Materials Today: Proceedings xxx (xxxx) xxx

Contents lists available at ScienceDirect

Materials Today: Proceedings

journal homepage: www.elsevier.com/locate/matpr

Synthesis of silver nanoparticle using marine red seaweed *Gelidiella acerosa* -A complete study on its biological activity and its characterisation

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ARTICLE INFO

Article history:
Received 30 June 2020
Accepted 13 July 2020
Available online xxxx

Keywords:
$AgNO_3$
Anti-bacterial
FT-IR analysis
Zeta potential and AFM

ABSTRACT

The study was to evaluate the biological activity and agar extraction from nano synthesized red seaweed *Gelidiella acerosa.* The algae were Collected from the coastal areas of Rameswaram, TamilNadu. Seaweed was subjected to pre-treatment process with Alkaline pre-treatment. The aqueous extraction of *Gelidiella acerosa* were extracted by decoction method and Synthesis of $AgNO_3$ was carried out. Silver nano synthesized aqueous extract was observed for the biological activities such as Anti-bacterial. Antibacterial activity of the nanosynthesised seaweed extract was checked for gram-positive and gram-negative bacteria. The *Pseudomonas aeruginosa* and *Bacillus subtilis* bacteria contains a good of zones of inhibition. Furthermore, characterization studies such as UV–spectrometry analysis, FT-IR analysis, Zeta potential and AFM.

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1. 1.Introduction

Nanotechnology is involved in the synthesis of nanoparticle of variable sizes, shapes, chemical compositions and controlled disparity and their potential use for human edges. Although chemical and physical strategies might with success turn out pure, well-defined nanoparticle, these strategies square measure quite expensive and probably dangerous to the surroundings. Use of biological organisms such as microorganisms, algal extract or algal biomass could be an alternative way to chemical and physical methods for production of nanoparticle in an eco-friendly manner [1,2]. The algae play a vital role in economically and ecologically import for the photosynthetic nature. The algae are unicellular are multicellular organism that found everywhere in the environment such as marine water, freshwater or in the surface of moist rocks. Alga is categorized mainly of two types as microalgae and macro algae. Nowadays, the algae take a major role in aquaculture, pharmaceutical, cosmetics and other applications [3].Fig. 1.

Gelidiella acerosa is a red seaweed which that abundantly growing in the coastal area in the southern part India. It has been mainly used as a gelling agent to make for the cookery ingredient. Seaweed is an important source of seaweed polysaccharide such as agar, carrageenan and alginates which can be isolated for various applications as food, biomedical, pharmaceutical and biotechnological [4].

DIVISION: Rhodophytaceae
CLASS: Florideophyceae
ORDER: Gelidiales
FAMILY: Gelidiellaceae
GENUS: *Gelidiella*

These seaweed polysaccharides are termed hydrocolloids for their ability to form a gelled solution upon dissolving in hot water. Seaweeds are measured as a source of bioactive compounds which they able to produce a variable of secondary metabolites, which that characterized by a wide range of biological activity such as anti-bacterial, anti-fungal, anti-tumor, anti-inflammatory and also displays substantial antioxidant activity [5,6]. Among all the biological materials, algae AgNPs are referred to as -bionanofactorie as a result of each the live and dead dried biomasses were used for the synthesis of AgNP's. The metallic synthesis of silver nanoparticle has widely used for its anti-microbial, anti-tumor, anti-viral, anti-inflammatory activities [7,8].

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https://doi.org/10.1016/j.matpr.2020.07.242
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Fig. 1. Gelidiella acerosa.

2. Materials and methods

2.1. Collection of samples

The marine red seaweed *Gelidiella acerosa* was collected from the southeast coast Mandapam coast of Gulf of Mannar, Tamil Nadu, India. The collected seaweed was washed with seawater to remove the epiphytes and sand particle. The collected seaweed was dried and packed. In laboratory the seaweed was washed with freshwater and shade dried. The shade dried seaweed was stored.

2.2. Sample identification

The marine seaweed *Gelidiella acerosa* were identified and authenticated by Dr. Ganesan, Senior Scientist, Central Salt and Marine Research Institute, Mandapam Camp, Ramanathapuram, Tamil Nadu, India.

