Green Synthesis of Silver Nano Particles from *Saracaasoka* and its Antimicrobial Activity Against *Listeria monocytogenes* and *Salmonella typhimurium*.

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Abstract: The present study focuses on the effect of different parameters like time, temperature and concentration of plant extract on the synthesis of silver nanoparticles (AgNPs) using leaf extract of *Saraca asoka* and their antibacterial activity against *Salmonella typhimurium* and *Listeria monocytogenes*. The surface plasmon resonance was measured at 413- 427 nm. The size of the synthesized AgNPs were assessed using FWHM and was found in the range of 50 -60 nm. Fourier Transform Infrared Spectroscopy (FTIR) analysis showed that the primary group contributing for the reduction of AgNPs were C-O stretches from alcohols, esters and ether groups. The synthesized AgNPs showed good antibiotic resistance against *Salmonella typhimurium* while it showed mild antibiotic resistance against *Listeria monocytogenes*.

***Index Terms*–Silver nanoparticles, antimicrobial activity, *Saraca asoka*.**

I. INTRODUCTION

Nano particles can be synthesized using various approaches including chemical, physical and biological. Chemicals used for nano particles synthesis known to produce toxic and non-ecofriendly byproduct. This warrants the need for a green option for the synthesis of nanoparticles. This has led to the increased interest in biological approaches which are free from toxic chemicals as byproducts. Various methods are applied for the green synthesis of nanoparticles. Microorganisms are used for the synthesis of nanoparticles (Das, et al., 2014). Plant extracts are also used for cost-effective synthesis of nanoparticles (Ibrahim, 2015, Khan, Tareq, Hossen, &Roki, 2018, Koparde& Gaikwad, 2018 and Shaik, et al., 2018). Other than silver various other metals like gold, iron, zinc and platinum have been exploited in the field of nano technology (Krishnadas, R, & S, 2017).

Plants provide a better way for nano particles synthesis as they are free from toxic chemicals as well as provide natural biomolecules for the synthesis of nano particles. Plant extracts is the most adopted method of green synthesis of nano particles. Synthesis of nano particles of different metals using a variety of plant extracts has been done, but the standardization of the shape, size and morphology of the synthesized nanoparticles has been a challenge. Various field of nanotechnology applications range from catalyst, micro and nano electronics (semiconductors and single electron transistors) nonlinear optic devices, photo electrochemistry to biomedicine, diagnostics, food and environmental chemical analysis and others (Conteseu&Putyera, 2009).

Silver nanoparticles have a great use in biology including antimicrobial activity (Sap-Lam, et al., 2010; Chandrappa, et al., 2017) due to this silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and medical devices. Silver is an effective antimicrobial agent which exhibits low toxicity. The most important application of silver and silver nanoparticles is in medical industry such as tropical ointments to prevent infection against burn and open wounds. Novel methods of ideally synthesizing nanoparticles are thus being thought which are formed at ambient temperature, neutral pH, low coasts, and environment friendly fashion. The present study focuses on the characterization of ideal microenvironment suited for the production of smallest silver nanoparticles, by analyzing the various factors such as concentration, time and temperature by using *Saraca asoka* leaves and assessing its antimicrobial activity against *Listeria monocytogenes* and *Salmonella typhimurium*.

II MATERIALS AND METHODS

2.1 Plant material collection and Extraction

Fresh leaves of *Saraca asoka* were collected from Kollam district, and washed several times with tap water and distilled water to remove dust particles and then shade dried to remove the residual moisture and grinded to form powder. Then plant extract was prepared by boiling 2g of the leaf powder in 40ml distilled water at 60^{o}C for about 10m. The supernatant was filtered using filter paper to remove the particulate matter. A light brown clear solution was obtained and stored at 4-8^{o}C.

2.2 Synthesis of silver nanoparticles

Silver nitrate salts were used for the preparation of AgNPs. 2mM solution of silver nitrate was prepared by dissolving 0.0085g of $AgNO_3$ in 25ml of distilled water. 1ml extract of *S. asoka* leaf was mixed with 5ml of 2mM $AgNO_3$ solution. A color change from pale yellow to colloidal brown indicated the formation of silver nano particles.

2.3 Characterization of Silver Nano Particles

The optical property of AgNPs was determined by UV-Vis-IR spectrophotometer (UV-3600 Shimadzu). The effect of various parameters such as concentration, reaction time and temperature on the synthesis of silver nano particles were examined. Effect of time was studied at regular intervals of 1hour, 2hour, 3hour and 4 hours and effect of temperature was studied by varying the temperature between 0 – 40^{o}C. The effect of concentration of plant extract on the formation of AgNPs was also studied systematically by mixing 2mM AgNO3 solution and S. asoka extract in different concentration ratios i.e. (5: 0.01, 5: 0.02, 5: 0.03, 5: 0.04, 5: 0.05) (ml: ml).

