INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 6, Issue 2 - 2019

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2019.06.02.006

Green synthesis of Silver Nano particles from Litchi seeds and its Antimicrobial activity

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Abstract

Plant mediated synthesis of metallic nanoparticles creating commercial demand in various field like health related products, chemistry and medicine. The green synthesis method is the best method for the synthesis of metallic nanoparticles. It is easy, effective and eco-friendly compared to chemical and microbial mediated synthesis. It has been reported that silver nanoparticles are non-toxic to human and most effective against bacteria, virus and other microorganisms. Nano ranged particles especially silver contained substances gives promising range of antimicrobial activity. In this present study silver nanoparticles were synthesized using litchi seeds extract because it has high medicinal value. Synthesized nanoparticles were characterized under UV-Vis spectral analysis, Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR) and antimicrobial activity of nanoparticles were examined. The result reviews UV-Vis spectroscopy absorption was recorded at 435nm which is a characteristics of silver nanoparticles. FTIR analysis supported the reducing property of silver nanoparticles and the spectral range between 1000-4000cm^{-1}. AFM study gave diameter of silver nanoparticles range of 80-120nm. The results of antimicrobial activity are, the *Staphylococcus* spp *& Streptococcus* spp showed high sensitivity and *E-coli* showed low sensitivity. In this work silver nanoparticles showed the greatest potentiality towards controlling the growth of bacteria.

Keywords: Silver nanoparticles, Atomic force microscope (AFM), Litchi seeds, Fourier Transform Infrared spectroscopy(FTIR).

Introduction

Nanoparticles plays an important role in various fields like medical science, bioelectronics and biochemical application and its studies existence in nature to fabricate new nano-devices. Illumination of plant mediated synthesis of nanoparticles is a very promising field of research. Green synthesis of noble material is important because they are eco-friendly and human health beneficial (Shafqat Ali khan et.al 2018).Some other methods like chemical and physical methods used for synthesis of noble metal nanoparticles. Many work on green synthesis of nanoparticles are carried on various metallic nanoparticles like silver, gold, lead, selenium. Selenium extraction from orange peel (Bala Krishna R et.al.2014). Silver nanoparticles are the metal of having capability to kill microorganisms effectively (Shrama NC, et al., 2007). Green synthesis advantages compared to other synthetic method as they are commercial and restrict the use of toxic chemicals as well as high pressure energy and temperature. Ultimately silver nanoparticles enhance the effect of the anti-cancer drug.

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In present study seed extract of litchi seeds used for the synthesis of silver nanoparticles. *Litchi chinensis* is scientific name of the litchi fruit. Due to their peculiar properties they have been used for several applications agents and ultimately enhance the tumor killing effect of anticancer drugs. It has been reported that silver nanoparticles are nontoxic to humans and most effective against bacteria, virus and other eukaryotic microorganisms at low concentrations and without any side effects [1]. The bactericidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial.

Materials and Methods

Collection of plant parts

Litchi chinensis fruits were purchased in the markets of Moodbidiri, Karnataka in the month of December 2017.They were cleaned with deionized water and allowed it to shade dry for 10-15 days. It was kept in hot air oven at 60^0C for 24-48 hours completely. Dried sample was grounded to fine power using motor and pestle (Figure 2).

Figure 1: Lychee seeds

Figure 2: Powdered seeds

Synthesis of silver nanoparticles from Litchi seeds (Gawthami Ramesh, et.al, 2017)

1 mM aqueous extract of silver nitrate was prepared, 2.5g of seed powder was mixed (Figure 3) with 100ml of silver nitrate solution kept in water bath and heated at 95^0C. A change in the colour of the solution (Figure 4) was observed after heating the mixture for 2hours. The extracts were stored at 4^0C for further use.

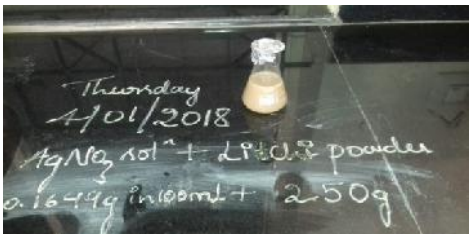

Figure 3 (Before incubation)

Figure 4 (After incubation)

Characterization of synthesized silver nanoparticles.