2.3. Preparation of extract

The aqueous extract was extracted by the decoction method. The alkaline pretreated sample were soaked with distilled water and placed in the water bath for 45mins at 55 °C. Kept it stand to cool down and then filtered the sample extract by Whatman's No.1 filter paper. Sample was stored under cold temperature for future use [9].

2.4. Synthesis of silver nanoparticle [AgNPs]

For biosynthesis of AgNP's 20 ml of *Gelidiella acerosa* seaweed aqueous extract was added with 80 ml of 1 Mm $AgNO_3$ Solution at room temperature. The stability was checked continuously for five days against acquired control. The bio synthesis of silver nitrate can be confirmed by visual observance and with UV –Spectrometer at 250–600 nm [10].

3. Characterizations of silver nanoparticle

3.1. Visual observance

The color change in the reaction after the treatment of seaweed extract with the 1 mM of $AgNO_3$ indicates the synthesis of silver nanoparticle [11].

3.2. UV–vis spectroscopy

The bio reduction in the silver ions in aqueous extract of seaweed was recorded using Shimadzu UV-Spectra instrument at the range of wavelength 200–600 nm [11].

3.3. Zeta potential

Zeta potential was analyzed by synthesis of silver nanoparticle to determine the charge present on the particle. The size and zeta potential of nanosynthesised sample were analyzed by Zetasizer Nano Series (Malvern Instruments, Malvern, UK) with 0.1–1000 μm [20].

3.4. Ft-Ir

The Fourier transform infrared spectroscopy was analyzed for silver nanosynthesised colloidal particle by scanning the spectrum range 550–4000 cm^{-1} [19].

3.5. AFm

The $AgNO_3$ seaweed extracted samples were visualized with an Atomic Force Microscopy [AFM]. The thin film of the sample was prepared on the glass slide by placing the sample on it and allowed it to dry for few minutes. The slide was then scanned with AFM [12].

3.6. Anti-bacterial activity

Anti-bacterial activity was done using an agar well diffusion test method. Nutrient media [NA] media was prepared then sterilized by autoclaving at 121 °C for 15 min.25 ml of the sterilized media was poured into the sterilized Petri dish and allowed to solidify at temperature. A sterile cotton swab was prepared for purpose of spreading the bacterial culture and punched the opening within the plates. for various concentration of AgNO3 extracted *Gelidiella acerosa* sample like 25, 50, 75 and 100 μl with 20 μl antibiotic [Gentamycin] as an impact. The plates were incubated for 24hrs at 32 °C. After 24hrs the zone of inhibition were taken [20].

4. Results and discussion

Aqueous extract of the red seaweed *Gelidiella acerosa* were made by the decoction method and treated with silver nanoparticle. The reduction of $AgNO_3$ was visually observed for the evident colour change [brownish –yellow colour] of the reaction mixture after 24hrs [Fig. 2]. This might be due to the surface Plasmon resonance effect due to excitation and the reduction of $AgNO_3$. The control sample without the silver nitrate showed no indication of any colour change. The AgNP's obtained were characterized by UV–visible spectroscopy with the characterisation peaks at 300–700 nm. It interpreted with the good peak observance at 404.0 nm [13].Fig. 3.

The stability of the $AgNO_3$ sample were checked over a week. For every 24hrs the UV readings were taken and the observance recorded were 404.0 nm at 24hrs, 48hrs the observance was 403.4 nm, 72hrs the observance was 410.7 nm, 96hrs the observance was 407.3 nm,120hrs the observance was 411.9 nm. Throughout a week the $AgNO_3$ synthesised sample maintained the stability of the sample with the particular standard peak of observance between 404 and 412 nm.[Table1]

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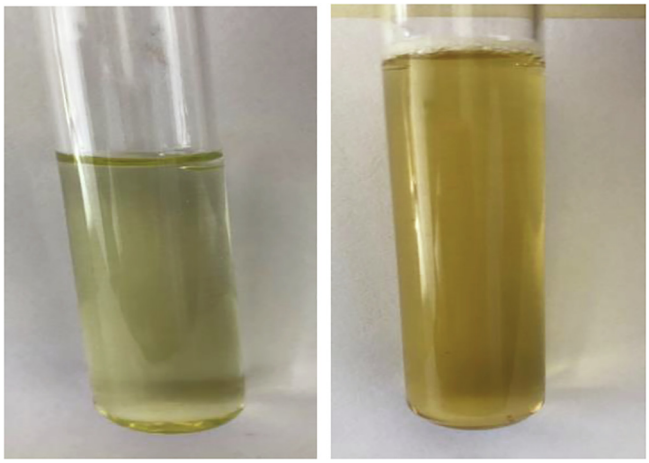

Fig. 2. Control (left) and silver (right) nanoparticle synthesized from Gelidiella acerosa.

