ=900 and a = 11.339 (3) Å, b = 11.146 (3) Å, c = 6.657 (3) Å & V= 840.2 Å3. The obtained lattice parameter values are in good agreement with the reported literature values [20]. Density of Gutt was calculated by using ρ= MZ/ NV, where M is the molecular weight, Z is the number of molecules per unit cell, N is the Avagadro number and V is the volume of unit cell. Density (ρ) is found 1.6535 g/cm3.

The X-ray diffraction experiment shows that GuAce crystal belongs to the tetragonal crystal system with the space group P4122. The cell parameters of GuAce crystal obtained are, α=β=γ=900, a = 6.999(3) Å, b = 6.999(3) Å, c = 19.67(3) Å and V = 963 Å3. [19] The powder XRD pattern of the grown crystals of GuAce is shown in Fig. 4.3.It exhibits the d spacing with respect to the hkl values. The Powder XRD peak values are indexed using the Treor program.

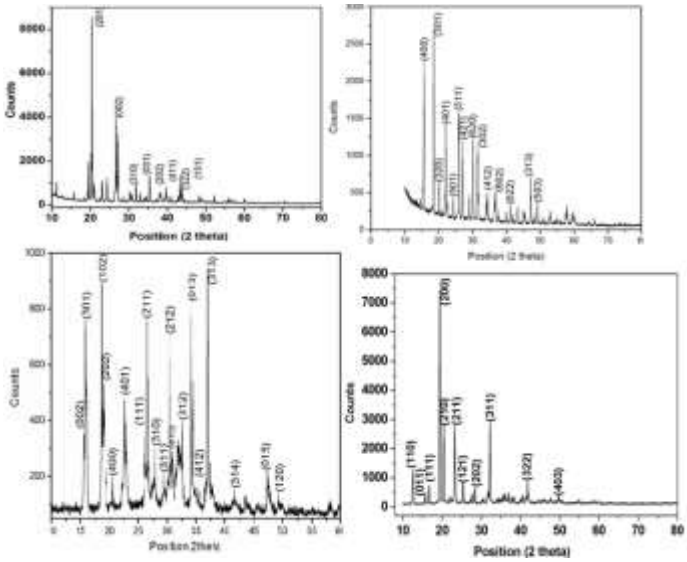

Figure 2: Powder XRD graph of a) Gutt single crystal; b) GuAce single Crystal; c) Gumal single crystal; d) GuAcr single crystal.

The grown Gumal single crystal belongs to orthorhombic system with the space group Pca21. The lattice parameters are α=β=γ=900 and a=19.197 Å, b= 3.679 Å, c= 11.324Å and V= 812 Å3 with four molecules per unit cell atom (Z= 4). The observed values are in well agreement with the already reported values of Sathya et al. [21]. All the observed reflection lines in XRD pattern are indexed using the Treor program.

The grown GuAcr single crystal belongs to orthorhombic system with the space group Pna21.The lattice parameters are α=β=γ=900 and a= 9.8351 Å, b=c= 8.2862 Å with four molecules per unit cell atom (Z= 4). Density of GuAcr was calculated by using ρ= MZ/ NV, where M is the molecular weight, Z is the number of molecules per unit cell, N is the Avagadro number and V is the volume of unit cell. Density is found 1.2752 g/cm3. The observed value has synchronized with the already reported values [22]. All the observed reflection lines in XRD pattern are indexed using the Treor program. Fig. 2d shows the Powder XRD graph of GuAcr single crystals.

3.2. SHG EFFICIENCY

The Nonlinear optical property of the as grown single crystals (Gutt, GuAce, Gumal, GuAcr) were carried out by kurtz and perry technique. The powdered sample with average particle size of 100-150µm was used to test the second harmonic generation property of Gutt. The second harmonic signal generated in the crystal was confirmed from the emission of green radiation by Gutt single crystal. The amplitude of the SHG output voltage was measured using a photomultiplier and a digitalizing oscilloscope assembly and it was found to be 1.2 times of KDP [20]. For GuAce single crystal,KDP crystal was taken as the reference material. The SHG efficiency of the GuAce is 0.8 times greater than that of KDP. This study confirmed the SHG by green radiation of GuAce. Hence, it can be used for applications in photonic and optoelectronic devices [19]. The output of Gumal is monochromotated at the intensity of 532nm emits green light. The emission of the Green light confirmed the presence of non linear optical property. Potassium dihydrogen phosphate (KDP) also powdered into the

identical size is used as reference material in this experiment. SHG value of Gumal single crystal is found to be 1.2 times greater than that of KDP [21]. For GuAcr single crystals, the value obtained in the oscilloscope was compared with the voltage output for a standard KDP sample. The ratio of the two values gives the relative Second harmonic generation efficiency of the GuAcr material (32.3 mV) is nearly 3.05 times that of KDP (10 mV). From the above observation, we confirm that the GuAcr is suitable for NLO applications [22].

