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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING SPINACH LEAF EXTRACT AND ASSESSMENT OF ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

This study reports the synthesis of silver nanoparticles by using Spinach leaf without the use of toxic chemicals. The formation of silver nanoparticles was confirmed visually by observing the colour changes from pale yellow to dark brown colour. The nanoparticles were characterized by using UV-visble spectrophotometer and SEM (Scanning Electron Microscope). The nanoparticles have diameter in the range of 30-90nm. Synthesized particles were spherical, triangular and cuboidal in shape. The antibacterial and antifungal activities of the nanoparticles were assessed and these nanoparticles were found to be toxic against the pathogenic microbial strains. This suggests that the plant resources can efficiently be used in the prouction of silver nanoparticles which could be utilized in various fields such as biomedical, nanotechnology etc.

INTRODUCTION

The word "nano" has been used to indicate one billionth of meter or "10-9". The term nanotechnology was coined to describe precision manufacturing of materials at the nanometer level [1]. Particles with a size up to 100 nm were usually referred as nanoparticles and they exhibited completely new properties based on their size, distribution and morphology [2]. [3] stated that the synthesis of nanoparticles using biological method was preferred over the physical and chemical methods and the term "Green nano synthesis" has been proposed for the biological synthesis of nanoparticles. The production through biological methods is considered to be safe and eco- friendly [4] because the use of high pressure, energy , temperature and toxic chemicals of conventional physical and chemical methods are completely avoided. Plants are free from toxic chemicals and provide natural capping agents so they can provide a better platform

for nanoparticle synthesis[5] extracts of different plant parts (seed, leaf as well as tuber) are used for the synthesis of metal nanoparticles [6]. [7] mentioned that silver has been used as an antimicrobial agent for centuries therefore silver nanoparticles have attracted intensive research interest because of their important applications as antimicrobial, catalytic, and antifungal activity [8-10].

[11-14] have studied antifungal and antibacterial properties of nanoparticles. It was suggested that nanoparticles have some properties viz. their high surface area to volume ratio and the unique chemical and physical properties, which increases their contact with microbes and nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. These naoparticles showed high toxicity against different multi drug resistant human pathogens. Therefore the nanaotechnology has amplified effectiveness of silver particles as antimicrobial agents in medical industries.

The present report deals with a low-cost, convenient, green synthesis approach to obtain large quantities of silver nanoparticles by reduction of silver ions using Spinach leaf extract. The Spinach plants were irrigated with treated, untreated waste water and with ground water of Bhiwadi industrial area , and leaves of all the three types of plants were used for nanoparticles synthesis.

Materials and methods :

P**repration of leaf extract by homogenization method** : 5 gm fresh leaves, each from plants grown in treated , untreated waters and ground water in Bhiwadi (Rajasthan , India) industrial area, were taken . Each sample was washed several times with deionized water, cut into fine pieces and were boiled in 50 ml distilled water than filtered through Whatman No.1 filter paper (pore size 25 µm). The filterate was collected and stored at 4˚C for further use.

Synthesis of silver nanoparticles : Prepared 1mM solution of silver nitrate (AgNo3) and used for the synthesis of silver nanoparticles.10 ml of leaf extract was added into 90 ml of aqueous solution of 1mM silver nitrate with constant stirring. The conical flask was exposed to the sunlight. The colour of solution changed from yellow to brown colour.

Characterization of silver nanoparticles :

UV-Vis spectral analysis : The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reduction media after diluting a small aliquot of the sample in distilled water. UV-Vis spectroscopy was carried out on UV-2450 (Shimadzu).