4.1. Zeta potentia

The zeta potential value of AgNPs increases from 50mv to −50mv. It was notable that AgNPs were more prominently found in the aqueous nanosuspension of sample at a peak value of −25 mV showed in Fig. 4. Seaweed extract mediated synthesized AgNPs has negative zeta potential value and thus is stable under a wide pH range. Thus, it indicates the particles are stable due to its electrostatic repulsion [14].

4.2. FT-IR spectrum analysis

FTIR spectroscopy is a combinate technique for characterization of silver nanoparticle from marine red seaweed *Gelidiella acerosa.* The improved development of high-resolution instruments and software render FTIR spectroscopy as a unique technique of characterization and analyzing of silver nanoparticle synthesized from seaweed extract. The FTIR spectrum reported from the freeze-dried powder of silver nanoparticle, formed after 72hr of incubation with seaweed aqueous extract. The characteristic bands are related to the chemical and physical changes of the OH stretching vibrations at ca. 3290.560096 cm^{-1}, the OH bending of absorbed water at ca. 1654 cm^{-1}, the OH stretching vibrations at ca. 3290 cm-1, the symmetric and asymmetric stretching of CH_2 at ca.2912 cm^{-1}, the O–H and N–H deformation vibration at ca. 1236 cm^{-1}, the CO stretching at ca. 1091 cm^{-1} and 985 cm^{-1}, the CC bond stretching at ca. 920 cm^{-1} and double bonded CO vibration at ca. 1240 cm^{-1} [15]. The bands seen at 3280 cm-1 and 2924 cm^{-1} were assigned to the stretching vibrations of primary and secondary amines, respectively, while their corresponding bending vibrations were seen at 1651 cm-1 and 1548 cm-1 respectively [16]. The bands observed at 1379 cm^{-1} and 1033 cm^{-1} can be assigned to the C-N stretching vibrations of aromatic and aliphatic amines respectively. Thus, the observation confirms the presence of protein in the samples of silver nanoparticles.

The presence of these bonds at their respective wavelengths strongly indicates the presence of functional groups such as alcohols, methylene, alkanes, amides, primary and secondary amines (or) alcohols, carbonyl and aromatic groups. The bands at wavelengths 900 cm-1, 1375 cm-1, 2900 cm-1, 2995 cm-1–4000 cm-1 are very compatibility to the state of the crystalline and amorphous regions [17]. The spectral features of aqueous seaweed extract and silver nanoparticle are quite like each other. The detailed spectral features are nonetheless quite different in the spectra of clusters. Obtained in the Fig. 5.

4.3. Atomic force microscopy

The silver nanosynthesised extracts of Marine seaweed *Gelidiella acerosa* were characterized by AFM for its detailed absorbance of size, morphology and agglomeration of $AgNO_3$. The sample were analyzed with the peaks of 0–630.7 nm. The particle average roughness is at 59.721 nm.the tip in contact for the redundancy is −0.24144. The surface skewness is −0.185085. Coefficient of kurtosis is 0.416182. The topographical image of seaweed silver nanoparticle is reported and the formation of silver nanoparticle and its agglomeration was clearly observed in Fig. 6 [12].