FTIR analysis

The chemical composition of the synthesized silver nano particles was studied by using FTIR spectrophotometer (IR Affinity I Shimadzu) the solutions were dried at 60ºC and the dried powders were characterized in the range 4000-400cm^{-1} using KBr pellet method.

2.4 Determination of antibacterial activity

The antibacterial activity of biogenic AgNPs synthesized using *S. asoka* leaf extract were assayed by disc diffusion method against two pathogenic bacterial species *Listeria monocytogenes* and *Salmonella typhimurium*. Petri plates were prepared by pouring 30ml of nutrient agar. The test organisms were inoculated on solidified agar plate, spread and allowed to dry for 10 min. A sterile cotton swab was used to spread S. typhimurium and Listeria monocytogenes on the plates. Using sterile forceps, the sterile discs contain the AgNPs (10 µg/ml, 20 µg/ml, 30 µg/ml), plant extract (30 µg/ml) and Tetracycline (30µg/ml) were laid down on the surface of inoculated agar plates. The plates were incubated at 37ºC for 24h. Each sample was tested for the zone of inhibition which was measured using scale after 24 h.

The zone at which no bacterial colonies can be grown are called inhibition zone. The inhibition zone depends upon the concentration of antibacterial compound. An increase in the concentration of antibacterial compound will cause an increase in the zone of inhibition.

III. RESULTS AND DISCUSSION

UV-Vis analysis

The formation of the Silver nano particles (AgNPs) was indicated by the change in the color of the solution from colorless to yellow and finally brown color (figure.1). The silver nanoparticles show strong surface plasmon resonance (Jensen, Malinsky, Haynes, & Van duyne, 2000). The formation of silver nanoparticles was examined by measuring the absorption spectra of synthesized AgNPs in the range of 300–700 nm for the reaction of silver salt with *S. asoka* leaf extract under different conditions. The effect of different parameters (time of reduction, concentration of the plant extract and temperature in which the reduction was carried out) on the formation of the AgNPs were evaluated using the absorption spectra in the range of 300–700 nm. Smaller nano spheres primarily absorb light and have the peaks near 400nm while larger spheres exhibit increased scattering and have peaks that broaden and shift towards longer wave lengths known as red shift. (Paramelle, et al., 2014). The size of AgNPs was compared using the equation (Jain &Mehata, 2017)

$$d = \frac{h v_f}{\pi \Delta E_{1/2}}$$

The results of the spectral analysis indicate that time of reduction, temperature and concentration of the plant extract used for reduction have significant effect on the size and rate of the formation of silver nano particles. The effect of concentration of plant extract on the formation of AgNPs was studied systematically by mixing 2mM $AgNO_3$ solution and *S. asoka* extract in different concentration ratios i.e. (5: 0.01, 5: 0.02, 5: 0.03, 5: 0.04, 5: 0.05) (ml: ml). As the plant leaf extract was mixed with the $AgNO_3$ solution, a color change from pale yellow to dark yellow and finally brown was observed within few minutes. The results of the effect of increasing concentration on the speed of the reduction reaction and the size of the nano particles measured using UV spectra are depicted in graph (figure. 2a). The effect of different incubation time (1h, 2h, 3h and 4h) and the effect of temperature (0 ºC, 10 ºC, 20 ºC, 30 ºC and 40 ºC) on the formation of AgNPs from S. asoka leaf extract are shown in figure. 2b and figure. 2c respectively.

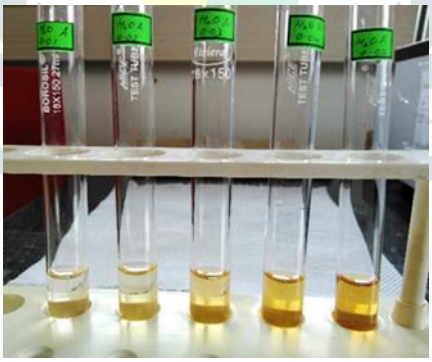

Figure 1. The color change observed while the formation of AgNPs using different concentrations of aqueous *Saraca asoka* leaf extract.

Sharp peaks were observed in 420nm wavelength indicating the production of AgNPs. ΔE1/2 values showed an increase with the increase in concentration of plant extract from 0.03ml to 0.05ml. As seen in the graph the rate of production of silver nanoparticles also enhanced with increasing concentration from 0.03ml-0.05ml. As FWHM showed an increase with increasing concentration this indicates that there is a decrease observed in the size of the synthesized silver nanoparticles. Concentrations below 0.02ml of plant extract indicated low production rate. The rate of the production of silver nanoparticles increased with the increase in the duration of the reaction time highest production was achieved at 4h. The maximum FWHM was observed at 2h so the smallest size of the nanoparticles was produced during the 2h incubation time. The morphology of the synthesized AgNPs influence its bioactivity (Dong, Ha, Binh, &Kasbohm, 2012). The best temperature of the production of AgNP preparation was 30 ºC. Below 30ºC no production of silver nanoparticles was observed at 40ºC the rate of the production drops. The smallest size of silver nanoparticles was also observed during 30ºC.

