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Synthesis And Characterisation Of Silver Nanoparticles Using *Brassica oleracea capitata* (Cabbage) And *Phaseolus vulgaris* (French Beans): A Study On Their Antimicrobial Activity And Dye Degrading Ability

Sushmita Deb

Department Of Biotechnology, Sathyabama University, Chennai, India

***Corres.author: :sush28june@gmail.com, Mob: 9840603066**

Abstract: The present study is aimed at rapid synthesis of silver nanoparticles using plant extracts of *Brassica oleracea capitata* (Cabbage) And *Phaseolus vulgaris* (French Beans) and to evaluate their antimicrobial activity. Their ability to decolorize the dye Congo red and Mordant Black 17 was also challenged. For detail and further characterization of nanoparticles, UV-vis spectroscopy, Fourier transform infrared spectroscopy measurements have been analyzed. The microscopic analysis of these particles shows that they are 20-50 nm in range and assembled in cubic morphology. In the present study, the antimicrobial activity of the nanoparticles was evaluated against the pathogenic strains of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi and Klebsiella pneumonia. MIC value of silver nanoparticles against pathogenic strains was observed as 1.08µg/ml. A significant decolourisation rate of 50-60% was observed for the dye Congo red, while a lower rate was observed for Mordant Black 17, due to its structural complexity.This green chemistry approach toward the synthesis of silver nanoparticles is cost efficient, alternative to conventional physical and chemical methods of synthesis and would be suitable for developing a biological process for large-scale production.
Keywords: Silver nanoparticles; *Brassica oleracea capitata; Phaseolus vulgaris;* SEM; TEM; Antimicrobial activity; Dye degradation.

Introduction And Experimental

The field of Nanotechnology is a rapidly growing science of producing and utilizing nano-sized particles.

The noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials[1]. The silver nanoparticles have found used in many applications in different fields-silver an effective antimicrobial agent[2]; and have diverse in vitro and in vivo applications[3]. Although there are many routes available for the synthesis of silver nanoparticles, such as thermal decomposition, electrochemical, microwave assisted process etc.; Biological methods of nanoparticles synthesis using microorganisms, enzyme, and plant or plant extract offer numerous benefits over chemical and physical methods due to their cost effectiveness, environmental friendly nature, and can be easily scaled up for large scale synthesis.

In this experiment, the rapid synthesis of stable silver nanoparticles has been demonstrated using the extracts *Brassica oleracea capitata* (Cabbage) and *Phaseolus vulgaris* (French Beans). Cabbage (*Brassica oleracea* or *B. oleracea var. capitata* [4], is a member of the genus Brassica and the mustard family, *Brassicaceae*. It is a leafy green or purple biennial plant, grown as an annual vegetable crop for its dense-leaved heads. Cabbage is used in

a variety of dishes for its naturally spicy flavor. Cabbage is a good source of vitamin K, vitamin C and fiber[5].Cabbage can also be included in dieting programs, as it is a low calorie food. French Beans are unripe or immature pods obtained from the bean plant belonging to common fabaceae family and known scientifically as Phaseolus vulgaris. They are very low in calories (31 kcal per 100 g of raw beans) and contain no saturated fat; but are very good source of vitamins, minerals, and plant derived micronutrients.Green beans contain excellent levels of vitamin A, and many health promoting flavonoid poly phenolic antioxidants such as lutein, zeaxanthin and ß-carotene in good amounts.

Materials And Methods

To achieve a "green" synthesis of the nanomaterials, reaction medium chosen was distilled water. The reducing agents used were plant extracts. The reagent used for the synthesis (AgNO3) was analytical grade and does not involve any toxic hazard on the environment. The plants used were *Brassica oleracea capitata* (Cabbage) and *Phaseolus vulgaris* (French Beans).