4.4. Anti-bacterial activity

The antibacterial activity of silver nanoparticle that locally kills bacteria or slow down the active of growth without being toxic to surrounding tissues. Silver nanoparticle antibacterial activity

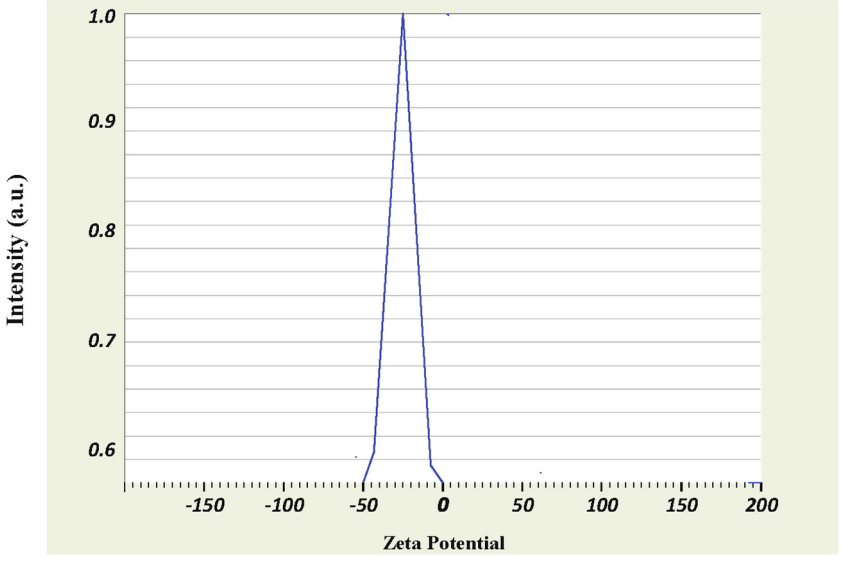

Fig. 3.

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Table1
UV–Visible Spectrometry analysis of $AgNO_3$ synthesised extract.

Hours	Wavelength (300–700 nm)
24hrs	404.0
48hrs	402.4
72hrs	410.7
96hrs	407.3
120hrs	411.9

depends on two main factors: physiochemical components of nanoparticle and type of bacteria. Based upon the bacterial cell wall structure, components and functions. Gram negative bacteria considered in this experiment are *Pseudomonas aeruginosa* and *Escherichia coli.* Gram positive bacteria considered are *Staphylococcus aureus* and *Bacillus subtilis* have interpreted in the Fig. 7 [a, b, c, d].

The structure of the cell wall plays a vital role in tolerance or susceptibility of bacteria in the presence of nanoparticle. Silver nanoparticle attach to the Gram-positive bacteria *Bacillus subtilis has* the greatest number of zones of inhibition in comparison to all the other bacteria. This might be due to its less complex cell wall composition [18]. Silver nanoparticle of concentrations 75 and 100 μl have the capacity to target the Gram-positive bacteria *Bacillus subtilis.* By contrast, the silver nanoparticle of concentration 100 μl was penetrative for Gram positive bacteria *Staphylococcus aureus.* This might be due to its cell wall composition that differed from *Bacillus subtilis* [21,22].

Gram negative bacteria *Escherichia coli* produced no zone of inhibition. This might be due to the bacteria defense mechanism against the silver nanoparticle [23–27]. The silver nanoparticle

