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Green Synthesis, Characterization, and Antibacterial Activity of Silver Nanoparticles using *Ocimum sanctum* (Tulsi) Leaf Extract

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Abstract

Green nanotechnology has now developed as a new distinct field of research in modern science and technology. The green nanotechnology, which can be also termed as "bionanotechnology" is based upon the nanoparticles synthesis from the natural sources. Among the various availability of natural sources the treatment of plant extract makes the process of synthesis of nanoparticles more easier, cost effective and eco-friendly. The leaves extract of *Ocimum sanctum* were used as a reducing and stabilizing agent for the synthesis of silver nanoparticles (AgNPs). The biosynthesized nanoparticles were characterized by the help of UV–VIS spectrophotometer, FTIR and SEM analysis and their antimicrobial activity was screened against *Micrcoccus luteus, Escherichia coli* and *Pseudomonas aeruginosa.*

Keywords: Biosynthesis; Plant extract; *Ocimum sanctum*– Silver nanoparticles; Antimicrobial activity.

Introduction

Nanotechnology is said as the field of engineering which deals with the fine tuning of matter at the atomic, molecular and sub molecular level. Nanoparticles are implicit to be equally natural and synthetic designed particles lesser than 100 nm. Green nanotechnology or nanobiotechnology has become apparent as a distinguish part of nanotechnology in which the nanoparticles are produces from the natural sources. Various natural sources including plants, algae and microbes have been widely used for the manufacture of nanoparticles. Nanoparticles manifest completely latest along with enhanced characteristics based on specific features as morphology, size and distribution, if compared with larger particles of the bulk material they are made of. These properties widely differ as such as mechanical, electrical conductivity, catalytic activity, melting point, thermal and, optical absorption. From the earlier period to till date, Indian greeneries have been widely explored for their medicinal properties. Recently, as the rapid development occur in the field of research, many such plants have been acquiring importance due to their unique constituents and their versatile application [1]. *Ocimum sanctum*, holy basil, or *tulasi* (also spelled *thulasi*), its an aromatic plant belongs to the family Lamiaceae which is native to the Indian subcontinent and worldwide as a cultivated plant all through the Southeast

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Asian tropics [2]. In a large scale polygeographical investigation of this species governed by utilizing chloroplast genome sequences, the plant originated from North Central India which have been propounded by a group of researchers from Central University of Punjab, Bathinda [2].

Basil is considered to be sacred, medicinal and has extensive application in the indigenous medicinal system in the Asian countries and also it having the well known medicinal properties such as antibacterial, antifungal, antiseptic, antipyretic, anticancer, and antioxidant [3]. Oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene (about 8%), β-elemene (11%) and germacrene D (about 2%) are some of the main chemical constituents present in tulsi.[4] *Ocimum sanctum* was generally used for the making of silver nanoparticles. Because of their large ratio of surface-to-bulk silver atoms silver nanoparticles are the kind of nanoparticles of silver composed of large amount of silver oxides. silver nanoparticles are even found to be better than the nanoparticles of other metals on various parameters. Both silver nanoparticles and gold nanoparticles are commonly used in the application of optical detection for their surface plasmon resonance effect [5]. The efficiency of Plasmon excitation of silver nanoparticles is known to be more distinct than that of gold nano particles, which has shown in their stronger, sharper plasmon resonance peaks at the same particle concentration. Thus silver nanoparticles can be provide better idea for certain applications like localized plasmon resonance or surface enhanced Raman scattering detection. When being used along with the fluorescence emission detection silver nanoparticles turn more advantageous than gold nanoparticles. The emission range of fluorophores at a wavelength above 500 nm but the absorbance range of Plasmon resonance of gold nanoparticles is primarily of 500-600 nm, and therefore satishfy the detectable fluorescence upto certain range, when the fluorescent dyes are in close proximity to the particle surface.By this concern the fluorescence quenching is minimized for silver nanoparticles, as their plasmon resonance absorbance is mostly underneath 500 nm, which is little convergence with the emission wavelength of mostly fluorescent dyes [6]. Conventionally, various types of physical as well as chemical methods were used for the manufacturing of the nanoparticles. The physical vapour condensation (PVC), arc-discharge method, laser ablation, evaporation-condensation, sputtering, and mechanical milling among which evaporation-condensation and laser ablation are the kind of common physical methods mostly used [7]. Physical methods for the synthesis process never involving toxic chemicals and becomes fast. To prevent against agglomeration these methods necessiatate the usage of stabilizers to secure the Ag nanoparticles so that pure silver nanoparticles can be produced. The chemical method includes variety of methods like pyrolysis (spray and aerosol), chemical etching, sputtering and sol-gel process amongst which chemical reduction using various reducing agents is most widely used [8]. For reduction of silver ions (Ag+) in aqueous or non-aqueous solutions different kinds of organic and inorganic reducing agents, such as sodium borohydride (NaBH4), sodium citrate, ascorbate, elemental hydrogen, Tollen's reagent, N,N-dimethyl formamide (DMF) and poly (ethylene glycol) block copolymers are commonly used [9]. The conventional methods have been used for a very long time and are still in use. But these methods are expensive, laborious and toxic. Hence, the alternative method such as biological method used for the synthesis of nanoparticles from natural source has been put forward that tend to be eco-friendly. The natural sources may be microbes, plants or algae [10]. But the plant extract involvement eliminates elaborate processes of maintaining microbial cultures. From the experimental Studies it have been manifested that *Alfaalfa* roots can bitterly absorb Ag (0) from agar medium and are also able to transport it to the plant shoot in the same state of oxidation. Various plants including M. *balbisiana*, *A. indica*, *Crataegus douglasi, and Acalypha indica* have been used for the manufacturing of silver nanoparticles [11]. Here we have been designed a rapid, convenient and eco-friendly green methodology i.e. for the synthesis of silver nanoparticles from silver nitrate by using leaf extracts of *Ocimum sanctum* (green tulsi). The plant mediated synthesized Ag NPs were properly characterized and studied in details with all of their properties which is most significant to current science and prevailing technologies as per the current research is concern.

