EXOPOLYSACCHARIDES COATED SILVER NANOPARTICLES GREEN SYNTHESIS USING CROTON SPARSIFLORUS MORONG LEAF AND THEIR ANTIMICROBIAL ACTIVITIES

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ABSTRACT: In Metal NanoParticles (MNPs) bio-fabrication process, one of the major developments is nanotechnology and Nanoscience. There are improved and new properties for Nanoparticles compare to bulk material larger particles according to different characteristics as morphology, distribution and size. Several methods are used to achieve the nanoparticles Production. The biological activity based leaf extraction is used in Silver NanoParticles (SNPs) synthesis. This paper presents, Exopolysaccharides coated silver nanoparticles Green synthesis using Croton sparsiflorus morong leaf and their Antimicrobial Activities. Several monosugar units are present in ExoPolySaccharides (EPS) named biopolymers and which are present in different structures. The nanobiocomposite characterization is done with the sequence of methods as UV-visible spectroscopic, FE-SEM (Field Emission Scanning Electron Microscopy) with EDX Spectroscopy (Energy Dispersive X-ray) and FT-IR (Fourier transform infrared) Spectroscopy. Especially, FESEM and EDX are used to identify the EPS-SNPs presence and well as surface morphology. The nanobiocomposite different concentrations are tested against Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 6538 and Escherichia. coli ATCC 11229 using 96-well plates for finding antibiofilm and antibacterial activity. Therefore, described paper explored the antimicrobial activities significance.

KEYWORDS: Green synthesis, Silver nanoparticles, Exopolysaccharides, FE-SEM, UV-visible spectroscopic, FE-IR, antimicrobial activities.

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I. INTRODUCTION

In Metal NanoParticles (MNPs) bio-fabrication process, one of the major developments is nanotechnology and Nanoscience [1].

In nanoparticles synthesis, metal nanoparticles are acts as stabilizing agents and as well as reducing agents with plant extracts because of particular properties of nanopartices as mechanical, chemical, magnetic, optical and electrical [2]. Smaller silver nanoparticles production facilitated by modern nanotechnology with greater efficacy towards bacteria and low toxicity for human [3]. Multi drug resistant bacteria against activity are improved by using attractive alternative of nanoparticles instead of antibiotics. Biocompatibility of nanoparticles are improved according to the important aspect of nanoparticles as their size and shape. Further modifications are enhanced according to different applications for various fields [4].

Recently, for environmental friendly use, great efforts are made on silver nanoparticles. Various fields are uses the Silver NanoParticles (AgNPs) because of special characteristics of it as mechanical, chemical, magnetic, medicinal, optical and electrical. Especially, cosmetic products, antimicrobial applications, biosensor

materials and electronic components are integrated with Silver nanoparticles [5].

Recently, one of the emerging research areas is nanoparticles green synthesis which is alternative to the conventional physical and chemical methods [6]. Green synthesis achieves easily scalable, environment friendly and cost effective, and there is no need of high toxic chemicals, high temperature, high pressure and high energy. Because of exclusive physicochemical characteristics, various applications are uses this nanoparticles synthesis method, therefore researchers are showing more interest on this area [7]. Nanoparticles greener synthesis is a smoother way provided by biological methods of synthesis which are really greater efficiency with slower kinetics, good stabilization, great control on crystal growth and offer better manipulation.

Biocompatible polymers coating on nanoparticles are increase the stabilizing activity in a polymeric matrix which is suggested by some authors [8]. Multiple strategies are involved for achieving hybrid polymer-nanoparticle materials, such as polymeric solutions adding to nanoparticles, silver ions reduction and at the same time polymerizing monomers [9]. Biomolecules with silver nanoparticles are having great capacity; therefore it is used in gene transfer, drug delivery systems, antibacterials and cellular engineering.

Different structures and conformations are having the several monosugar units which are involved in ExoPolySaccharides (EPS) based biopolymers. These biopolymers are collected basically from fungi, plants, bacteria and microalgae. EPS biological function is closely matches to its configuration and bacterial growth is inhibited ability is found in one of the biological functions of ExoPolySaccharides (EPS).

Many natural products are uses the Croton sparsiflorus morong plants which are having high medical uses. These plants are used in treatment of high blood pressure controlling, skin diseases treatment, curation of cuts and wounds because of rutin named active principle component presence. The screening of Croton sparsiflorus morong with phytochemically called secondary metabolites according to antimicrobial properties [10]. Without any additional chemicals, little amounts of leaf extracts are used to prepare the Silver nanoparticles. Therefore, silver nanoparticles green synthesis of described paper uses the Croton sparsiflorus morong leaf extract. Both organic and inorganic constituents are contained in this plant.

