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Green synthesis of silver nanoparticles using aqueous extract of *Gracilaria corticata* J.Ag. (Red algae) in Hare Island, Thoothukudi, Tamil Nadu, India

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ABSTRACT
Development of an ecofriendly, reliable and rapid process of biological synthesis of nanoparticles is an important role in nanobiotechnology. Antimicrobial activity and medicinal value of *Gracilaria corticata* J.Ag. fascinated to utilize it for biosynthesis of silver nanoparticles (AgNPs). The present study revealed the utility of aqueous extract of *Gracilaria corticata* J.Ag. for the green synthesis of AgNPs. Effect of various parameters on synthesis of nanoparticles was monitored by UV-Visible spectrometry. Characterization of nanoparticles was done by Fourier Transform Infra-Red (FTIR) and X-Ray diffraction (XRD) analyses. Fourier Transform Infra-Red (FTIR) and X-Ray diffraction (XRD) pattern revealed the formation of silver nanoparticles, which showed the bond formation and crystal nature.

I. INTRODUCTION
Nanotechnology is one of the most effective fields for research. Nanoscience is a rapidly developing field in research work, which contributes to produce a wide range of various synthesized Silver Nanoparticles [1]. Nanotechnology is the branch of science and technology that deals with the production of substances in size less than 100 nm scale as Nanoparticles and the metal Nanoparticles have their individuality among the other Nanoparticles over the last few decades because they have larger surface area per weight [2]. Silver nanoparticles have opened up a wide variety of new disciplines in biomedical protocols, since this marked reactivity of nano-silver due to their larger surface area-to-volume ratios [3]. The properties of the Nanoparticles make them attractive tools for research work i.e. Physical, Chemical, biological, thermal, dielectric, electrical, mechanical, electronic, magnetic, and optical [4].

The physically and chemically synthesized of AgNPs by different approaches producing pure and characterized nanoparticles but have some disadvantages as being expensive in addition to their hazard effects on the environment. Generally, biogenic synthesis of silver nanoparticles becomes necessary via green chemistry concepts to produce silver nanoparticles with enhanced stability has no disadvantages and there is no hazard effect on the environment [5]. Silver Nanoparticles are being considered as a worldwide accepted and successful research area for diagnostic and therapeutic applications, such as cancer detection and cancer treatment, biological imaging, biomedicine and drug delivery. The prominent applications of silver nanomaterials include detection and diagnostics, pharmacological activities including analgesic, antinociceptive, anti-implantation, and antimicrobial activities, catalysis and biolabeling [6]. Hence the present study was aimed to synthesis and characterize the silver nanoparticles using the aqueous extract of *Gracilaria corticata* J.Ag.

II. METHODOLOGY
The plant materials used in the present study were *Gracilaria corticata* J.Ag. belonging to Rhodophyceae (red algae). The plant materials were collected from Hare Island, Thoothukudi, located in Thoothukudi district, Tamil Nadu, India, during the month of December, 2016 and confirmed by the manual of Seaweeds of the south east coast of Tamil Nadu, India written by John Peter Paul and Patric Raja (2014) [7]. The plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered

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samples were then stored in the refrigerator for further use.

NANOBIOTECHNOLOGICAL STUDIES
SYNTHESIS OF SILVER NANOPARTICLES

For the synthesis of silver nanoparticles, 1g dried powder of the *Gracilaria corticata* J.Ag. was taken in a 100ml Erlenmeyer flask with 30ml of sterile distilled water and then boiled the mixture for 2 minutes. After boiling, the mixture was filtered in the Whatmann No.1 filter paper. 1mM solution of silver nitrate solutions was prepared. 5ml of plant extract was mixed with 25ml of 1mM solutions separately. The formation of reddish brown colour was observed respectively and λ max at different time intervals were taken for 8 hours using a UV-Visible spectroscopy. Then the solution was stored in room temperature for 24 hours for the complete settlement of nanoparticles. After 24 hours centrifuge the reaction mixture, discard the supernatant. Add 1ml of distilled water to the pellet and wash by using centrifugation. Collect the pellet by using acetone/ethyl acetate/Alcohol. Dry in the watch glass and store the nanoparticles.