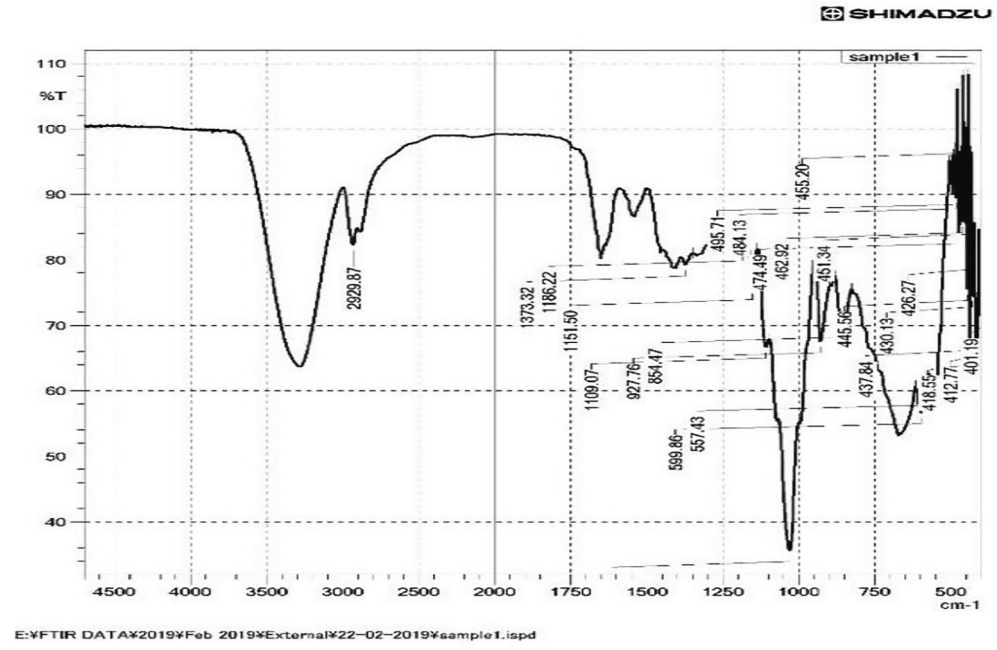

Fig. 4. Zeta potential distribution graph of AgNO3 synthesized marine seaweed Gelidiella acerosa.

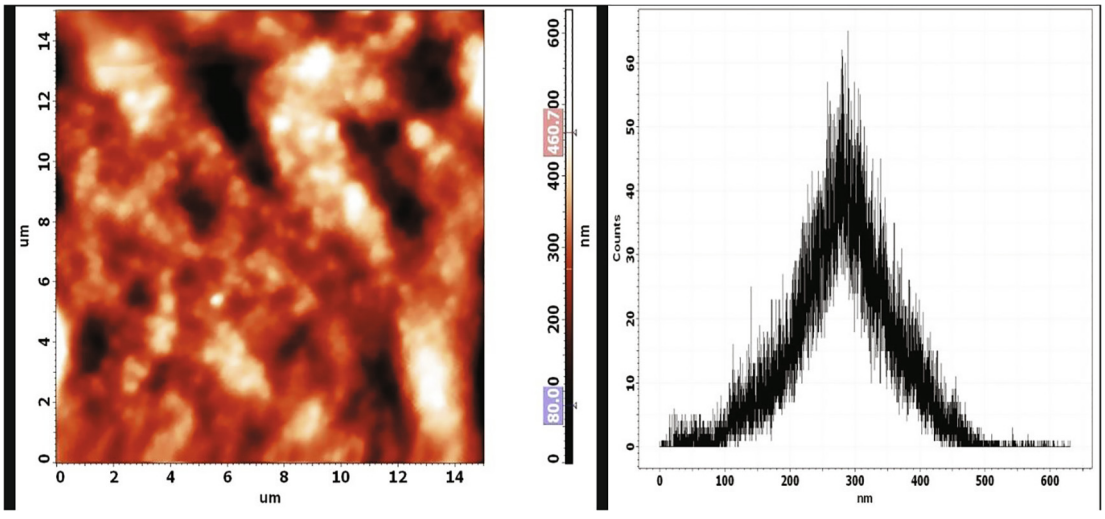

Fig. 5. FT-IR analysis of the Biosynthesized AgNP's using Gelidiella acerosa extract.

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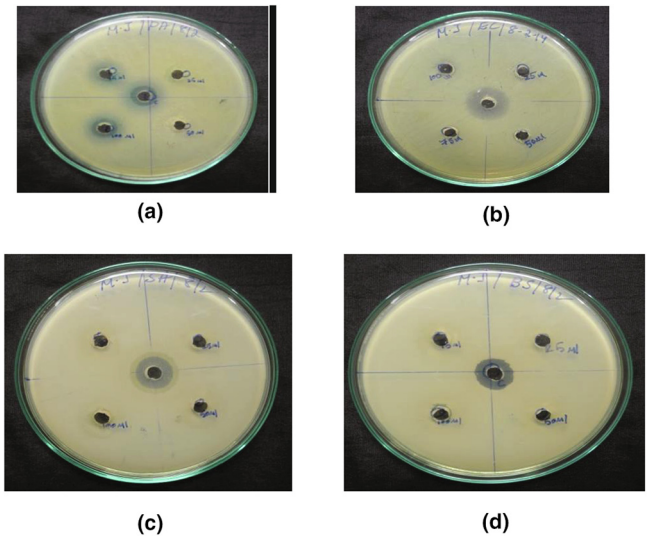

Fig. 6. The Atomic Force Microscopy analyses of biosynthesised AgNO3.

inhibited the growth of the gram-negative bacteria in *Pseudomonas aeruginosa*, showed an inhibition in the concentration of 50, 75 and 100 μl but has no effect against *Escherichia coli.* The bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis* have the greatest number of silver nanoparticle concentrations of inhibiting their growth. These bacteria absorb nanoparticle on the cell surface and decrease the amount of bacteria concentration on the bacterial culture plate. Therefore, silver nanoparticle synthesized from the seaweed *Gelidiella acerosa* can be regarded as promising candidates for inhibiting bacterial infections [28–36]. Showed in the table 2.