Remaining paper is prearranged as follows: survey of literature is presented in Section II, Section III introduces the described green synthesis of Silver nanoparticles, experimental results elaborated in Section IV, and finally paper is concluded with Section V.

II. LITERATURE SURVEY

B. Paul, A. Haque, M. A. -A. Mamun, M. Paul and K. Ghosh, et. al. [11] presented NanoconJugates (NJs) optical and structural properties with the inclusion of biomolecules based pure Zinc Oxide (ZnO) NanoParticles (NPs). Pure ZnO structure high phase is illustrated by the NPs and NJs XRD patterns. In photoluminescence spectrum, visible green emission is mainly combined with oxygen vacancies of ZnO nanostructure surface. Glucose biomolecules reduces this green emission considerably. From 531 eV, core level in NJs increases in X-ray photoelectron spectroscopy and this increment is depends on higher solution

glucose concentration during synthesis. New kinds of NJs development is achieved by using described promising platform and their multifunctionality and integrity are forwarded by the interfacial properties investigation.

T. K. Son, N. V. Tien et. al. [12] presents green chemical method for copper nanoparticles with polyester filter cloth and also evaluates their antimicrobial property. In aqueous solution, $[Cu(OH)_4]^{2-}$ (Copper hydroxide) ions are reduced by ascorbic acid which can affect the commercial PolyEthylene Terephthalte (PET) filter cloth in situ pieces for the generation of Copper nanoparticles. Synthesized copper particles sizes are varies from less than 100 nm to over 500 nm by using the Scanning Electron Microscope (SEM) images analysis. Phosphate-Buffered Saline (PBS) and sterilized milk with Escherichia coli NBRC (National Biological Resource Center) 14237 suspensions through these modified filters are used to analyze the Antibacterial activity of the modified polyester filters.

Y. P. Yew et al., [13] produced green approach for synthesis of superparamagnetic magnetite nanoparticles (Fe_3O^{4-}) (Ferrus Oxide) which is environmental friendly approach. In this synthesis process, Seaweed Kappaphycus alvarezii (K. alvarezii) extract is used as bio-stabilizer. TEM and XRD are used to analyze the synthesized Fe_3O_4 NPs/K. alvarezii morphology and structure. EDX spectroscopy, Raman spectroscopy and FTIR are used to identify the Fe_3O_4–NPs presence. Vibrating sample magnetometer is used to analyze the K. alvarezii/Fe_3O_4–NPs magnetic properties and it is resulted as 24.85 emu/g. 53.57 mV is zeta potential value for very stable K. alvarezii/Fe_3O_4–NPs.

M. B. Ahmad, R. F. Elsupikhe, N. Zainuddin, N. A. Ibrahim, K. Shameli, et. al. [14] developed silver nanoparticles (Ag-NPs) using eco-friendly photochemical method in a natural polymer (κ-carrageenan). The synthesis of Ag-NPs using photochemical method included silver nitrate and κ-carrageenan as a reducing agent, UV-irradiation, a silver precursor and stabilizer with different irradiation time at room temperature. UV-vis spectroscopy is improves the Ag-NPs formation in which the maximum surface plasmon absorption is observed at 420-430 nm. UV-vis spectra used in accordance with size and data distribution of Transmission Electron Microscopy (TEM). The κ-carrageenan surface is changed by the data illumination of Scanning electron microscope. Ag-NPs and κ-carrageenan between interactions are indicated by Fourier transform infrared spectra.

S. A. Ojo et. al. [15] presented Bacillus safensis LAU 13 extract based silver-gold alloy (Ag-Au) nanoparticles green biosynthesis with strain (GenBank accession No: KJ461434). The characterization of biosynthesized AuNPs and Ag-AuNPs uses FTIR spectroscopy, UV-Vis spectroscopy, TEM. Antifungal activities, malachite green degradation, blood anti-coagulation, human blood clot thrombolysis are evaluated according to the biosynthesized nanoparticles. Maximum absorbance of AuNPs and Ag-AuNPs is obtained at 561 and 545 nm, respectively.