UV-Vis spectrometer analysis (Geetha Watal et.al, 2013).

The reduction of pure Ag^+ ions was mentioned by measuring the UV-Vis spectrum of the reaction medium at 6 hours after diluting as small aliquot of the sample into distilled water .UV-Vis spectral analysis was done by using UV-Vis spectrometer silver nanoparticles are known to exhibit a UV-Visible absorption maximum in the range of 300-600nm.

AFM (Atomic Force microscope) analysis of silver nanoparticles.

Atomic force microscopic analysis was done using AFM machine. Thin film of the sample was prepared on a carbon coated grid by dropping a very small amount of the grid, extract solution was removed using blotting paper and then film on the AFM grid was allowed to dry putting it under a mercury lamp for 5 minutes. The size and shape of nanoparticles was observed.

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FTIR Fourier Transform Infrared Spectroscopy).

Fourier Transform Infrared spectroscopy (FTIR) was used to recognize the functional groups bound to the silver surface and involved in the formation of silver nanoparticles. The lyophilized powder sample was used and spectrum at the special range of 1000-4000nm.

Antimicrobial activity.

Antimicrobial activity of synthesized nanoparticles was tested against the *Streptococcus* spp, *Staphylococcus* spp and *E. coli* by agar well diffusion method. Petri dishes were poured with Muller Hinton agar media. The test organisms were then spread over the surface of the media using sterile swab stick and 7mm of cork borer was used. Different concentration of silver nanoparticles i.e, 20µl, 40µl and 60µl respectively dispensed into the wells using a micro pipette and allowed the extract to diffuse for 30 minutes at room temperature. The plates were incubated at 37^0 c for 24 hours. The zones of inhibition were measured.

Results

Synthesis of Silver Nanoparticles

Formation of silver nanoparticles by the reduction of silver ions during the Litchi seed extract can be easily monitored from the change in colour [Figure 5(a)] of the reaction mixture from light brown [Figure 5(b)] to dark brown due to the excitation of Surface Plasmon vibrations. The change in the colour of the reaction mixture was observed after 1 hour of incubation which indicated the formation of silver nanoparticles. The formation indicates that the silver ions in reaction mixture medium have been converted to elemental silver having the size of nanometricrange.

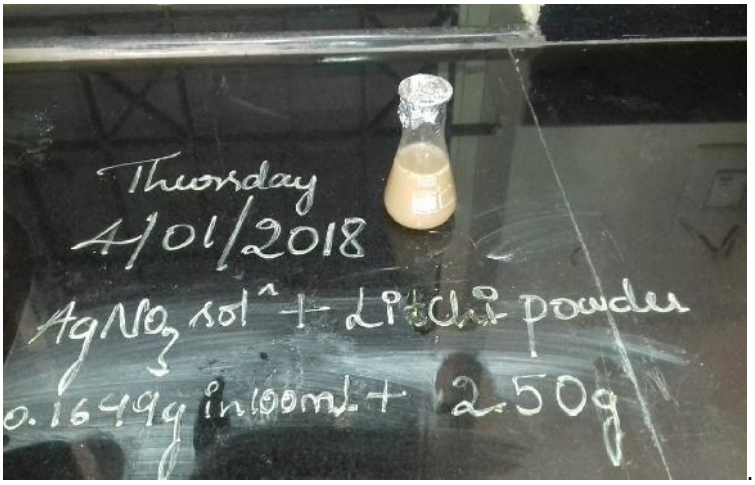

Figure 5 (a): Colour before incubation

Figure 5 (b): Colour changing after incubation

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Ultraviolet- Visible (UV- Vis spectroscopy) Spectra analysis.

UV- Visible absorption maximum in the range of 300-600 nm. In this study the formation of silver nanoparticles was initially confirmed by using UV-Visible spectroscopy due to Surface Plasmon Resonance phenomenon. Absorption (Figure 6) was at 435nm which is a characteristic of silver nanoparticles.