The characterization was made to understand the potentiality of nanoparticles which can be done by having a understanding knowledge of their synthesis along with applications. The characterization techniques included UV-VIS spectroscopy, further, anti-microbial testing was performed using the three bacteria namely, *Escherichia coli*, *Micrcoccus luteus* and *Pseudomonas aeroginosa*. In response to the antimicrobial activity of the plant extract the zone of inhibition was obtained which was evaluated by disc diffusion method. From the obtained results from analysis

of the antimicrobial property of the plant extracts make sure that they are safe to be released into the environment and hence suitable to be concerned for pollution remediation.

Materials and Methods

Collection of Plant materials

O. sanctum leaves were taken from the botanical garden of Department of Biotechnology, GIET University Gunupur, odisha, India. The reagents such as Nutrient Agar was supplied by (Cat. No.: M001, Himedia, Mumbai and Silver nitrate (Cat.No.: 209139, Sigma Aldrich, India). Bacterial cultures (*Micrcoccus luteus* MCC 2408; *Pseudomonas aeruginosa*. MCC 2511; *Escherichia coli* MCC 2155) were procured.

Preparation of O. sanctum leaf extract and 1 mM $AgNO_3$

Fresh leaves of O. sanctum (100 g) were transferred to sterile 250 mL conical flask and diced into fine pieces. The leaves were washed with tap water and distilled water. The leaves were incubated in oven for four hours at 800C and then grinded into fine powder. 100 mL leaf powder solution was prepared and heated at 60 0C for one hour to prepare the aqueous extract. Then the extract was filtered using Whatman No. 1 filter paper and the filtrate was stored at 4^0C for further use. Silver nitrate ($AgNO_3$, Sigma Aldrich, USA), 0.0421 gm was added to 100 mL of double distilled water and dissolved thoroughly. To prevent the auto oxidation of silver, the solution was preserved in amber coloured bottle.

Determination and synthesis of silver nanoparticles

The aqueous leaf extract of O. *sanctum* and 1 mM $AgNO_3$ were mixed in the ratio of 1:10 and was kept in water bath shaker at 60^0C for 30 min until change in color was observed. The generation of silver nanoparticles in leaf extract solution is confirmed by changes in color.

UV–visible spectrometric analysis.

Samples (1mL) of the AgNPs solution were collected sporadically to observe the completion of bioreduction of Ag+ in solution, which is directed by dilution of the samples with 2 ml of de ionized water and consequent scan in UV-visible (vis) spectra, of wave length 450 nm in a UV-vis spectrometer (UV-1800 spectrophotometer, Shimadzu, Japan), having a resolution of 1 nm. UV-VIS spectra were recorded at intervals of 0 min, 30 min and 24 h.

Fourier transform infrared (FTIR) analysis of silver nanoparticles.

The uncapping ligands of 200 ml residual solution of AgNPs can be done by centrifuging at 10,000 rpm for 30 min and the precipitate was kept in 10 ml ethanol and distilled water and the process was repeated 3-4 times. The powder of purified AgNPs was prepared by drying in oven and then analyzed by Fourier Transform Infrared (PerkinElmer, MA, USA).

