GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM ULVA FASCIATA DELILE.

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ABSTRACT

This study confirmed that the aqueous extract of seaweed *Ulva fasciata* is capable of producing Silver nanoparticle (AgNPs) by reducing $AgNO_3$ solution and is proved to be an efficient, eco-friendly and simple method. We have characterized the synthesized AgNPs using several techniques. The characteristic absorption peak at 249 nm in UV-Visible spectrum confirmed the formation of AgNPs. Crystalline nature of AgNPs is evident from the characteristic peaks in the XRD pattern. FESEM images revealed that the synthesized NP_S are nearly spherical with size in the range 68.79-103.20 nm. The Characteristic peaks in the FTIR spectrum revealed the presence of Alcohols, Phenols, Alkanes and Amides, which is responsible for the formation of AgNPs. The outcome of this study is to know the methodology of green synthesis of silver nanoparticles and its characterization.

IndexTerms: *Ulva fasciata*, UV-Visble Spectroscopy, X-ray diffraction Measurement, Field Emission Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy, Energy Dispersive X ray, Particle size analysis.

1. INTRODUCTION

Nanotechnology is a branch of science and engineering dedicated to materials which is having dimensions in the order of 100^{th} of nm or less (Salata, 2004). Being the term is new; it has been widely used for the development of more efficient technology. In recent years, nanotechnology has been widely used by industrial sectors due to its applications in the field of electronic storage systems (Kang *et al*., 1996), biotechnology (Pankhurst *et al*., 2003), magnetic separation, preconcentration of target analyses and targeted drug delivery (Dobson, 2006; Rudge *et al*., 2001). Consequently these particles have capacity to make a significant impact to the society. As the field of nanotechnology advanced, novel nanomaterials become apparent and have different properties as compared to their larger counterparts. The difference in the physiochemical properties of nanomaterials can be attributed to their high surface-to-volume ratio. Because of its unique properties, they became excellent candidate for biomedical applications as variety of biological processes occur at nanometer scales (Mody *et al*., 2009).

Silver nanoparticles are the particles of silver with 1-100 nm size. Some of the silver nanoparticles are composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms. Like gold nanoparticles, ionic silver has a long history and was initially used to stain the glass for yellow. Currently, there is also an effort to incorporate silver nanoparticles into a wide range of medical devices, including bone cement, surgical instruments and surgical masks. Moreover, wounds are treated by using right quantities of ionic silver (Qin 2005).

Physical and chemical method of synthesizing nanoparticles is expensive, labour-intensive and also hazardous to the environment and living organisms. Whereas, Green synthesis of metal nanoparticles using organisms and plants or plant extracts has emerged as a nontoxic and eco-friendly method. It has been suggested as valuable alternatives to physical and chemical methods.

Antimicrobial properties of silver nanoparticles caused the use of these nanometals in different fields of medicine, industries, animal husbandry, packaging, accessories, cosmetics, health and military. Silver nanoparticles from plants show potential antimicrobial effects against infectious organisms such as *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Syphilis typhus* and *Staphylococcus aureus* (Cho *et al*., 2005; Duran *et al*., 2007; Rakkimuthu *et al*., 2018).

Ulva fasciata (Ulvaceae), also known as Limu palahalaha or sea lettuce, is common green algae used for consumption in many parts of the world. High nutrients and fresh water are often indicated by its presence. In this study, silver nanoparticles are synthesized using the aqueous extract of green algae *Ulva fasciata* and it is characterized by biophysical instruments.

2. MATERIALS AND METHODS

2.1 Collection and Identification:

The green algae *Ulva fasciata* Delile. *(Ulvaceae*) was collected from Kanyakumari Coast, Tamilnadu, South India. The algae was identified and authenticated by Botanical Survey of India, Southern Region, Coimbatore.

Figure 1: Habit of *Ulva fasciata* Delile.

2.2 Preparation of seaweed extract:

100 gms of sample were washed thoroughly with 250 ml of distilled water to remove extrageneous materials and log of the washed seaweed was finely cut into small pieces and stirred with 100 ml sterile Milli Q water for 1min and kept in a water bath at 4^0C for 20 minutes. Finally, the extract was filtered with Whatman no.1 filter paper and it is used for further analysis.