The maximum extinction coefficient was in the range of 390nm to 430nm which indicated the formation of silver nanoparticles with a size range of 30-60nm. The spectra also confirm that there is no aggregation in the formed silver nano particles as no secondary peaks were observed in longer wave lengths. The signature optical property of noble metal nanoparticles is the localized surface plasmon resonance (Jensen, et al., 2000). The wavelength corresponding to the extinction maximum is highly dependent on the size, shape, and dielectric properties of the metal nanoparticles (Kreibig& Vollmer, 1995).

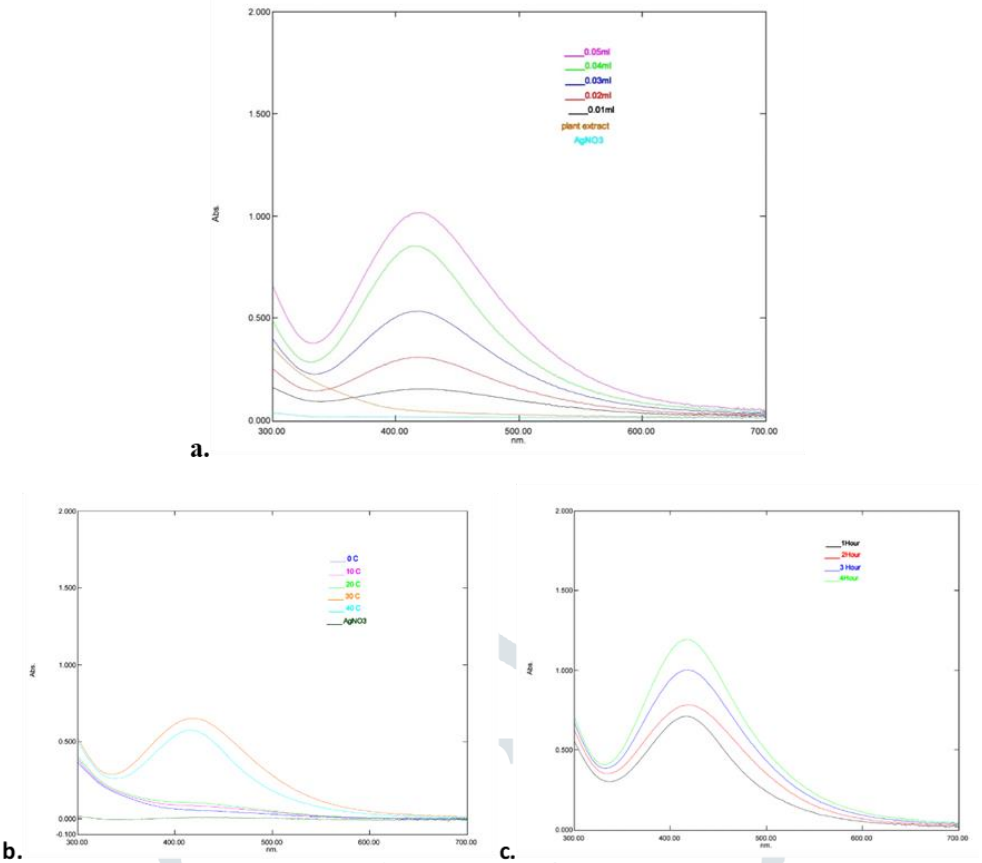

Fig. 2 UV-Vis absorption spectra of biosynthesized AgNPs recorded a. by using different concentrations of *Saraca asoka* leaf extract b. At different time intervals c. At different temperatures

FTIR

The FTIR spectra of the AgNPs synthesized using S.asoka plant extract (figure 3) as reducing agent, showed peaks at around 1300 and 450 cm^{-1}. The prominent peak around 1300 cm-1 is the characteristic feature of C-O vibrations found in alcohols, ethers, esters, carboxylic acids and anhydrides. The plant extract is rich in phytochemicals but the FTIR results indicate that in *S.asoka* leaf extract the major contribution in the reduction of the silver salt is provided by C-O group. Previous works have reported the role of groups like C=O, C-Cl, -OH (Bagherzade, Tavakoli, &Namaei, 2017), NH2, and COO- in the reduction of silver salts by different plant extracts (Khalil, et al., 2014).