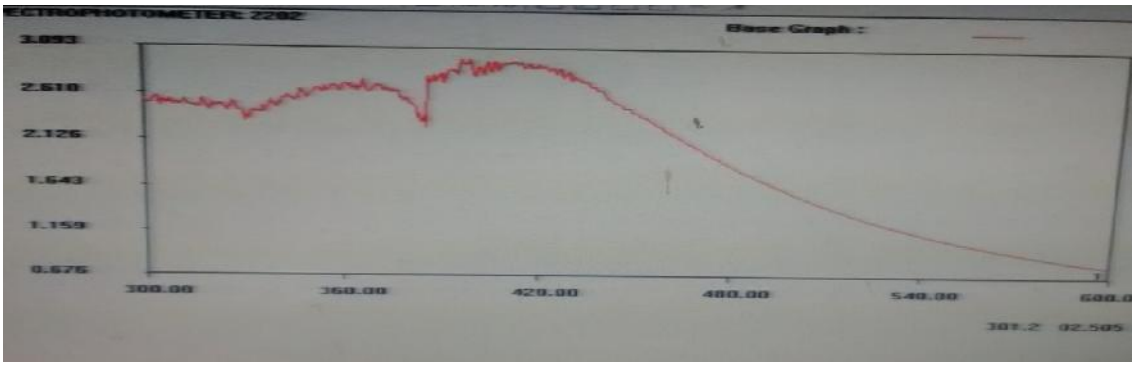

Figure 6: UV-Visible spectroscopy

AFM (Atomic Force Microscope) analysis of silver nanoparticles.

Synthesized silver nanoparticles were evident that the morphology of the synthesized silver nanoparticles were hexagonal in shape with the diameter range of 80-120nm. The diameter size confirms the presence of silver nanoparticles (Figure 7).

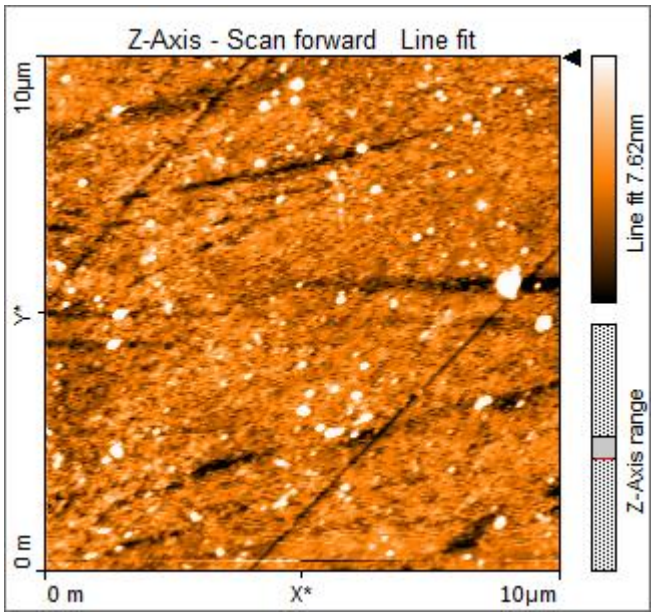

Figure 7: Atomic force microscope

Fourier Transform Infrared Spectroscopy (FTIR)

The lyophilized nanoparticle samples were analysed in FTIR to identify the possible biomolecules responsible for the reduction of the silver ions by cell filtrate. The representative spectra of nanoparticles obtained manifests absorption peaks (Figure 8) using the spectral range between 1000-4000cm^{-1}.The absorption peaks were observed at 3546.42cm^{-1}, 3470.32cm^{-1}, 1629.97cm^{-1}, 1567.56cm^{-1}, 1414.35cm^{-1} and 763.68cm^{-1} can be assigned as absorption bands of –NH group of amines, -OH group of phenols, C-H aromatic stretch of groups, NHCO group of amides and C-CL functional groups. The FTIR analysis supported the reducing property of silver nanoparticles by seed extract which in turn imparted the high stability of the synthesized silver nanoparticles.