CHARACTERIZATION OF SILVER NANOPARTICLES
UV-VISIBLE SPECTRA ANALYSIS

The reduction of pure silver ions was observed by measuring the UV-Visible spectrum of the reaction at different time intervals taking 2ml of the sample, compared with 2ml of 1mM silver nitrate solution used as blank. UV-Visible spectral analysis has been one by using An Elico spectrophotometer at a resolution of 1nm from 200 to 1100 nm.

FT-IR ANALYSIS

FT-IR spectrum in the range 4000 to 400cm^{-1} at a resolution of 4cm^{-1} using Perkin-Elmer spectrometer was used to detect the silver nanoparticles. The sample was mixed with KCl procured from Sigma. Thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier Transform Infra Red (FTIR) for the analysis of the nanoparticles.

XRD ANALYSIS

X-ray diffraction (XRD) analysis of drop-coated films of silver nanoparticles in sample was prepared for the determination of the formation of silver nanoparticles by XPERT-PRO software and X-ray diffractometer operated at a voltage of 40kv and a current of 30mA with Cu Kα radiation.

III. RESULTS AND DISCUSSION

SYNTHESIS OF SILVER NANOPARTICLES

Reduction of silver ion into silver particles during exposure to the plant extract could be followed by colour change. Silver nanoparticles exhibited dark brown colour in aqueous solution due to the surface Plasmon resonance phenomenon. The appearance of the dark brown colour indicated the formation of silver nanoparticle synthesis in the reaction mixture, as it was well known that silver nanoparticles exhibit striking colours (yellow to dark brown) due to excitation of surface plasmon vibrations in the particles. It was reported that some amount of OH- groups tended to promote the reduction of silver ions in some chemical methods

UV-VISIBLE SPECTRUM OF SILVER NANOPARTICLES

UV-Visible spectra of the reaction media of the *Gracilaria corticata* J.Ag. were taken at different time intervals. The Surface Plasmon Resonance (SPR) vibrations were found in the reaction media of *Gracilaria corticata* J.Ag. between 424 to 433.5nm with λmax at 423nm with absorption of 0.701 which was pink colour shifted at 15min. The light green colour shifted at 422nm with absorption of 0.914 at 30min, blue colour shifted at 425.5nm with absorption of 1.323 at 1h, red colour shifted at 426nm with absorption of 1.824 at 2h, followed by black colour shifted at 431nm with absorption of 2.761 at 4h which was related to an increase the amount of silver nanoparticles (Figure-1).

The position and the number of peaks in the absorption spectra were dependent on the shape of the particles. For an ellipsoidal particle there are two peaks whereas for spherical particle there was only one peak [8]. The present study reveals the synthesis of the silver Nanoparticles.

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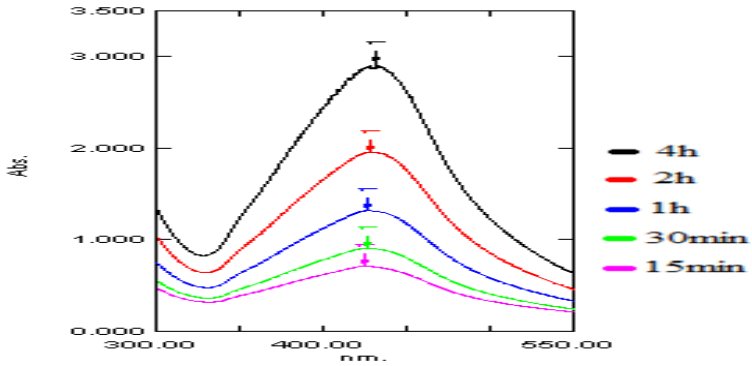

Figure 1: UV-Visible spectrum of silver nanoparticles using *Gracilaria corticata* J.Ag.

FT-IR SPECTRUM ANALYSIS OF SILVER NANOPARTICLES

FT-IR spectrum of silver nanoparticles synthesized by *Gracilaria corticata* J.Ag. was shown in Figure-2 and Table-1. This spectrum showed the presence of bands at 823.55, 1383.83, 1760.89, 2339.49 and 2359.74cm^{-1}. The bands at 823.55cm^{-1} corresponds to 1,2,4-trisubst benzenes (CH out-of-plane deformation), 1383.83cm^{-1} to sulfonyl chlorides (SO_2 antisym stretch), 1760.89cm^{-1} to anhydrides (C=O sym stretch), the band at 2339.49cm^{-1} was assigned to phosphines (P-H stretch) and 2359.74cm^{-1} was assigned to phosphines (P-H stretch).