J. Hamedi, A. Dehnad, F. Derakhshan-Khadivi, R. Abuşov, et. al. [16] presented gold nanoparticles biosynthesis using Arthrobacter genus member isolated from north-west of Iran of Andaliyan gold mine. Gold nanoparticles formation is explained by the aqueous medium UV-vis and XRD (X-Ray Diffraction) spectra with the strain

and 1 mM $HAuCl_4$ (Chloroauric Acid) for 24 h. spherical shape gold nanoparticles intra-extracellular production are shown by TEM micrographs with average size of 40 nm. Isolate was belonged to Atrhrobacter is revealed by molecular and morphological test results and it is 100% similarity in 16Sr Ribonucleic acid (RNA) gene sequences to Arthrobacter nitroguajacolicus.

W. Li et al., [17] described a novel method for silver nanoparticles synthesis by using selected solvents and materials. In an aqueous medium, it is a straight forward and green method. Renewable resources based three reagents (glucose, tollens' reagent, dextran) are used in this green approach. Silver nanoparticles storage stability and formation based on effect of tollens' reagent concentration is also discussed in this study. The characterization of silver nanoparticles uses UV-vis spectroscopy, Energy-Dispersive X-ray Spectroscopy (EDS), Transmission Electron Microscopy (TEM). Results indicate that, the silver nanoparticles distribution, formation rate and size are significantly affected by reaction parameters.

N. A. Zamanhuri, R. Alrozi, M. S. Osman and M. A. M. Ariff, et. al. [18] presented pink guava tree waste fruit extract based gold (AuNPs) and silver (AgNPs) nanoparticles green synthesis by using chloroauric acid and silver nitrate solutions respectively. For the synthesis, according to different aging time and temperatures, the size of gold and silver nanoparticles is different. Synthesized nanoparticles of gold and silver are characterized by using instrumental techniques as UV-vis.

R. K. Dutta, P. K. Sharma, P. K. Singh, M. Kumar, V. N. Singh, A. C. Pandey et. al. [19] presents, a chemical technique for synthesis of Nanophosphors of $ZnO:Cu^{2+}$ (Zinc oxide :Copper) based on coprecipitation method. In several steps at 100°C, nanophosphors are sunthesized at different temperatures (100-400°C) for 4 h. ZnO nanoparticles are remarkably stable which are surface-modified. Intrinsic impurities and surface defects removing process make the occurrence of reduction in annealing temperature with luminescence. At the same time unsaturated bond density is reduced by citric acid and surface is passivated which results surface trap sites numbers reduction for nonradiative recombination processes and enhances the luminescence intensity. $ZnO:Cu^{2+}$ nanophosphors are also used in bioimaging systems, drug delivery industries and biosensors besides citric acid capped.

R. J. Ranasinghe, E. Torres-Chavolla, E. C. Alocilja, et. al. [20] presents gold nanoparticles (AuNPs) tunable physicochemical properties. Depending on their evolving excellent properties as high surface-to-volume ratio, conducting properties and biological compatibility, these are recognized as ideal candidates in biosensing platforms of biological recognition events for electronic signal transduction. 30-60 nm average sized AuNP are obtained. The characterization of AuNP uses particle-size distribution, transmission electron microscopy analysis, UV-Vis spectra. Biosensing application uses are enhanced by using production of green-chemistry AuNP and these applications as electroactive labels or transducers, electrochemical DNA (DeoxyRibonucleic Acid) detection systems based on nanoparticles.

III. EXOPOLYSACCHARIDES COATED SILVER NANOPARTICLES GREEN SYNTHESIS

Materials:

From Sigma–Aldrich Chemical Company, all the chemicals, reagents and silver nitrate

(AgNO3, 99.9%) are collected. By using dilute HNO_3 (Nitric acid), all glassware were washed and dried in oven. From botanical garden of Annamalainagar Cuddalore district, Tamil Nadu, the leaves of Croton sparsiflorus morong are collected. Used Bacterial strains are as Pseudomonas aeruginosa ATCC (American Type Culture Collection) 27853 (P. aeruginosa), Staphylococcus aureus ATCC 6538 (S. aureus) and Escherichia coli ATCC 11229 (E. coli).

The Scientific Name of collected Plant is Croton sparsiflorus morong, and its Common Name in general called as Croton Bonplandianum. Collected plant is under the family of Euphorbiaceae. Work flow of Exopolysaccharides coated silver nanoparticles Green synthesis using Croton sparsiflorus morong leaf and their Antimicrobial Activities is represented in below Fig. 1.