Scanning electron microscopy of silver nanoparticles.

The AgNPs pellet was dehydrated in an oven and thin films of dried samples (10 mg/mL) were arranged on carbon coated copper grid and analyzed for size determination. The SEM analysis was done to determine the particle size and texture of nanoparticles and the presence and formation of silver nanoparticles.

Minimum inhibitor concentration (MIC) and minimum bacteriocidal concentration (MBC) studies

The MIC and MBC studies were done to find out the concentration of biosynthesized silver nanoparticles showing growth inhibition of bacterial strains.

Results and Discussion

AgNP characterization

UV-vis analysis

As a result of surface Plasmon vibration Silver nanoparticles (AgNPs) appear yellowish brown in color in aqueous medium [12]. By the addition of leaf extracts to aqueous solution of silver nitrate, the color of the solution converted from faint light to yellowish brown to reddish brown and finally becames colloidal brown which indicating formation of AgNP. Similar changes in color also have been detected in previous studies [13] and therefore confirmed that completion of reaction occured between leaf extract and $AgNO_3$. After some time intervals of 15 min, 30 min, 45 min, 60 min and 24 h from the initiation of reaction are shown in Figure 1, the UV-VIS spectra recorded. Due to the surface Plasmon resonance of AgNPs, formation of absorption spectra of AgNPs occured in the reaction media with the absorption maxima

in the range of 450 nm. From the UV-VIS spectra, it has been indicated that most rapid bioreduction denoted by broadening of them peak which implied the formation of poly dispersion of large nanoparticles due to slow reduction rates [14]. And also the UV-VIS spectra also exhibited that formation of AgNPs occurred more rapidly within the first 15 mins and the AgNPs remained stabilized in the solution even after 24 h of completion of reaction.

FTIR analysis

From the FTIR analysis it has been characterized that the AgNPs acquired from tulsi plant extract (Curve A) which has shown in Figure 2. Prominent bands of absorbance were observed at around cm^{-1} in all the AgNPs solution. The stretched bands, vibrational bands responsible for the existence of compounds like flavonoids and terpenoids [15] and also may be responsible for stabilization of obtained AgNPs and efficient capping.

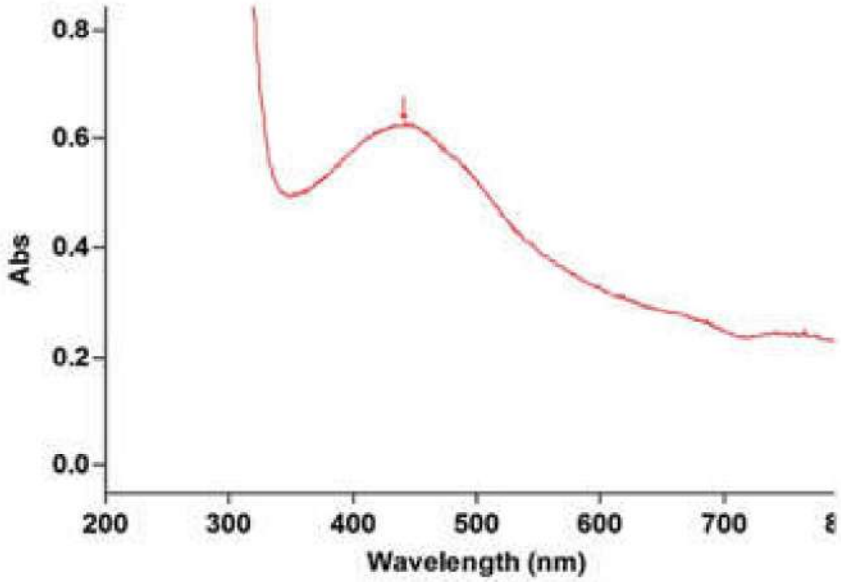

Fig. 1: UV-VIS absorption maxima of silver nano particles. From the data it has been estimated that based upon the presence of absorbance peak of AgNPs solution at the wavelength range of 300-800 nm the absorption maxima were found to be 451 nm.