2.3 Biosynthesis of AgNPs:

50ml aqueous seaweed extract was mixed with 50ml of 1mM $AgNO_3$ solution, stirred well for 1 minute, kept in a water bath at 60^0C for 1 hour and then it is incubated in dark at room temperature under static condition. A control set up was also maintained without seaweed extract. The bioreduction of $AgNO_3$ into AgNPs can be confirmed visually by the change in colour from yellow to brown.

2.4 Characterization techniques:

2.4.1 UV – Visible Spectroscopy analysis:

The reduction process of the formation of AgNPs in solution was monitored on a Perkin – Elmer UV-VIS Spectrophotometer (Lambda - 35, Germany) to know the kinetic behavior of the AgNPs. After the addition of $AgNO_3$ to the plant extract, the spectras were taken in different time intervals upto 24 hrs between 200 and 800 nm at a scan speed of 480nm/min. to study the effect of time duration on NP formation.

2.4.2 X-ray diffraction measurement (XRD):

The phase evolution of cleaned powder as well as that of sintered samples was studied by X-ray diffraction technique (Philips PAN analytical, The Netherland) using Cu kα radiation. The generator voltage and current was set at 35 KV and 25 mA respectively. The Ag samples were scanned at 15 to 70^0C range in continuous scan mode. The scan rate was 0.04/sec.

2.4.3 Field Emission Scanning Electron Microscopy (FESEM)

FESEM was used to characterize mean particle size and morphology of the AgNPs. The powder sample and freeze dried sample of the AgNPs solution was sonicated with distilled water; small drop of this

sample was placed on glass slide and allowed to dry. A thin layer of platinum was coated to make the samples conductive Jeol. JSM-6480 L V FESEM machine was operated at a vacuum of the order of 10-5 torr. The accelerating voltage of the microscope was kept in the range 10-20kv.

2.4.4 Fourier Transform Infrared (FTIR) Measurement:

FTIR measurements were carried out to investigate and predict any physicochemical interactions between different components in a formulation in the dried biomass of the extract treated with $AgNO_3$ to find out the compound responsible for the synthesis of $AgNP_S$. FTIR measurements were taken for the AgNPs synthesized after 0 hr, 1 hr, 6 hrs, 12 hrs and 24 hrs of reaction. These measurements were carried using a FTIR PERKIN ELMER instrument with a wavelength range of 400 to 4000 nm where the samples were incorporated with KBr pellets to acquire the spectra.

2.4.5 Energy Dispersive X ray (EDX) Analysis observation of AgNPs:

EDX was carried using JEOL -2100 High Transmission Electron Microscope to confirm the presence of Ag in the particles as well as to detect other elementary compositions of the particles.

2.4.6 Particle Size Analysis (Diffuse Light Scattering (DLS) method):

Mie-scattering theory (Thiele and French, 1998) provides rigorous solutions for light scattering by an isotropic sphere embedded in a homogeneous medium. In order to find out the particles size distribution, the Ag powder was dispersed in water by horn type ultrasonic processor (Vibronics, VPLPI). The data on particle size distribution were extracted in Zeta sizer version 6.20 Mall052893, Malvern Instruments.

3. RESULTS AND DISCUSSION

3.1 UV –Vis Spectrophotometer Analysis:

The formation of AgNPs was visually evident from the colour change of reaction mixture from light yellow to dark brown, indicating the formation of AgNPs (Figure 2). Intensity of brown colour increased in direct proportion to the incubation period. It may be due to the excitation of surface Plasmon resonance (SPR) effect arising due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field and reduction of $AgNO_3$ (kannan *et al*., 2013).

Figure 2

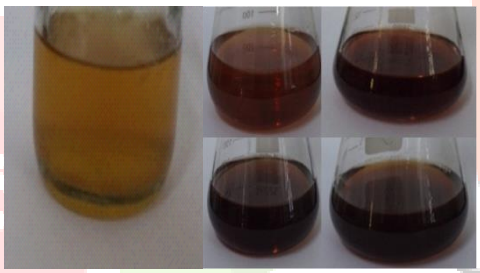


Figure 2 The colour intensity of the *U. fasciata* extract incubated with Silver ions at 0 hr, 1 hr, 6 hrs, 12 hrs and 24 hrs in clock-wise direction.