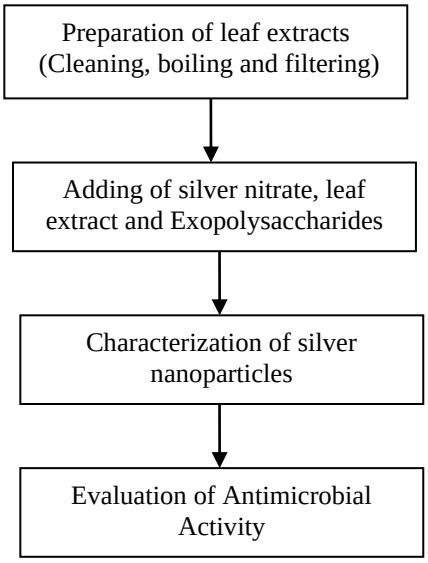

Fig. 1: WORK FLOW OF EXOPOLYSACCHARIDES COATED SILVER NANOPARTICLES GREEN SYNTHESIS

Preparation of leaf extracts:

The collected leaves are gently washed using tap water and again with distilled water. 100 mL sterile distilled water is boliled at 80^0C for 15 min with the addition of 10 g of leaves and then cool it under room temperature. After some time this extract is filtered out by using Whatman filter No. 1 paper stored at 4^0 C for further synthesis.

Synthesis of Silver nanoparticles:

The silver nanoparticles synthesis is started with a 1.6 mL tube in which 380 µL of EPS (1000 ppm), 20 µL of Croton sparsiflorus morong leaf extract and 600 µL of silver nitrate (10 mM) are collected and mixed well. This collected mixture is incubated at 60ºC for 1 hour with 3000 rpm (rotation per minute). The monitoring of SNPs formation is observed after the color change from yellow/brownish to dark brownish. Absolute ethanol (2:1 relation; absolute ethanol: synthesis reaction) is added to the synthesized solution incubated at -20ºC for 12 hours. Polymer coating is allowed in this step and forms a silver nanoparticles matrix. In the next step, this mixture is centrifuged for 10 minutes at 9500 rpm 4ºC. This discharges the supernatant and evaporates the remaining ethanol at room temperature. At last EPS-SNPs nanobiocomposite is obtained by lyophilized the product for 12 hours.

Characterization of silver nanoparticles:

The test plant extract supernatant in the solutions used to reduce the Ag+ ions. Silver nanoparticles presence characterization uses UV–Visible (UV-Vis) spectroscopy with the help of aqueous component (2.0 mL). In aqueous solution, silver ions bio-reduction was monitored by using solution UV–Vis spectrum and is observed in the range of 300–800 nm.

FE-SEM is used to find the sample Morphological characterization in which the carbon tape is coated with pinch of dried sample. Again this tape is auto fine coated with platinum then this subjected to FE-SEM analysis. A carbon tape with reduced silver placed on a copper stub is dried for EDX analysis and FE-SEM attached with an EDX equipment. Different EPS-SNPs functional groups presence is identified by allowing the FT-IR spectra analysis. Lyophilized EPS samples, nanobiocomposite and EPS-SNPs functional groups are determined through FT-IR spectrophotometer, reading the 400- 4000 cm−1 region.

Any free biomass residue is removed after reaction of residual solution centrifuged for 20 min at 4000 rpm and then redisperses the resulting suspension in 10 mL sterile distilled water. Three times repetition of centrifuging and redispersing processes results the final sample and it is palletized with KBr (Potassium Bromide) and analyzed using FT-IR.

Evaluation of Antimicrobial Activity:

The microdilution method is used to examine the antibacterial performance. Different treatment EPS and EPS-SNPs concentrations are included in incubation of bacterial suspensions. Bacterial culture of 100 µL is inoculated into 20 mL LB or TSB media to an overnight at 37ºC with 150 rpm. This process is continued up to it reaches exponential phase with 107 cells/mL. In the next step, dilute the culture as 1:1000 in 1.5 mL tubes and 96- well plate added to 100 µL of this dilution for the treatment. Each well contained a final volume of 200 µL with 105 cells/mL concentration. The final EPS-SNPs concentrations are: 5.3, 10.6, 21.2, 42.4, 84.8, 169.7, 339.4, 678.8, 1357.5 and 2715 nm. Incubate the each plate later at 37ºC for 20 hours with 150 rpm. Corresponding growth and sterility controls are performed by triplicate examination.