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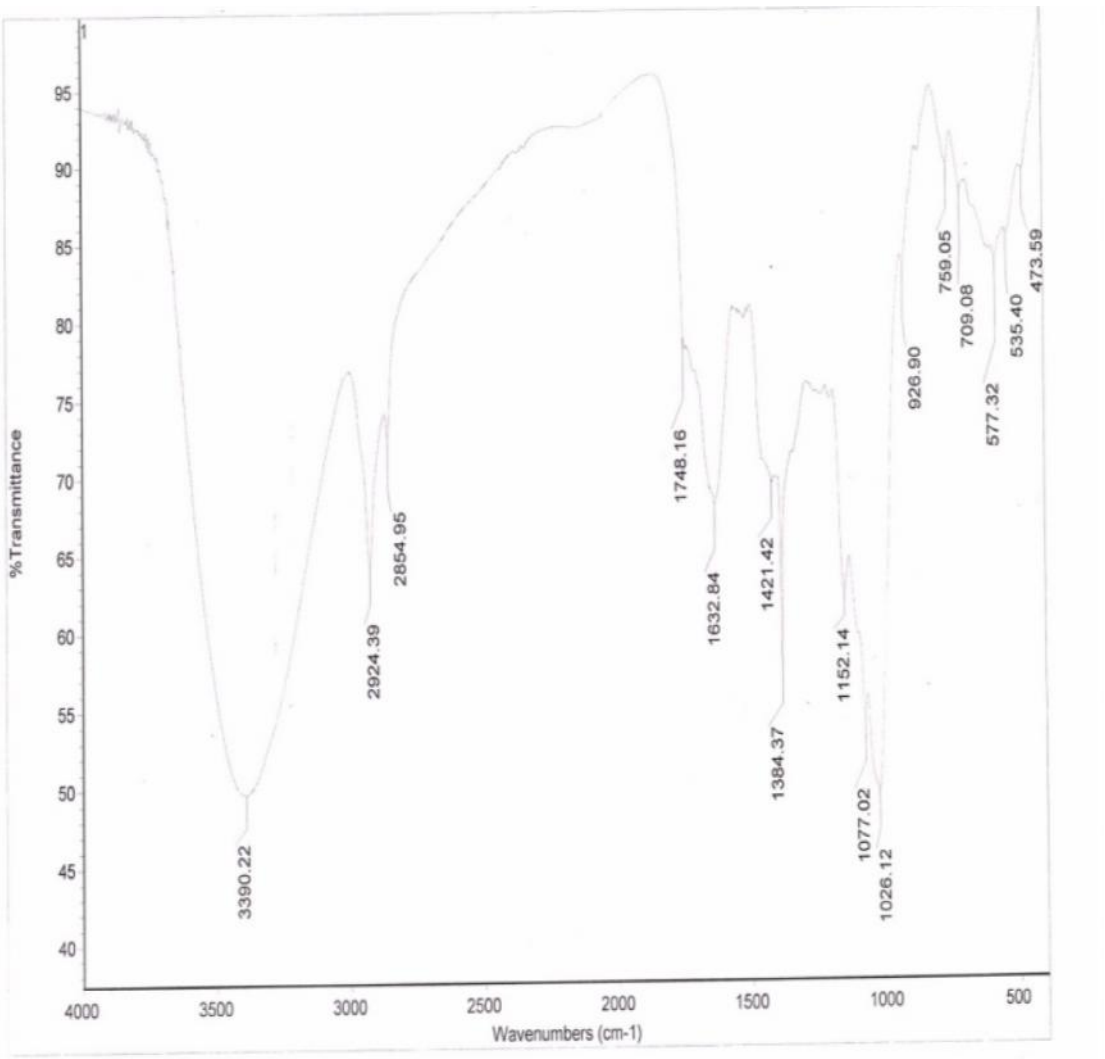

Figure 8: Fourier Transform Infrared Spectroscopy

Antimicrobial activity for different concentration of the nanoparticles extract

The synthesized nanoparticles showed inhibition zone against *Staphylococcus.* spp, *Streptococcus.* spp and *E. coli.* Different concentration of 20µl, 40µl and 60µl of synthesized silver nanoparticles were used for the study of antibacterial activity (Table:1).The result shown in Figure:10, it indicates the zone of inhibition against the test organisms. *Staphylococcus.spp* and *Streptococcus.* spp shown high sensitivity in all different concentration (20µl,40µl and 60µl). Minimum inhibition zone was observed against *E. coli* (Figure 10).