UV-Visible Spectroscopy is an important technique to ascertain the formation of metal NPs. The peaks obtained are at 0 hr (Figure 2-A 201 nm), 1 hr (Figure 2-B 432 nm), 6 hrs (Figure 2-C 435 nm), 12 hrs (Figure 2-D 207 nm) and 24 hrs (Figure 2-E 249 nm). A sharp intense peak at 24 hrs (249nm) in the UV-Visible spectra (Figure 2 -E) confirmed the formation of AgNPs.

Figure 2 -A (0 hr)

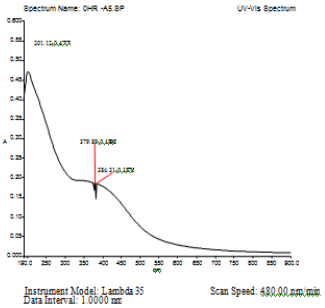


Figure 2 -B (1 hr)

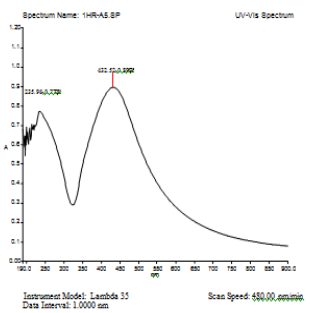


Figure 2-C (6 hr) Figure 2-D (12 hr) Figure 2-E (24 hr)

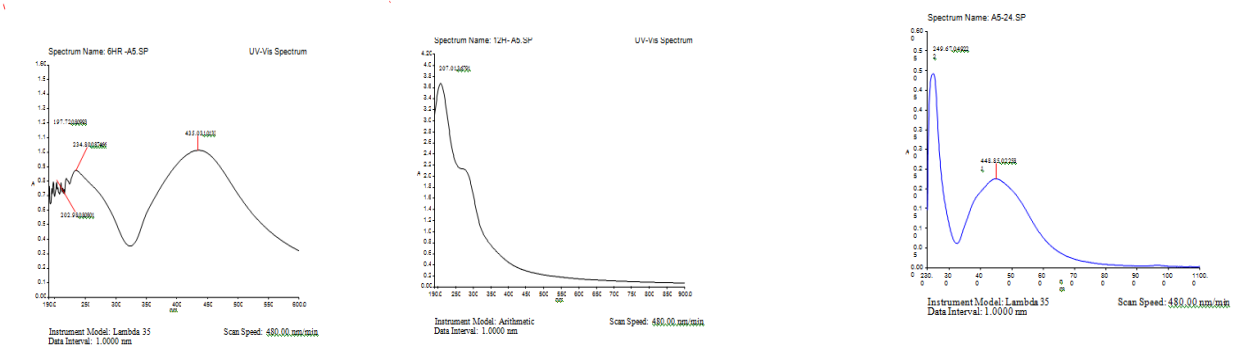


Figure 2A-2E: UV– Visible Spectrum of AgNPs formed using *Ulva fasciata*

3.2 Field Emission Scanning Electron Microscopy (FESEM):

FESEM has been used to identify the size, shape and morphology of NPs. The morphology and size of the green synthesized AgNPs were studied by FESEM (Figure 3). The size of the silver nanoparticle thus formed was 103.20 nm.

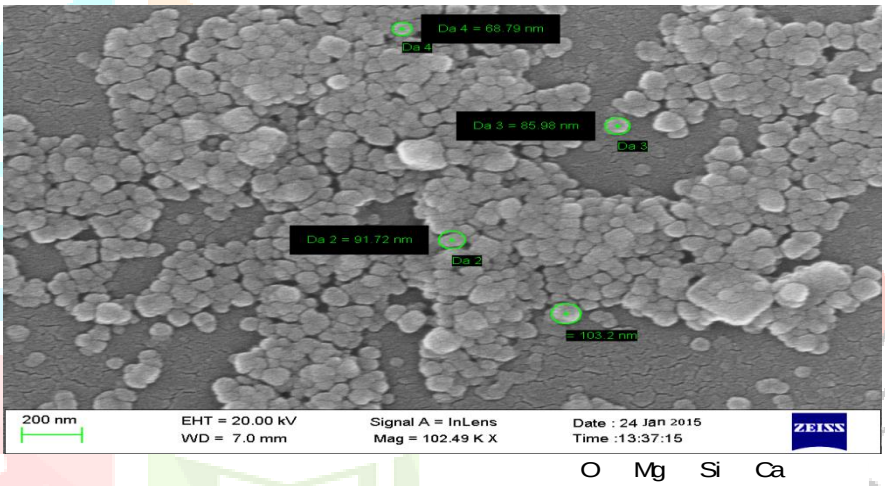
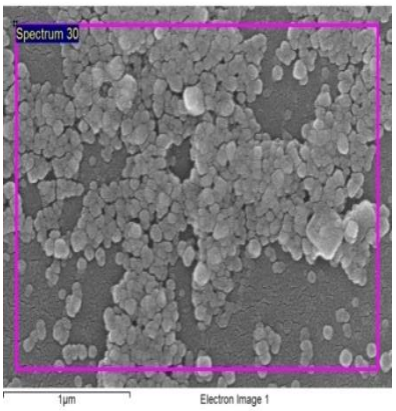