SEM analysis : Scanning Electron Microscopic (SEM) analysis was done by using EVO 18 Scanning Electron Microscope. Thin films of the samples were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid , extra solution was removed using a blotting paper and a thin film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Anti bacterial assay : The antibacterial assay were done on *Escherichia coli* (MTCC 1687), *Pseudomonas aeuroginosa* (MTCC 424*),* *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96) by well diffusion method. Nutrient agar media was used to cultivate the bacteria . Prepared the innoculum (20 µl) and spreaded it on media . Cut the wells in media and loaded 40 µl of the samples and standard solution in wells. Streptomycin was used as a standard solution**.**

Antifungal assay : The antifungal assay were done on *Fusarium oxysporium (*NCIM 2480) and *Penicillium funniculosum* (NCIM 1170) by well diffusion method. Potato dextrose agar (PDA) media was used to cultivate fungus. Prepare the innoculum (10 µl) and spreaded it on PDA plates. Cut the wells in media and loaded 40 µl of the samples and standard solution in wells. Ketonazole was used as a standard solution.

RESULTS AND DISCUSSION

Table 1 : shows Inhibition zone (IZ) and Activity index (AI) of silver nanoparticles against different bacteria and fungi.

Plant sample	Bacteria								Fungus			
	E.coli		*Staphylococcus aureus*		*Bacillus subtilis*		*Pseudomonas aeruginosa*		*Penisillium funniculosum*		*Fusarium oxysporium*	
	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
Ground water	18	0.6	22	0.63	4	0.6	4	1.5	Nil	0	11	0.343
Treated	22	0.73	21	1.5	6	0.75	8	0.75	21	0.75	12	0.375
untreated	21	0.7	20	1.42	3	0.37	6	1	2	0.071	14	0.437
Streptomycin	30	-	14	-	8	-	6	-				
Ketoconazole (Standard)									28	-	32	-

As the plant extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from pale yellow to dark brown due to reduction of silver ion which indicated formation of silver nanoparticles. Earlier [15] observed that as the plant extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from yellowish brown colour in solution due to excitation of surface

Plasmon vibrations in silver nanoparticles. [16-17] reported rapid synthesis of stable silver nanoparticles by bioreduction [18]. According to [19] the probability of reduction of AgNO3 to silver might be illustrated due to the absorption of light by the photosynthesizing organism that produced carbohydrates which were utilized by the cell as glucose by glycolysis.

UV–visible spectroscopy is an important technique to determine the formation and stability of metal nanoparticle in aqueous solution [20]. In the present report UV-Vis spectra recorded from the reaction medium after 4 hrs the strong surface Plasmon resonance band appear were at the range of 440-480 nm. Absorbance peak was observed at 459 nm ,457nm, and 448 nm of nanoparticles synthesized by leaves of plants irrigated with ground water, treated water and untreated water respectively.

The SEM image showed that the formed nanoparticles were spherical, spherical and cuboidal , triangular and cuboidal in shape and the size ranged from 30-60nm, 40-80nm, 60-90 nm of nanoparticles formed by the leaf of plant irrigated with ground water ,treated water and untreated water respectively. Similar results were obtained by [7,14,20-22].

The antibacterial activity of silver nanoparticles was tested against four strains of bacteria viz., *Escherichia coli, Pseudomonas aeuroginosa, Bacillus subtilis and Staphylococcus aureus*. The zone of inhibition (ZOI) and activity index were measured with 40 µl concentration of all the three water samples ground water, untreated and treated. Streptomycin was used as control in same concentration. The highest zone of inhibition (22 mm) was found against *E.coli* and *Staphylococcus aureus* , showed by silver nanoparticles synthesized from plants leaves irrigated with treated water and ground water respectively. The lesser zone of inhibition (3mm) was found against *Bacillus subtilis*, showed by silver nanoparticles synthesized from plants leaves irrigated with untreated water . [Table 1]

In case of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, nanoparitcles of ground water sample and treated water sample showed inhibition zone higher than that the standard (strepetomycin) antibiotic. Earlier [23] it was suggested that the antimicrobial activity of silver nanoparticles was related to the formation of free radicals and subsequent free radical induced memebrane damage. These free radicals might be derived from the surface of silver

nano particles and were responsible for the antimicrobial activity [24].