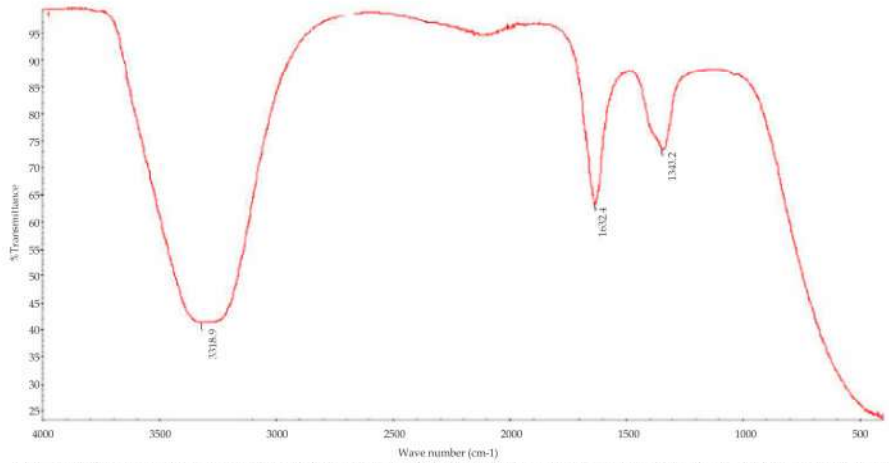

Fig. 2: FTIR graph of synthesised Silver Nano particle. The peaks are obtained at 1343.2,1523.4 and 3343.9.

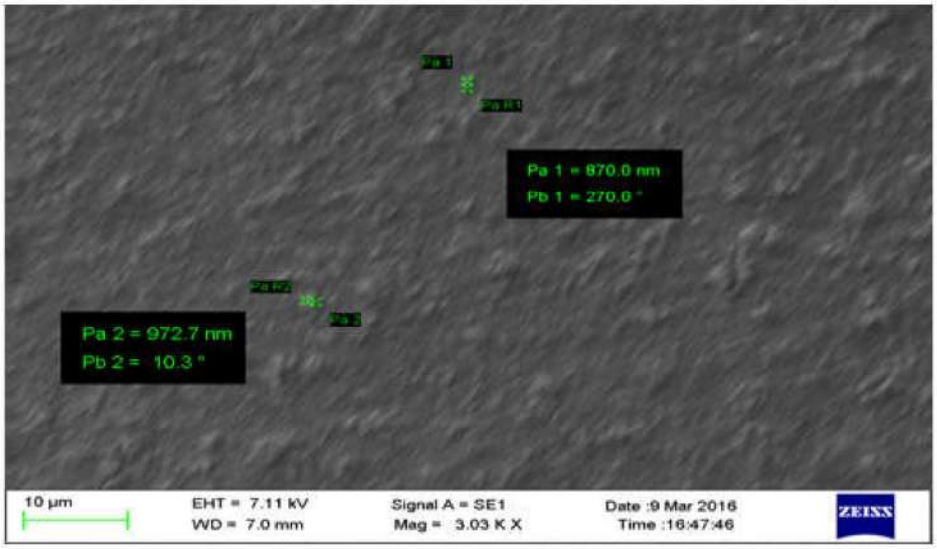

Fig. 3: SEM Analysis

Table 1: Antibacterial Properties

Extracts	Bacteria	Zone of inhibition in (mm)				
		By Leaf extract	By AgN	By AgNPs 1:4	By AgNPs 1:10	By AgNPs 1:20
Tulsi (leaf)	M. luteus	7 ± 0.03	6 ± 0.04	8 ± 0.004	13 ± 0.043	14 ± 0.002
	P. aeruginosa	7 ± 0.04	6 ± 0.002	7 ± 0.03	14 ± 0.008	17 ± 0.6
	E. coli	7 ± 0.15	6 ± 0.025	4 ± 0.006	3 ± 0.01	4 ± 0.022
Mehndi (leaf)	M. luteus	5 ± 0.07	6 ± 0.008	19 ± 0.1	13 ± 0.3	13 ± 0.04
	P. aeruginosa	5 ± 0.004	6 ± 0.05	11 ± 0.04	6 ± 0.009	15 ± 0.013
	E. coli	5 ± 0.023	6 ± 0.016	7 ± 0.021	10 ± 0.019	8 ± 0.009
Bramhi (leaf)	M. luteus	9 ± 0.004	6 ± 0.04	24 ± 0.02	19 ± 0.06	16 ± 0.002
	P. aeruginosa	9 ± 0.06	6 ± 0.015	7 ± 0.005	7 ± 0.003	10 ± 0.011
	E. coli	9 ± 0.05	6 ± 0.027	12 ± 0.4	11 ± 0.05	9 ± 0.035

SEM analysis

The AgNPs, SEM images has been shown in Figure 3. It has been estimated that AgNPs obtained in case of Tulsi leaf extracts appear to be cuboidal in shape and can be utilized as reducing as well as capping agents, availability of different quantity and nature of capping agents sustained in the leaf extracts. The shifts and difference in areas of the peaks obtained through FTIR analysis.