Figure 3: FESEM showing size, shape and morphology of NPs

3.3 Energy dispersive X-ray Spectroscopy (EDX) analysis of silver nanoparticles

The elemental composition of the green synthesized sample was determined by EDX analysis. The intense signal at 3kev strongly suggests that Ag was the element, which has an optical absorption in this range due to the (SPR) (Figure 4). Notably, the other signals in the range of 0.0-4 kev represent the typical absorption of carbon, oxygen, sodium, magnesium, calcium and potassium, thus indicates the presence of the algal extract (as a capping ligand) on the surfaces of the NPs.



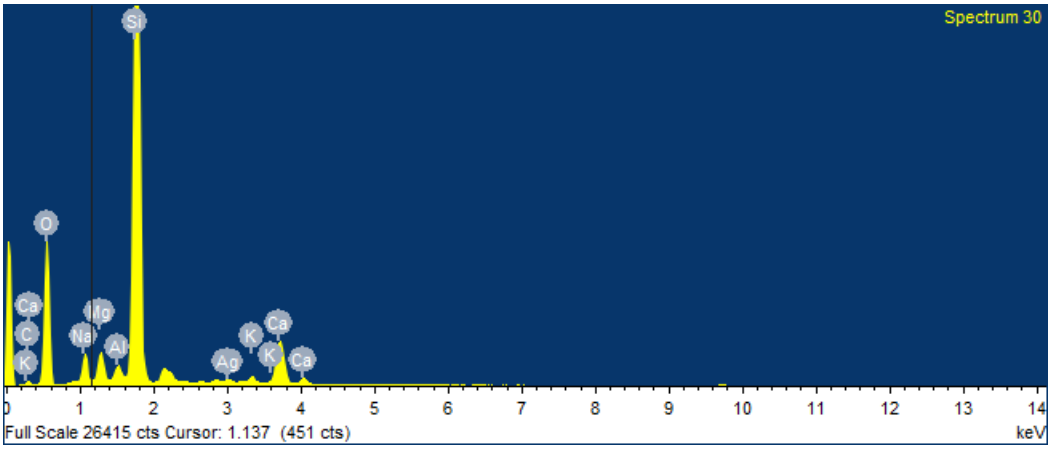


Figure 4: EDX spectrum of synthesized AgNPs

3.4 Particle size Analyzer (Diffuse light scattering (DLS) method):

A laser diffraction method with a multiple scattering technique has been used to determine the particle size distribution of the powder. It was based on Mie-Scattering theory (Thiele and French, 1998). The data on particle size distribution were extracted in Zeta sizer Ver.6.20 (Mal1052893, Malvern instruments) (Tables 1&2 and Figure 5A -5D).

Table 1: Summary of particle size analysis (Refering Figures 5 -A, 5-B and 5-C)

Record No	Count rate (kcps)	Z-Average (d.nm)	Size (d.nm)	Intensity (%)	St Dev (d.nm)	Pdl / Intercept
1	326.1	111.8	145.1 5209	98.7 1.3	56.00 468.9	0.331/0.876
2	315.3	113.5	154.7	94.3	67.15	0.351/0.874
3	318.5	116.3	141.5 5052	97.6 2.4	55.14 580.3	0.310/0.874

Table 2: Summary of Zeta Potential analysis (Figure 5-D)