The antifungal activity of silver nanoparticles was tested against two strains i. e. *Fusarium oxysporiu* and *Penisillium funniculosum* The zone of inhibition (ZOI) and activity index were measured with 40 µl concentration of all three samples (ground water, untreated and treated . Ketonazole was used as control in same concentration. The highest zone of inhibition (21mm) was found against *Penisillium funniculosum*, showed by silver nanoparticles synthesized from plants leaves irrigated with treated water whereas lesser zone of inhibition (Nil) was found against *Penisillium funniculosum* showed by silver nanaoparticles synthesized from plants leaves irrigated with ground water .[Table 1]

[25] observations were mentioned by [18] according to which the bactericidal effect of silver and AgNPps could be attributed to the attachment of AgNPs to the surface of the cell membrane disturbing permeability and respiration functions of the cell smaller AgNps having the large surface area available for interaction would give more bactericidal effect than the larger AgNps . [26] were of the view that it is also possible that Ag Nps not only interact with the surface of membrane, but can also penetrate inside the bacteria.

[27] observed that Ag-NPs exhibited potent antifungal effects on fungi tested, probably through destruction of membrane integrity. According to them SEM analysis also confirms the interaction between Ag-NPs and the membrane structure of *Candida albicans* and *Saccharomyces cerevisiae* cells. They found that during Ag-NPs exposure, the significant changes to their membranes, were recognized by the formation of ‘‘pits’’ on their surfaces, and finally, resulting in the formation of pores and cell death. The authors concluded that Ag-NPs had considerable antifungal activity, however, it is to be investigated further for clinical applications.

The present study we deals with a simple eco- friendly method for synthesis of silver nanoparticles from plant source. This method could be further used for industrial production of nanoparticles at room temperature and with a single step. These Nanoparticles showed antimicrobial activity against bacteria and fungus hence have a great potential in the field of medicine and the toxicity of silver nanoparticles on human pathogen might be utilized for manufacturing a new range of antibacterial agents.

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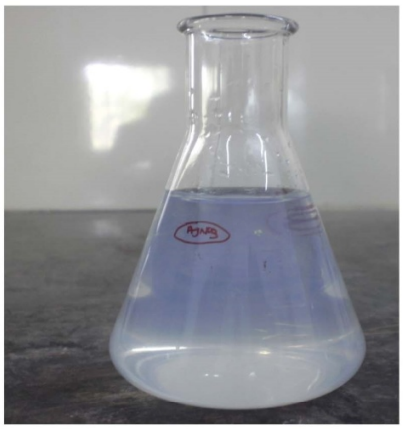
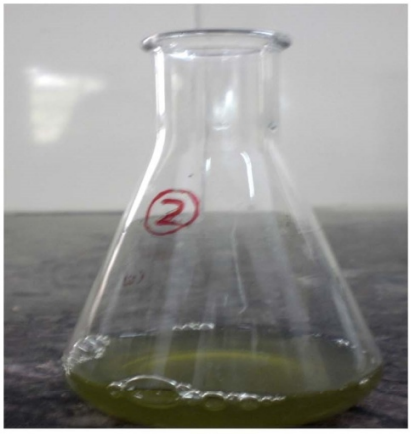