Table 1: Comparison of crystals and their space group and SHG efficiency

Single Crystal	**Space group**	**Lattice Parameters**	**SHG efficiency**
Guanidine tartarate (Gutt)	$P2_12_12_1$	a = 11.339 (3) Å, b = 11.146 (3) Å, c = 6.657 (3) Å	**1.2**
Guanidine Aceatate (GuAce)	$P4_122$	*a = 6.999(3) Å, b = 6.999(3) Å, c = 19.67(3) Å*	**0.8**
Guanidine Maleate (Gumal)	*Pca21*	*a=19.197* Å, *b= 3.679* Å, *c= 11.324*Å	**1.2**
Guanidine Acrylate (GuAcr)	*Pna21*	a= 9.8351 Å, b=c= 8.2862 Å	**3.05**

3.2. ANTIBACTERIAL ACTIVITY

The power of the natural guanidine single gems presented to antibacterial medication was considered in contrast to four bacterial strains. The aftereffects of the fundamental screening acquired are introduced in table 2.

The outcomes show that Guanidine acetic acid derivation displays the most noteworthy zone of restraint against the Pseudomonas aeruginosa and Enterobacter aerogens. This shows that response of guanidine with the acidic corrosive assumes a significant part in upgrading its antimicrobial movement. Acidic corrosive is utilized (dependent upon the situation) in our copies unit at a convergence of 2.5% and has been episodically seen to decrease bacterial burdens while being all around endured by patients [23]. Pseudomonas aeroginosa were chosen to survey the antibacterial impact of the gem material found. Extraordinary consideration was given on Pseudomonas culture as a result of its high pathogenisity by causing illnesses like pneumonia, septic shock, urinary parcel disease, Gastro digestive contaminations, skin and delicate tissue contaminations [24]. Guanidine acrylate shows the greatest zone of hindrance against Escherichia coli and Proteus vulgaris. When contrasted with the standard kanamycin, Guanidine acrylate shows high zone of hindrance. It looks like Guanidine acrylate against to the microbes E. coli and P. vulgaris.

As per the above said results, Guanidine subordinates had a considerable antibacterial action against these microorganisms [25]. Guanidine subsidiaries and standard kanamycin were seen to areas of strength for show against these pathogenic microbes.

Table 2: antibacterial activity of guanidine derivatives against Escherichia coli, Enterobacter aerogens, Pseudomonas aeruginosa and Proteus vulgaris.

Sample ID	Zone of inhibition (mm)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Enterobacter aerogens*	*Proteus vulgaris*
1-Guhtt	*20*	25	24	13
2- GuAce	21	36	34	25
3-Gumal	20	27	25	20
4-GuAcr	32	31	30	28
5 – Gutfb	24	26	24	17
Standard Kanamycin (30 µg)	25	26	25	27