Record No	Count rate (kcps)	Z-Potential (mV)	Mean (mV)	Area (%)	St Dev (mV)	Z-Deviation (mV)	Conductivity (mS/cm)
1	186.4	-20.9	-20.6	98.4	7.55	7.96	0.355

Figure 5-A

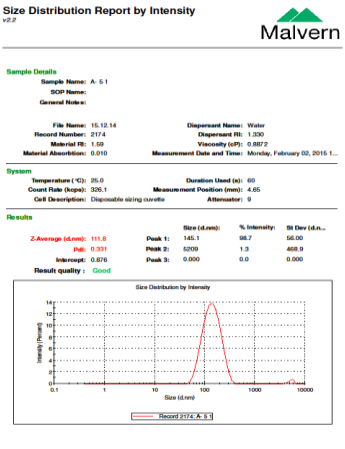


Figure 5-B

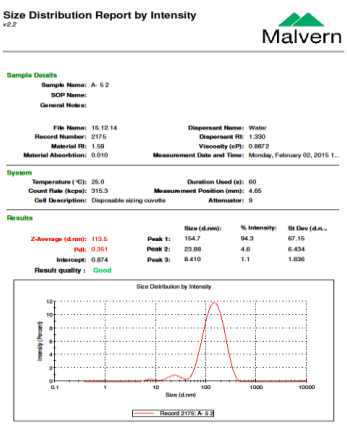
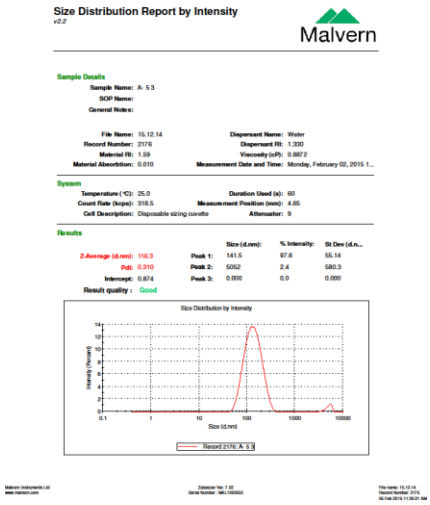
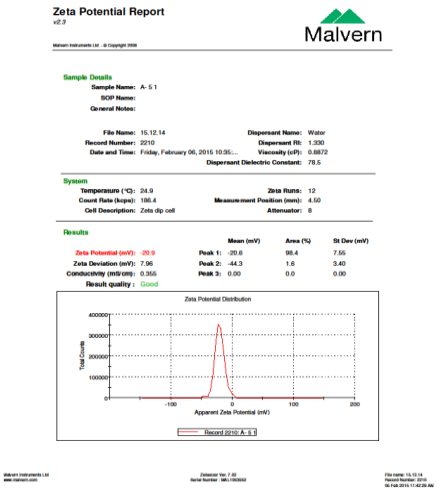


Figure 5-C

Figure 5-D





3.5 XRD analysis of Silver nanoparticles:

The crystalline nature of AgNPs was further confirmed from X-ray diffraction (XRD) analysis. The XRD spectrum (Figure 6) indicates the face–centered cubic structure of the AgNPs. There are six distinct reflections in the diffractogram at 27.93^0(14), 32.4^0(37), 38.2^0(129), 44.4^0(35), 46.33^0(16), 50.24^0(124), 64.6^0(30) and 78^0(19) (Table 3). The intense reflection at 129, in comparison to the other five may indicate the growth direction of the nanocrystals. On the basis of the half-width (^) of the 129 reflection in the powder pattern, the average grain size L-determined by broadening of the 129 reflection by the Debye – Scherer formula $D=K\lambda/\beta\cos\Theta$

Where K is the Scherrer constant and its value is .094, λ is the wavelength of the Xray, β is the full width at half maximum and Θ is the Bragg angle. From the Scherrer equation the average crystalline size of AgNPs is found to be 42nm. The absence of any additional reflections other than the reflections belonging to the Ag lattice clearly suggests that the green synthesized AgNP lattice was unaffected by other molecules in the algal extract