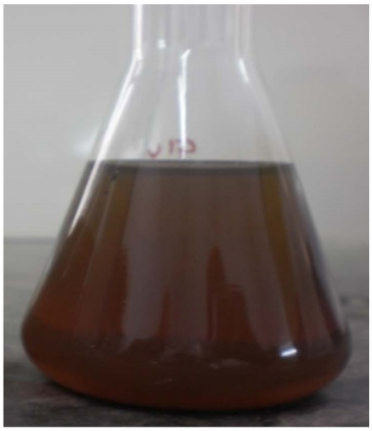
A



B

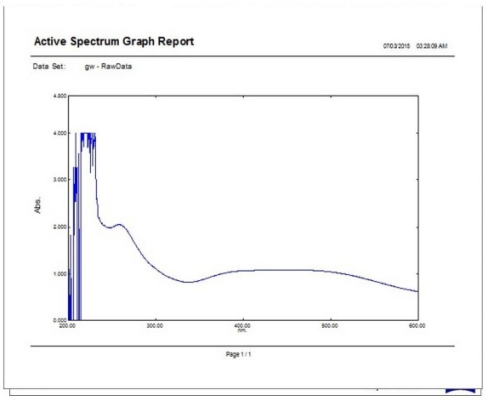
C

D

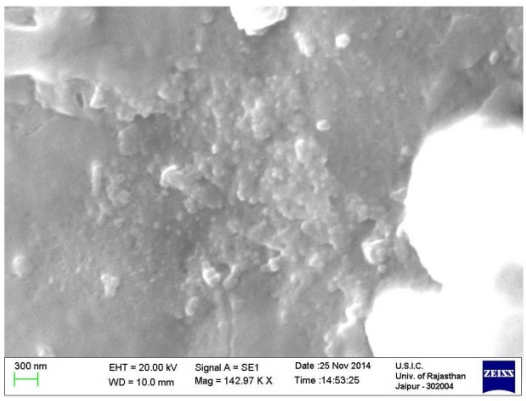


E

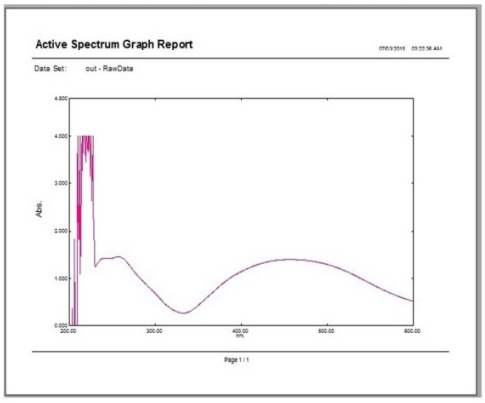
A : silver nitrate solution
B : plant leaf extract
C: mixture of both (A,B) solutions
D ,E : mixture after 4 hrs and 24 hrs respectively



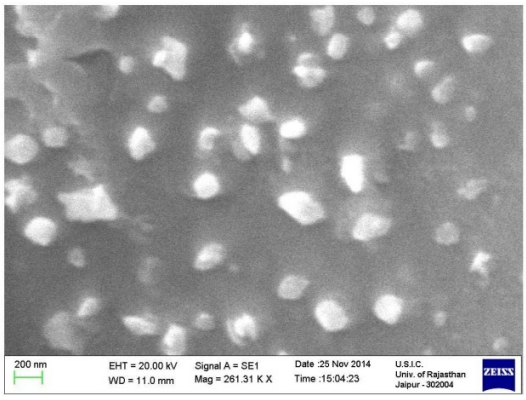
A



D



B



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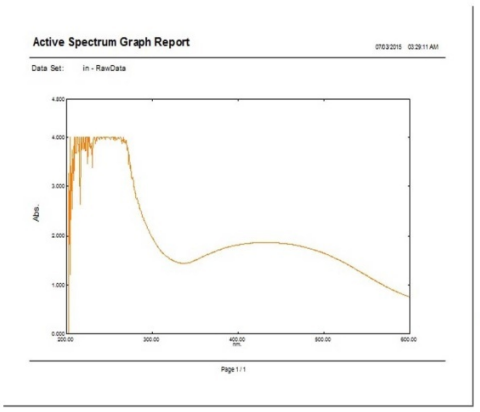
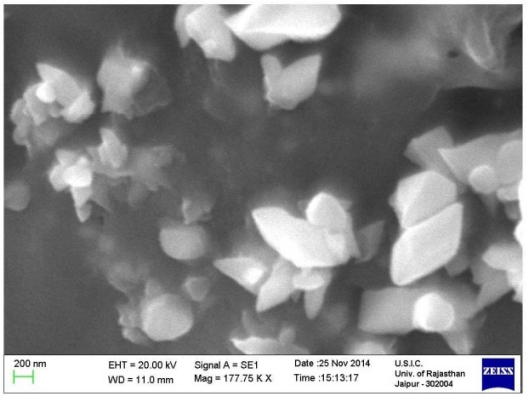