IV. RESULT ANALYSIS

The EPS-SNPs formation is identified by color changing to dark brown from its original color from pale yellow and this change is because of surface plasma of silver ions resonance and reduction by Croton sparsiflorus morong leaf extract. According to Ag NPs surface Plasmon resonance, kmax with single broad peak is observed at 474 nm. Total EPS-SNPs UV–Visible spectrum recorded at 24 h in this study. The explored results are indicating that, Ag+ ions reduction is possible with extracellular as shown in Fig. 2. Therefore these results conforms the Croton sparsiflorus morong leaf extract based Ag NPs. Earlier kmax values of typical EPS-SNPs for visible range of 450–480 nm.

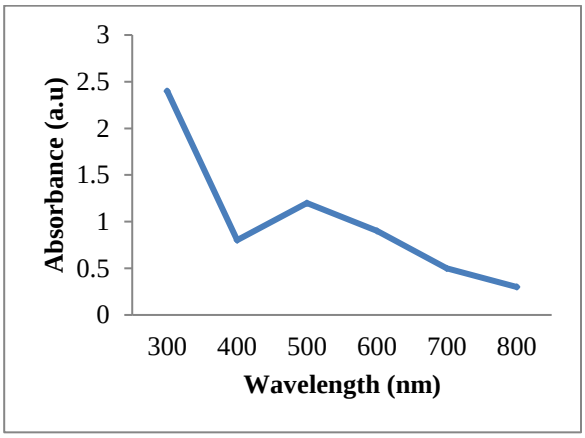

Fig. 2: EPS-SNPs UV–VIS SPECTRUM

Different magnifications 30,000 and 50,000 of FE-SEM are shown in Fig. 3A and B, respectively and this analysis is used to observe the SNPs by adoption of spherical morphology with smooth surface. EPS-SNPs accumulated on to the surface of FE-SEM images because of interactions of hydrogen bond and as well as electrostatic relations among number of molecules of bioorganic capping with EPS-SNPs.

Crystalline spherical EPS-SNPs are observed at FE-SEM micrograph previously described earlier. Fig. 3A and 3B shows the particle sizes in the range from 22 to 52 nm respectively. Ag NPs elemental compositions information is analyzed by using EDX analysis and is depicted in Fig. 4. At 3 keV silver strong elemental signal existence is confirmed by EDX spectrum which is fundamental for metallic silver nanocrystallites absorption because of surface Plasmon resonance. The highest existence of lipids, proteins and chlorine related compounds are identified with EDX apart from silver peaks. EPS-SNPs formation is confirmed by the strong Surface Plasmon Resonance (SPR) peak. EPS and EPS-SNPs functional groups are identified by performing FT-IR spectra analysis. SPR absorbance is extremely sensitive to shape, size and nature of formed particles along with inter particle.