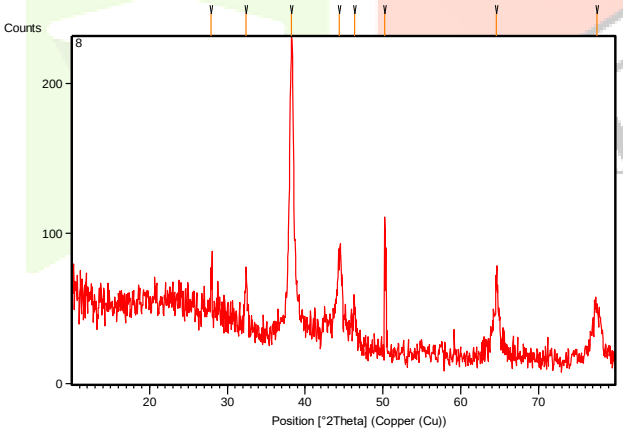


Figure 6: XRD pattern of AgNPs

Table: 3 Peak list of XRD

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
27.93(3)	14(16)	0.2(1)	3.19220	10.80
32.4(2)	37(67)	1(1)	2.76257	28.87
38.2(3)	129(290)	1(1)	2.35192	100.00
44.4(2)	35(74)	0.5(4)	2.03794	27.54
46.33(5)	16(37)	0.3(1)	1.95803	12.49
50.247(3)	124(10)	0.081(9)	1.81430	96.13
64.6(2)	30(155)	0(2)	1.44123	23.31
78(16)	19(781)	1(28)	1.23047	14.83

3.6 FTIR Analysis of Silver Nanoparticles:

FTIR measurements were carried out to identify the possible biomolecules in *Ulva fasciata* extract which is responsible for capping leading to efficient stabilization of the AgNP. The IR spectrum (Figure 7) of AgNPs synthesized using *Ulva fasciata* manifests prominent absorption bands located at 3875, 3787, 3324, 2350, 2064, 1285, 1403 and 673cm^{-1}. The broad spectrum at 3875cm^{-1} shows the O-H stretch, free hydroxyl of Alcohols and Phenols. The very strong absorption band at 3324cm^{-1} corresponds to the O-H stretch, H–bonded of Alcohols and Phenols and that at 1285cm^{-1} corresponds to the C –H bend of Alkanes. IR spectroscopic study confirmed that the *U. fasciata* extract has the ability to perform dual functions of reduction and stabilization of silver nanoparticles.

Figure 7-A (0 hr)

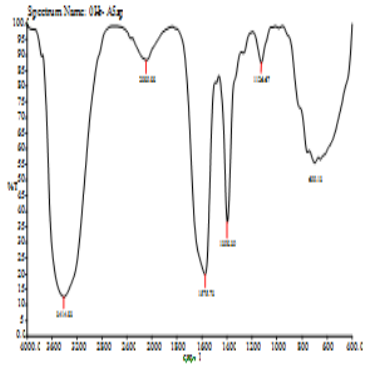


Figure 7-B (1 hr)

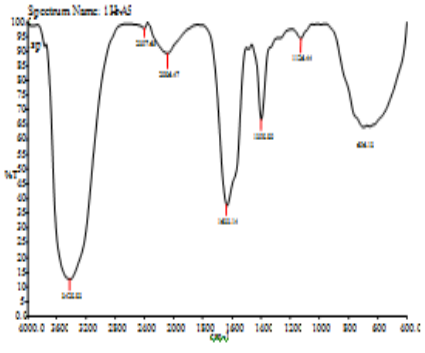


Figure 7-C (6 hr)

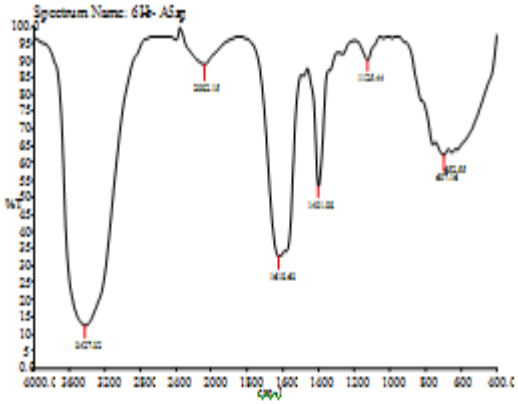


Figure 7-D (12 hr)