The positions of these bands were close to that reported for native proteins [9]. This evidence suggests that the protein molecules could possibly perform the function of the formation and stabilization of silver nanoparticles in the aqueous medium.

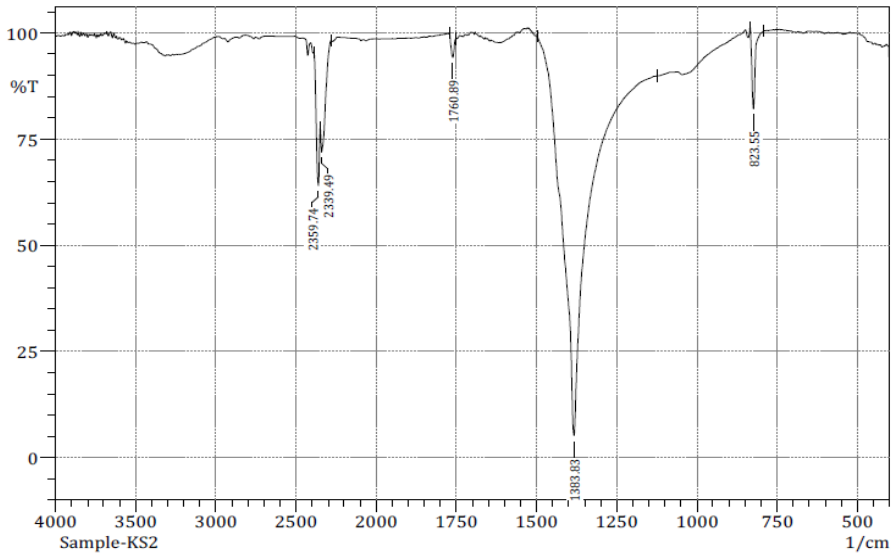

Figure 2: FTIR spectrum analysis of silver nanoparticles using *Gracilaria corticata* J.Ag.

Peak value	Functional group	Assignment
2359.74	Phosphines	P-H stretch
2339.49	Phosphines	P-H stretch
1760.89	Anhydrides	C=O sym stretch
1383.83	Sulfonyl chlorides	So_2 antisym stretch
823.55	1,2,4-trisubst benzenes	CH out-of-plane deformation

Table 1: FTIR spectrum analysis of silver nanoparticles using *Gracilaria corticata* J.Ag.

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X-RAY DIFFRACTION STUDIES

XRD pattern taken using *Gracilaria corticata* J.Ag. powder X-ray diffractometer instrument (XRDML) in the angle range of 10˚C-80˚C of the silver nanoparticles at 2θ, scan axis: Gonio. A number of Bragg reflections corresponding to 32.34, 35.32, 48.10, 54.40 and 67.10 sets of lattice planes were observed which can be indexed to face-centered cubic silver (Figure-3 and Table-2).

The peaks matched with the Joint Committee on Powder Diffraction Standards (file No. 04-0783), which further proved the formation of crystal silver nanoparticles [10]. The peaks were identified as AgNPs according to XPERT-PRO software (PDF#030921). The XRD pattern thus clearly showed that the silver nanoparticles were crystalline in nature [11].

The diffracted intensities were recorded from 10° to 80° at 2 theta angles. The diffraction pattern corresponded to pure silver metal powder. The XRD pattern indicated that the nanoparticles had a spherical structure. No peaks of the XRD pattern of Ag_2O and other substances appear and it can be stated that the obtained silver nanoparticles had a high purity. The observed peak broadening and noise were probably related to the effect of nanosized particles and the presence of various crystalline biological macromolecules in the plant extracts. The obtained results illustrate that silver ions had indeed been reduced to AgO by the extracts under reaction conditions [12].