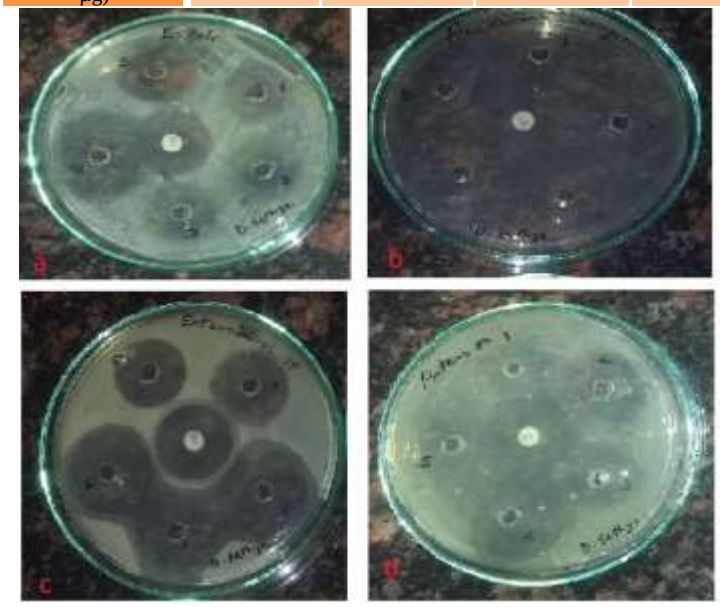

Fig. 1: Antibacterial activity of Guanidine derivatives against A) Escherichia coli, B) Pseudomonas aeruginosa, C) Enterobacter aerogens and D) Proteus vulgaris.

The Guanidine maleate and Guanidine tartarate shows a moderately low action against E. coli microbes. Guanidine tartarate shows low action against Enterobacter aerogens microbe. Guanidine tartarate shows least action against Pseudomonas aeruginosa and Proteus vulgaris. Fragrant mixtures apply antimicrobial action by modifying the construction and the development of pathogenic microorganisms. The instrument answerable for impervious to microorganisms can likewise incorporate catalyst restraint by the oxidized mixtures, potentially through response with carboxyl gatherings or through additional vague connections with proteins [26]. The amino N-molecules are engaged with intra chain hydrogen bonds.

The action of the Guanidine subordinates towards Escherichia coli diminishes in the request GuAcr > Kanamycin > GuAce > Guhtt= Gumal. In the interim, the antibacterial exercises of the

powders against E. coli are superior to S. aureus [27]. A similar movement of Pseudomonas aeruginosa diminishes in the request GuAce > GuAcr > Gumal = kanamycin > Guhtt. The comparative action of Enterobacter aerogens GuAce > GuAcr > Gumal= kanamycin = Guhtt. Relative exercises of Proteus vulgaris is GuAcr > Kanamycin > GuAce > Gumal> Guhtt. Antibiotic medication was utilized as standard for the examination of bacterial outcomes and the recently CT complex have applied huge inhibitory action against the development of the tried bacterial strains and information uncover that CT complex have critical effect on the antibacterial profile of S. aureus and B. subtilis and furthermore the CT complex showed inhibitory outcomes against E. coli and P. arenginosa [28, 29]. Maruthu et al. [30] has explained the slow evaporation solution growth method was used to grown a single crystals of amino acids in the presence of sodium acid phthalate. Based on single crystal X-ray diffraction investigation, the title crystals confirm to the orthorhombic structure of the space group B2ab. For our outcomes the kanamycin utilized as standard and it gives comparative and less movement against the chose pathogenic microscopic organisms. From this review, the Guanidine derivates are shown and displays fantastic disposal of all tried bacterial microorganisms. Especially, the GuAce and GuAcr were uncovered better antibacterial movement when contrasted with standard kanamycin and other Guanidine subsidiaries. Prior examinations[18] exploring the antibacterial impact of acidic corrosive, this investigation is regularly involved disinfectants with the impact of acidic corrosive in a wide range of neurotic microscopic organisms. In this article, we have summed up a scope of approaches that lead to Guanidine subordinate single precious stones that are possibly appropriate for natural applications are germicide and microorganism safe way of behaving.

IV. CONCLUSION

Good quality single crystals of Gutt, GuAce, Gumal and GuAcr were grown by temperature gradient slow evaporation technique. Powder XRD Single crystal XRD confirms that the crystals belong to the tetragonal system. SHG efficiency of the grown single crystals confirms by Kurtz and Perry technique. A focal message of this article is that the responsive idea of Guanidine subsidiary single precious stones gives new chances to develop NLO materials that can report designated natural associations. The aftereffects of the starter antimicrobial screening against four pathogenic microbes species demonstrate that the Guanidine subordinates is modestly dynamic and could be additionally separated vitro against a wide scope of microorganisms.

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AUTHORS
D. SATHYA
Department of Physics, Sadakathullah Appa College, Rahmath Nagar, Tirunelveli-627011, Tamilnadu, India
S. BALASUBRAMANIAN
Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam- 629502, Tamilnadu, India.
S. ANBU RADHIKA*
Dept of Zoology, PMT College, Melaneelithanallur- 627953, Sankarankovil, Tamilnadu, India
E. Mail: sanburadhika90@gmail.com