Table: 1: Antimicrobial activity at different concentration

Organisms	Zone of inhibition for different concentration of the silver nanoparticles extract in millimeter (mm)				
	20µl	40µl	60µl	Positive control	Negative control
Staphylococcus sp	2	5	7	1.5	-
Streptococcus sp	2	3	7	1.5	-
E.coil	2	4	7	1	-

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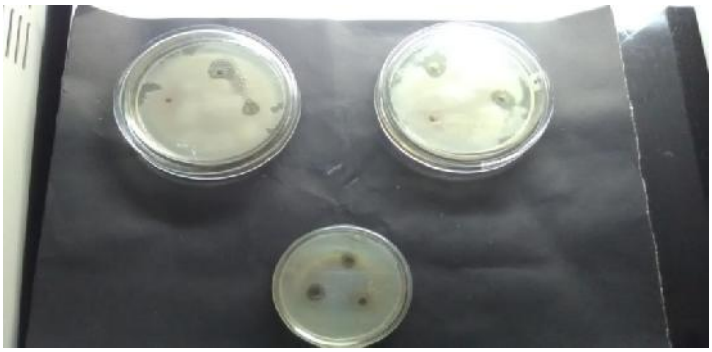

Figure 10: Antimicrobial activity at different concentration.

Discussion

In our present study, colour change indicates the presence of silver nanoparticles which was further confirmed by UV – Vis spectra analysis. Similar researches were carried by Sougata Ghosh et.al. 2017, Gawthami Ramesh, et.al. 2017, Chikdu.D, et .al 2015 and Iqbal MJ, et.al 2018 where synthesized silver nanoparticles shows highest UV Spectrum absorption peak at 540 nm, 450-470 nm, 420nm and 417nm respectively. In our present study the maximum absorption at 435nm.Insame research synthesized Silver nanoparticles of size 194.2nm and 41-55nm respectively. In our present study silver nanoparticles size of range 80-120nm.Similar type research done by Gawthami Ramesh et.al. 2017, where synthesized silver nanoparticles shown high sensitivity against *E. coil* and Uzma Murad, et. at 2018 heremethanol extract silver nanoparticles were shown high significant activity at 50mg/kg, in our present study Silver nanoparticles shows high significant at 60µl against *Streptococcus* spp *and Staphylococcus* spp and *E. coil* shown low sensitivity.

Conclusion

Nanoparticles have wide applications in various fields. The synthesis of nanoparticles by using plant extracts can be advantageous over other biological processes. The green synthesis approaches toward the synthesis of silver nanoparticles have many advantages. It was concluded that plant mediated synthesis of silver nanoparticles possess potential antimicrobial activities. The characterization analysis such as AFM, FTIR and UV-Visible spectra analysis proved that the particle so produced in nano dimensions would be equally effective as that of antibiotics and other drugs in pharmaceutical applications. This work indicates that the seed extract could be used as an efficient and potential green material for the reliable synthesis of silver nanoparticles. The synthesized nanoparticles have exhibited a wide range of activities to the bacteria strains and reveals high efficiency of silver nanoparticle as a strong antibacterial agent. Thus, this nanoparticles has the potential for the development of drugs for various diseases and also useful in biomedical application.

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DOI: 10.22192/ijcrcps.2019.06.02.006

How to cite this article:
Nayana. V, Leela. G. R, Kalpana.C, E. Jenitta Emima Packiyam, Shwetha D Shetty. (2019). Green synthesis of Silver Nano particles from Litchi seeds and its Antimicrobial activity. Int. J. Curr. Res. Chem. Pharm. Sci. 6(2): 30-36.
DOI: http://dx.doi.org/10.22192/ijcrcps.2019.06.02.006

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