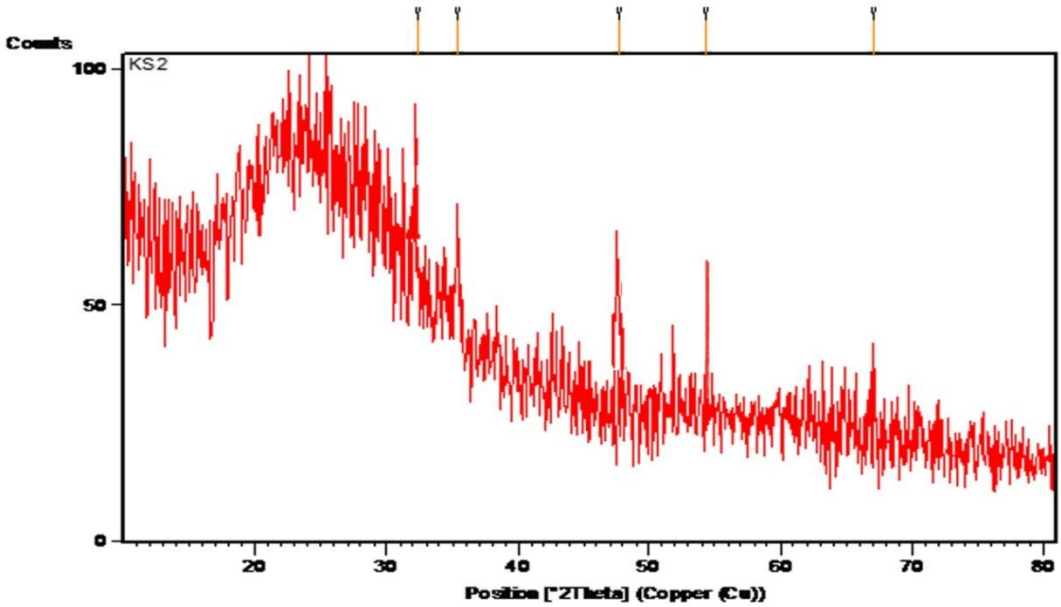

Figure 3: XRD spectrum analysis of silver nanoparticles using *Gracilaria corticata* J.Ag.

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
32.34	65.00	0.1542	3.21834	41.52
35.32	46.12	0.3170	2.77950	98.29
48.10	81.49	0.1432	2.78214	65.25
54.40	74.09	0.2210	1.96429	55.72
67.10	28.69	0.2530	1.60037	18.28

Table 2: XRD spectrum analysis of silver nanopartlcles using *Gracilaria corticata* J.Ag.

IV. SUMMARY AND CONCLUSION

Silver is one of the basic elements that make up our planet. It is a rare, but naturally occurring element, slightly harder than gold and very ductile and malleable. Silver nanoparticles of many different shapes (spherical, rod-shaped, truncated, triangular nanoplates) were developed by various synthetic routes. The silver Nanoparticles plays a major role in medicinal and preservative properties have been known for over 2000 years, especially in antimicrobial activity compared to

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other salts due to their extremely large surface area, which provides better contact with microorganisms. Silver ions and Nanoparticles are highly toxic and hazardous to microorganisms.

Silver Nanoparticles are also used in textile fabrics, as food additives and in package and plastics to eliminate microorganisms. Because of such a wide range of applications, various methods concerning the fabrication of silver nanoparticles, as well as various silver based compounds containing metallic silver (AgO) have been developed [13]. Silver nanoparticles used in environmental friendly antimicrobial nano paint [14], antimicrobial nature of silver ions plays a prominent role in food packaging systems. Silver nanoparticles have antibacterial properties mediated by silver ions [15], it used as preservative in food and various food related products [16], Silver nanoparticles are reported to show better wound healing capacity, better cosmetic appearance and scar less healing when tested using an animal model [17]

From the present study, it was concluded that the aqueous extract of *Gracilaria corticata* J.Ag. was used to synthesis of silver Nanoparticles. The silver nanoparticles were characterized by UV–visible Spectrophotometer, FT-IR and XRD. The peak are initiated from the 424nm indicating the formation of silver nanoparticles in spherical shape using UV–Visible Spectrophotometer. FT-IR spectrum of silver nanoparticles shows the presence of bands at 823.55, 1383.83, 1760.89, 2339.49 and 2359.74cm^{-1}. XRD pattern of silver nanoparticles shows a number of Bragg reflections corresponding to 2θ of 32.35, 35.31, 47.97, 54.44 and 67.02 sets of lattice planes of silver. EDX and SEM analysis showed the impurities and the size of synthesized silver nanoparticles about 18–45nm.

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