Antibacterial property analysis

By the supplement of AgNPs on nutrient agar culture media, its antimicrobial property investigated against *Micrcoccus luteus*, *Escherichia coli* and *Pseudomonas aeruginosa*. A control culture plate was separately maintained for the microorganisms from water. Results achieved has been shown in Table 1. The obtained zone of inhibition indicates that maximum antibacterial activity occurred in the prepared test sample. Results achieved in previous studies [16] also bear the antibacterial potential of AgNPs. In contrast to $AgNO_3$ and AgNPs,there is no such prominent antimicrobial activity found when crude form of plant extracts used and in control no zone of inhibition was acquired.

Conclusion

The green synthesis and characterization of AgNPs was done and confirmed by UV–VIS spectrophotometer. The nanoparticles appeared to be in shape with. The growth inhibitory value against bacterial species exhibited by MIC and MBC of the AgNPs. In summary, the extract of *O. sanctum* intervene the efficient synthesis of silver nanoparticles and provides additional property such as bacteriocidal efficiency and might act as long searched substitute and could be the response to antibiotic resistance.

References

1. Banerjee, Mantosh Satapathy, Aniruddha Mukhopadhya and Papita Das, Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis, Bioresources and Bioprocessing 2014;1:3.
2. Warrier PK. Indian Medicinal Plants. Orient Longman. 1995.p.168.
3. T. Harrach, F. Garbin, E. Munzig, K. Eckert and H. R. Maurer. P182 Bromelain: An immune modulator with Anticancer Activity. European Journal of Pharmaceutical Sciences. 1994;2(1-2):164-67. doi:10.1016/0928-0987(94)90355-7.
4. Kothari S.K., Bhattacharya A.K., Ramesh S., Garg S.N., Khanuja S.P.S. Volatile Constituents in Oil from different Plant Parts of Methyl Eugenol-Rich *Ocimum tenuiflorum* L.f. (*syn. O. sanctum L.*) Grown in South India. Journal of Essential Oil Research. 2005 Nov-Dec;17(6):656–58.
5. V. Kumar and S. K. Yadav. Plant-Mediated Synthesis of Silver and Gold Nanoparticles and Their Applications. Journal of Chemical Technology and Biotechnology. 2009;84(2):151-57. doi:10.1002/jctb.2023.
6. L.P. Hale, P.K. Greer, C.T. Trinh and C.L. James. Proteinase Activity and Stability of Natural Bromelain Preparations. International Immunopharmacology. 2005;5(4):783-93. doi:10.1016/j.intimp.2004.12.007.
7. Xu H, Käll M. Morphology effects on the optical properties of silver nanoparticles. J Nanosci Nanotechnology. 2002;4:254-59.
8. R. Hussain, S.J. Cillard and P. Cillard. Hydroxyl Radi- cal Scavenging Activity of Flavonoids. Phytochemistry. 1989;26(9):2489-91. doi:10.1016/S00319422(00)83860-1.
9. Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH. Interaction of silver nanoparticles with HIV-1. J Nanobiotechnology. 2005;3:6.
10. Song JY, Kim BS. Rapid biological synthesis of silver nanoparticles using plant leaf extract. Bioprocess Biosyst Eng. 2009;32:79–84.
11. S. Shankar, A. Ahmad and M. Sastry. Geranium Leaf Assisted Biosynthesis of Silver Nanoparticles. Biotechnology Progress. 2003;19(6):1627-31. doi:10.1021/bp034070.
12. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core Ag shell nanoparticles using Mehndi (Azadirachta indica) leaf broth. J Colloid Interface Sci. 2004;275:496–502.
13. Song JY, Kim BS. Rapid biological synthesis of silver nanoparticles using plant leaf extract. Bioprocess Biosyst Eng. 2009;32:79–84.
14. Dimitrijevic NM, Bartels DM, Jonah CD, Takahashi K, Rajh T. Radiolytically induced formation and optical absorption spectra of colloidal silver nanoparticles in supercritical ethane. J Phys Chem B. 2001;105:954–59.
15. Naik RR, Stringer SJ, Agarwal G, Jones S, Stone MO. Biomimetic synthesis and patterning of silver nanoparticles. Nat Mater. 2002;1:169–72.
16. Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. J Nanoparticle Res. 2011;13:2981–88.