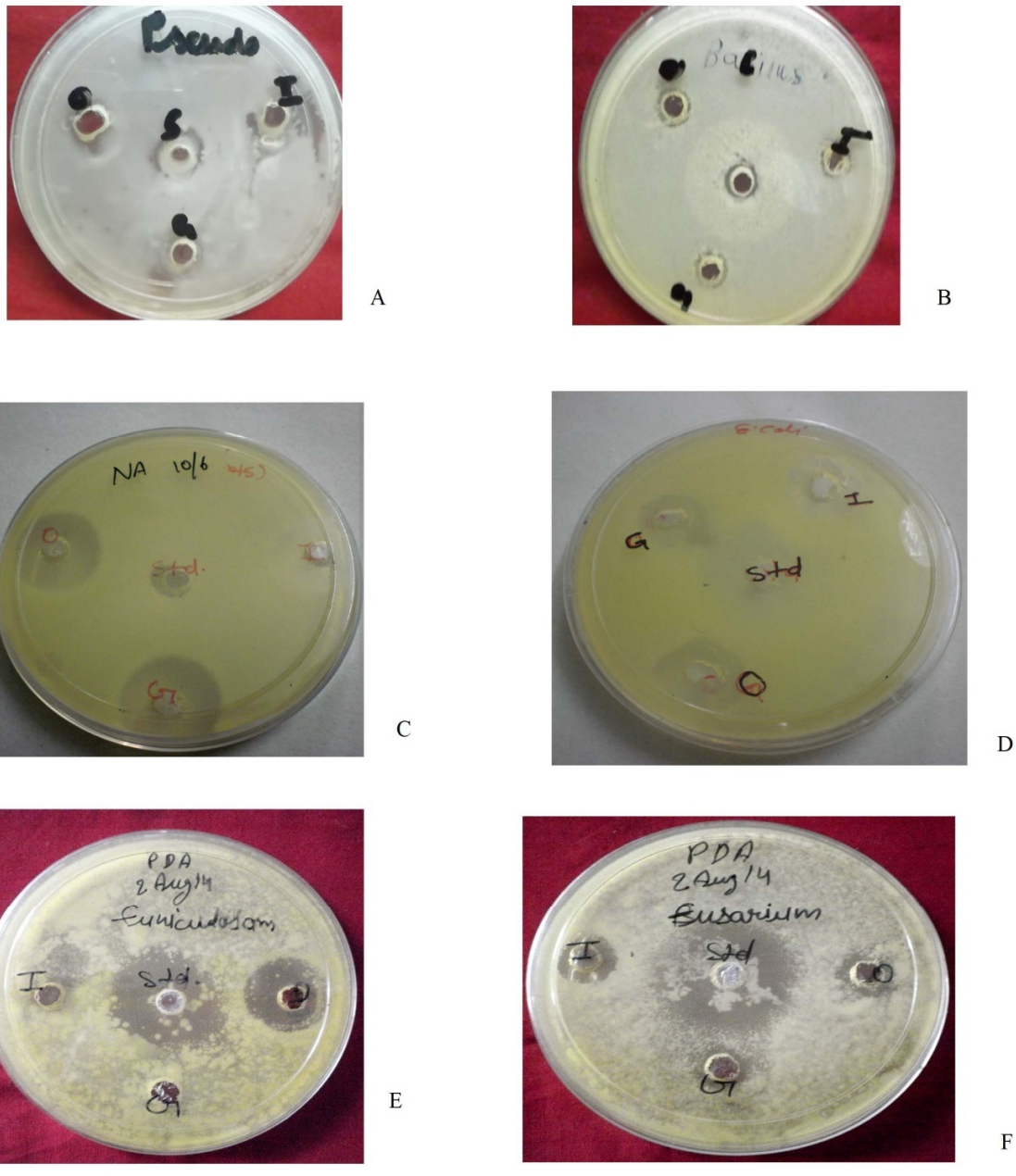
C



F

Figure : showing absorbance peak (A,B,C) and SEm images at 200nm of nanoparticles of plants irrigated with ground water, treated and unreated water respectivelv

SEM image (D E F)



A B C D E F

Figure : showing antibacterial (A,B,C,D) and antifungal activity (E,F) of nanoparticles against different strains of bacteria and fungus.