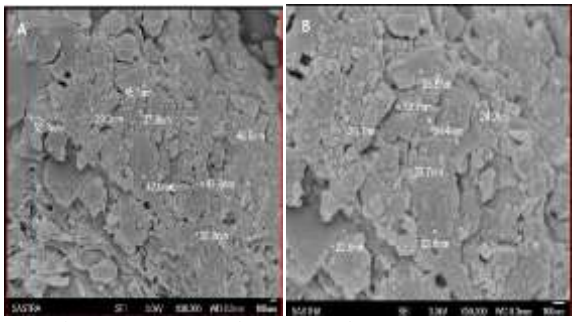

Fig. 3: FE-SEM (A AND B) OF EPS-SNPs

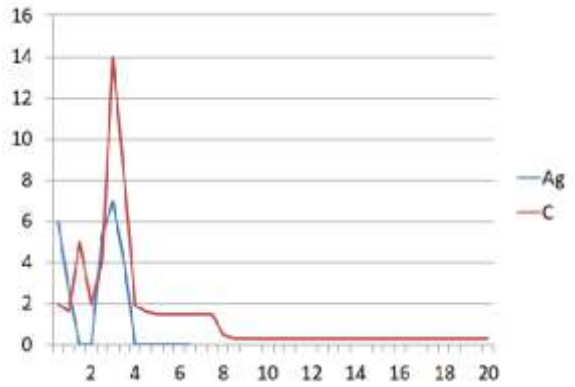

Fig. 4: EDX SPECTRUM OF EPS-SNPs

EPS and EPS-SNPs several functional groups are identified by performing FT-IR spectra analysis. The bands are revealed at 3328, 2920, 1645, 1365, and 1000-1200 cm^{-1} which are used to denote the hydroxyl groups stretching vibrations from a polysaccharide, Methylene (CH2) , enol and amide groups, C-C stretching, carboxyl groups, C-O-H, C-O, C-O-C and polysaccharides deformation vibrations. The sample is a polysaccharide indicative by the peak at1019 cm^{-1}. Presence of a polysaccharide is indicated by observed absorption peaks at the range of 1200-1000 cm^{-1}. Functional groups with negative charges as hydroxyl, carboxyl and amide are existed in exopolysaccharides and these are reacted with metallic cations. At the peaks of the C-O, COOH and O-H groups, reduced intensity is observed in the comparison of FT-IR spectrums of both EPS and EPS-SNPs. distinctive of glycosidic bands are existed in absorption peaks found in the 1200-1000 cm^{-1} which indicates that EPS were integrated into the nanoparticles.

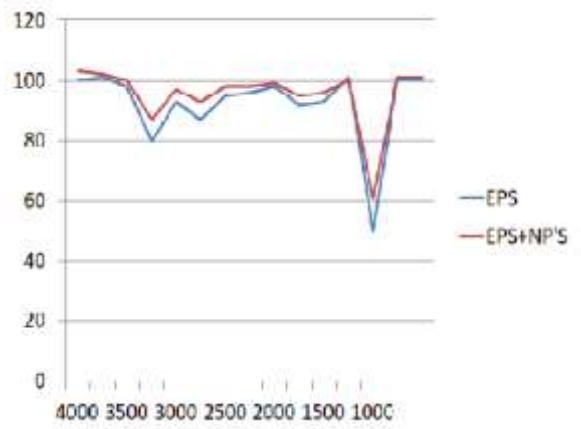

Fig. 5: EPS AND EPS-SNPs FOURIER TRANSFORM INFRARED SPECTRA

The ability to kill both Gram-positive and as well as Gram-negative bacteria of EPS-SNPs is determined by studying the antibacterial effects in which different treatments with exposing to bacteria. In

these experiments, S. aureus was susceptible at concentrations between 169.7 and 2715 nM of EPS-SNPs. Higher bacterial reduction EPSSNPs from 339.4 to 2715 nM is shown by E. coli and P. aeruginosa. The test bacteria visible growth is null from results as observed lowest concentration of Minimum Inhibitory Concentration (MIC). From Table 1, S. aureus ATCC 6538 has MIC value as 1357.5 nM, whereas, P. aeruginosa ATCC 27853 and E. coli ATCC 11229 has same MIC values for both strains as found to be 678.8 nM.

Table 1: MINIMUM INHIBITORY CONCENTRATION OF EPSSNPs

Strain	EPSSNPs (nM)	EPS (Nm)
E. coli ATCC 11229	678.8	1214
P. aeruginosa ATCC 27853	678.8	1145
S. aureus ATCC 6538	1357.5	2614

Interestingly, the activities of both antibiofilm and antimicrobial are better for EPS-SNPs than EPS. EPS-SNPs has more sensitive for E. coli ATCC 11229 and P. aeruginosa ATCC 27853 strains than S. aureus ATCC 6538.

V. CONCLUSION

In this paper, Exopolysaccharides coated silver nanoparticles Green synthesis using Croton sparsiflorus morong leaf and their Antimicrobial Activities is described. this study have shown that the silver nanoparticles green synthesis using Croton sparsiflorus morong leaf extract. The silver nanoparticles synthesis is started with a 1.6 mL tube in which 380 µL of EPS (1000 ppm), 20 µL of Croton sparsiflorus morong leaf extract and 600 µL of silver nitrate (10 mM) are collected and mixed well. The nanobiocomposite characterization is done with the sequence of methods as UV-visible spectroscopic, FE-SEM with EDX Spectroscopy and FT-IR Spectroscopy. Final sample is palletized with KBr and analyzed using FT-IR. Total EPS-SNPs UV–Visible spectrum recorded at 24 h in this study. EPS and EPS-SNPs several functional groups are identified by performing FT-IR spectra analysis. The test bacteria visible growth is null from results as observed lowest concentration of Minimum Inhibitory Concentration (MIC). S. aureus ATCC 6538 has MIC value as 1357.5 nM, whereas, P. aeruginosa ATCC 27853 and E. coli ATCC 11229 has same MIC values for both strains as found to be 678.8 nM. EPS-SNPs is more efficient biocide for the clinical imported labs because of their properties. Therefore, it is concluded that, synthesized nanopaticles are used in many applications as electronics development industries, drug delivery systems, cosmetics production fields, pharmacology, Cancer treatments, antifungal and fungal drug industries and etc.

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