5. Conclusion

Silver nanoparticles have been synthesized from marine red seaweed *Gelidiella acerosa* and characterized using Zeta potential and FTIR analysis. The presence of silver nanoparticle is determined through UV analysis stability test and the changes in silver nanoparticle frequency is investigated through FTIR analysis proving that changes in frequency is due to its dimensional changes in the bond lengths of corresponding sites. The silver nanoparticle in the present subject is of nano size (10^{-9}) and have a positive charge of improving their stability in in the cations and the bacterial activity because of interaction with negatively charged biological membrane. The silver nanoparticle synthesized seaweed can inhibit the growth of bacterial activity. The antibacterial agents in medicine is reason for the high activity of antibacterial activity thus, it can be broadly anticipated for the silver nanoparticle.

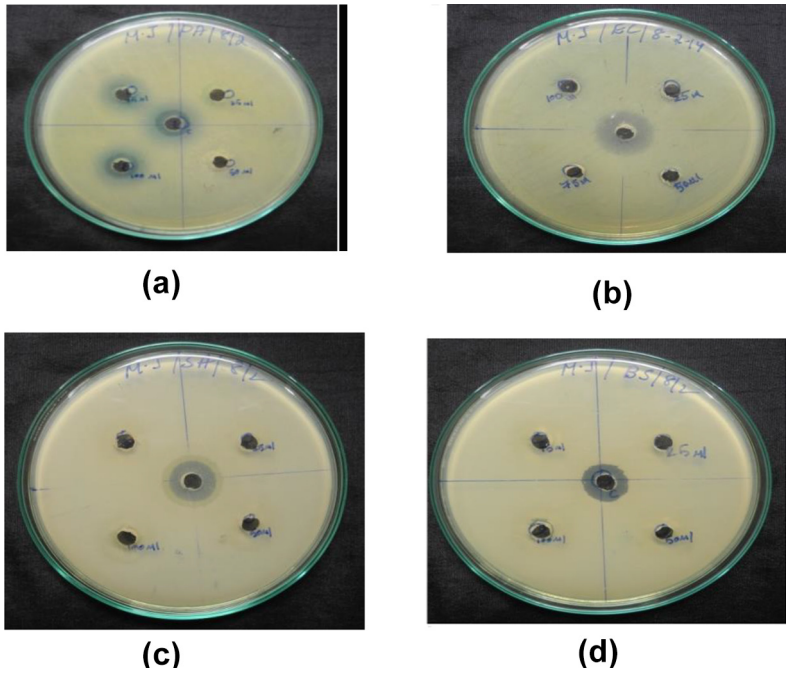

Fig. 7. a) Pseudomonas aeruginosa b) Escherichia colic) Staphylococcus aureus d) Bacillus subtilis.

Table 2
The zone of inhibition [mm] of $AgNO_3$ nano synthesized against isolated bacterial strain.

Organism	Concentrations (zones of inhibition in mm)				
	Control	25 μl	50 μl	75 μl	100 μl
Pseudomonas aeruginosa	1.9 ± 0.3	–	0.5 ± 0.3	0.6 ± 0.2	0.7 ± 0.2
Escherichia coli	2.3 ± 0.2	–	–	–	–
Staphylococcus aureus	2.6 ± 0.2	–	–	–	0.6 ± 0.2
Bacillus subtilis	1.9 ± 0.3	–	–	1.3 ± 0.2	1.6 ± 0.3

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Authors contributions

P. Jayashree, Kannan Mirunaalini and Thiruchelvi R have conceived and designed the experiment work.

P. Jayashree and Kannan Mirunaalini performed the experiment.

P. Jayashree and Kannan Mirunaalini worked together on manuscript writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The Authors are thankful to the Assistant professor and management of Vels Institute of Science, Technology and Advanced Studies for providing a facility to carry out this work.

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