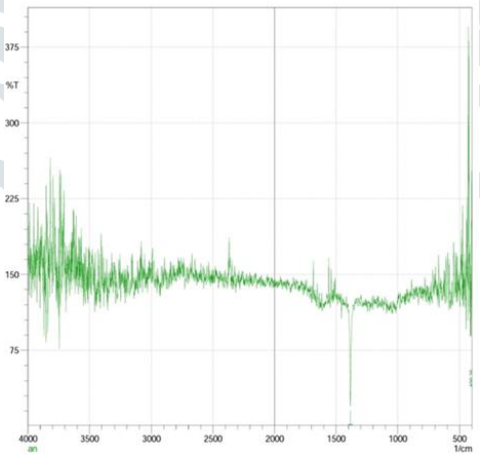

Figure 3. FTIR spectra of AgNPs synthesized by using *Saraca asoka* leaf extract as reducing agent.

Antibacterial Property

The antibacterial property of AgNPs synthesized from the *Saraca asoka* leaf extract was assessed using disc diffusion method against *L. monocytogenes* and *S. typhimurium*. Earlier studies have shown that the interaction of AgNPs with phytochemical constituents like flavonoids enhance their anti-microbial activity (Jain &Mehata, 2017). The results indicate that (Table 1 & 2) AgNPs synthesized using *S.asoka* extract showed a concentration-based increase in the antibacterial activity compared to pure *S. asoka* plant extract against *S. typhimurium*. The zone of inhibition achieved using the highest concentration that is 30µg/ml of the plant extract was 2mm while that of the 30µg AgNPs was 4mm which was close to the 5mm zone of inhibition produced by the standard 30µl tetracycline. The antibacterial activity of AgNPs against *Listeria monocytogenes* was equal to that of the plant extract in both cases the zone of inhibition was 2mm while the standard tetracycline showed 9 mm zone of inhibition. Although the zone of inhibition achieved by green synthesized AgNPs in the range 2-5mm at concentrations as small as 30µg/ml shows that at higher concentration these can be used as potential antibiotics. The results indicate that AgNPs from the*S. asoka* leaf extract have much more antibacterial activity against *S typhimurium* than *Listeria monocytogenes*.

Table 1. The zone of inhibitions achieved for different concentrations of the synthesized silver nano particles, plant extract and standard against *Salmonella typhimurium.*

Serial no	Compound	Concentration µg/ml	Zone of inhibition in mm
1	AgNPs	10	1.5
2	AgNPs	20	3.5
3	AgNPs	30	4.5
4	*S. asoka* Extract	30	2
5	Tetracycline standard	30	5

Table 2. The zone of inhibitions achieved for different Concentrations of the synthesized silver nano particles, plant extract and standard against *Listeria monocytogenes.*

Serial no	Compound	Concentration µg/ml	Zone of inhibition in mm
1	AgNPs	10	1
2	AgNPs	20	1.5
3	AgNPs	30	2.2
4	*S. asoka* Extract	30	2
5	Tetracycline standard	30	9

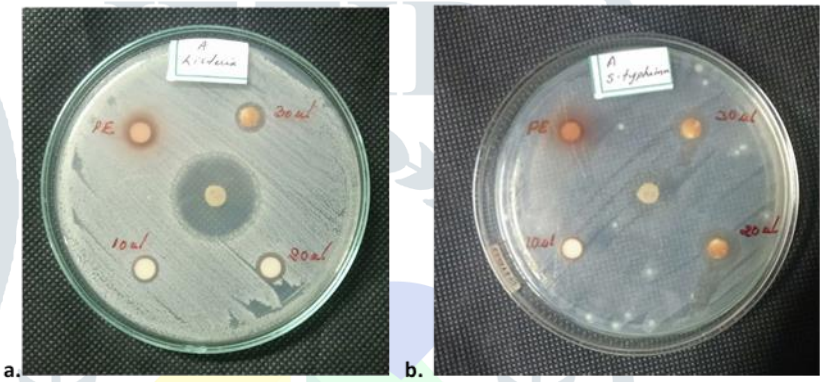

Figure 4. Inhibition zone around the well impregnated with various concentration of biogenic AgNPs against *Listeria monocytogenes* and *Salmonella typhimurium*.

Conclusion

It can be concluded that the *Saraca asoka* leaf extract can be used to achieve the green synthesis of silver nano particles in the size range of 50 - 60 nm. The particle shows potential antibacterial activity against *Listeria monocytogenes* and *Salmonella typhimurium* and can be used as an antibacterial agent. The spectroscopic analysis revealed that most efficient synthesis of the silver nano particles can be achieved by using higher plant extract concentrations ant 30^{o}C temperature and by incubating for durations of 4hours. So, *Saraca asoka* leaf extracts can be considered as eco-friendly substitute for the production of silver nano particles.

IV. ACKNOWLEDGMENT

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