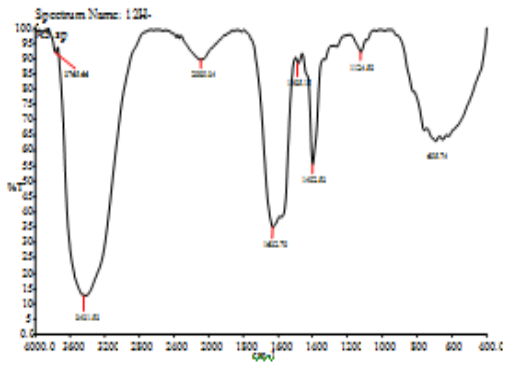
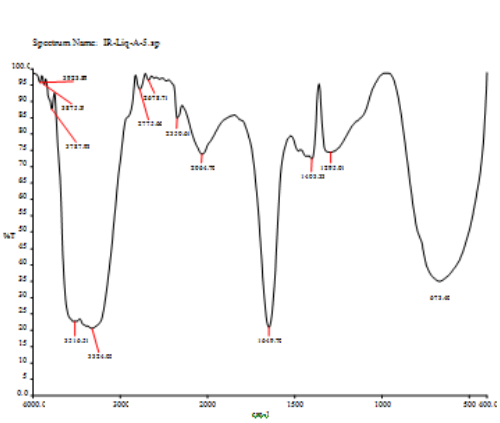


Figure 7-E (24 hr)

Figure 7-F (IR)



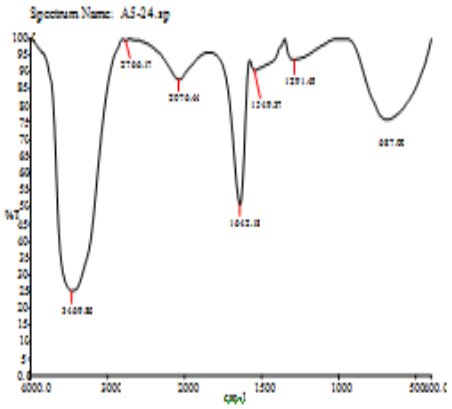


Figure 7: FTIR analysis

4. REFERENCES

[1] Cho, K., Park, J., Osaka, T. and Park, S. 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochimica Acta, 51(5): 956–960.

[2] Dobson, J. 2006. Gene therapy progress and prospects: magnetic nanoparticle-based gene delivery. Gene Therapy, 13(4): 283–7.

[3] Durán, N., Marcato, P. D., de Souza, G. I. H., Alves, O. L. and Esposito, E. 2007. Antibacterial effect of silvernanoparticles produced by fungal process on textile fabrics and their effluent treatment. Journal of Biomedical Nanotechnology, 3(2): 203–208.

[4] Kang, Y. S., Risbud, S., Rabolt, J. F. and Stroeve, P. 1996. Synthesis and characterization of nanometer-size Fe3O4 and γ-Fe2O3 Particles. Chemistry of Materials, 8(9): 2209–11.

[5] Kannan, R. R. R., Stirk, W. A. and Van staden, J. 2013. Synthesis of silver nanoparticles using the seaweed *Codium capitatum* P. C. Silva (Chlorophyceae). South African Journal of Botany, 86: 1-4.

[6] Mody, V. V., Nounou, M. I. and Bikram, M. 2009. Novel nanomedicine-based MRI contrast agents for gynecological malignancies. Advance Drug Delivery Reviews, 61(10): 795–807.

[7] Pankhurst, Q. A., Connolly, J., Jones, S. K. and Dobson J. 2003. Applications of magnetic nanoparticles in biomedicine. Journal of physics D: Applied physics, 36(13): R167.

[8] Qin, Y. 2005. Silver-containing alginate fibres and dressings. International wound journal, 2(2):172–6.

[9] Rakkimuthu, R., Dhanya, K., Bennita, L. and Naveenraj, B. 2018. Phytochemical analysis, synthesis of silver nanoparticles and its antibacterial activity from *Cyperus brevifolius* L. International Journal of Creative Research Thoughts. 6(1): 610-617.

[10] Rudge, S., Peterson, C., Vessely, C., Koda, J., Stevens, S. and Catterall, L. 2001. Adsorption and desorption of chemotherapeutic drugs from a magnetically targeted carrier (MTC). Journal of Control Release, 74(1-3): 335–40.

[11] Salata, O. 2004. Applications of nanoparticles in biology and medicine. Journal of Nanobiotechnology, 2:3.