Preparation Of The Extracts And Synthesis Of Silver Nanoparticles

Aqueous extract of *Brassica oleracea capitata* and *Phaseolus vulgaris* were prepared using 20 gm each. They were thoroughly washed in distilled water, dried, cut into fine pieces and were boiled in 150 ml of sterile distilled water for 5-10 mins at an interval of 30secs.The extract was filtered through Whatman No.1 filter paper (pore size 25 µm) and used for further experiments. 1mM aqueous solution of Silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 2.5 ml of the extract was added into 60 ml of aqueous solution of 1 mM Silver nitrate for reduction into Ag+ ions. The beaker was wrapped with silver foil and kept in dark for 24 hours until the colour darkens, after which the pH reading was taken.

Characterization Of Silver Nanoparticles

Synthesized silver nanoparticles were observed by UV–vis spectroscopy in 400 to 600 nm. The absorption spectra of the synthesized nanoparticles were measured using a Perkin-Elmer Lamda-45 spectrophotometer in 300–1000nm range. Detailed analysis of the morphology, size and distribution of the nanoparticles was documented by various instrumental analyses like Scanning Electron Microscopy using Hitachi S-4500 SEM machine and Transmission Electron Microscopy using a TEM, JEM- 1200EX, JEOL Ltd., Japan. The possible photochemical involved in the synthesis and stabilization of nanoparticles was identified by performing FTIR analysis.

Antimicrobial Studies

The silver nanoparticles synthesized using *Brassica oleracea capitata* and *Phaseolus vulgaris* extract was tested for antimicrobial activity by agar well diffusion method[6] against different pathogenic microorganisms *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi* and *Staphylococcus aureus* and by determining the Minimum inhibitory concentration (MIC). Briefly Luria Bertani (LB) broth/agar medium was used to cultivate the bacteria. These were cultured and maintained at 4^0C on nutrient agar slants. Pure cultures were sub cultured in nutrient agar broth for 24 h at 32^0C. Each strain was swabbed uniformly into the individual plates using sterile cotton swabs. 6 wells of equal diameter were made on the agar plates using gel puncture. Using sterile micropipette 10, 20,30,40,50 and 100 µl of the sample of nanoparticles solution were loaded onto each of the wells at the centre in all the plates. After incubation at 32^0C for 24 h, the different levels of zone of inhibition were measured.

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. In the present study, minimum inhibitory concentration was determined by standard broth dilution method. Bacterial strains were grown overnight on MHA plates at 37^0C before being used. The MIC was determined in Luria Bertani (LB) broth Hi-Media (Mumbai, India) using Ag-NPs in varying concentrations. Six test tubes, each containing 5ml of the nutrient broth were prepared. Microbial inoculums were prepared by sub culturing microorganisms into broth. Using sterile pipettes, Ag-NPs in concentration of 10,20,30,40 and 50 µl were added, while one was maintained as blank. The tubes were then incubated at 32^0C for 24 h. After incubation, the MIC was determined as the lowest concentration that inhibited the visible growth of the test microorganism.

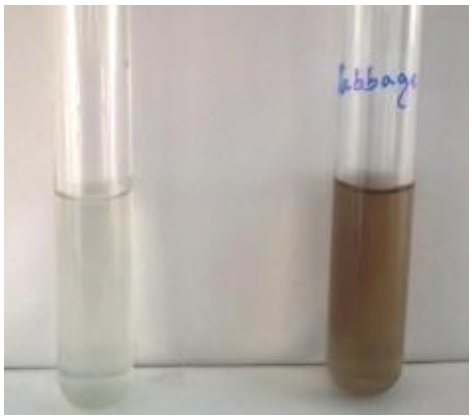
Dye Degradation Using Silver Nanoparticles

In this experiment, an attempt was made to decolourize the dye Congo red and Mordant black 17 using silver nanoparticles, synthesized from plant extracts of *Brassica oleracea capitata* and *Phaseolus vulgaris*. For decolourization study, 1ppm concentration of Congo red and Mordant black 17 were prepared. Five test tubes, each containing 5ml of Congo red (concentration of 1ppm) were prepared. Using sterile micropipettes, Ag-NPs of varying concentration 100, 50, 25 and 10 µl were added, while one was maintained as blank. The test tubes were incubated at 32^0C for 24hr. After incubation, samples were withdrawn and analyzed spectrophotometrically using UV-Visible spectrophotometer at 400-560nm.A similar procedure was applied for the decolourization of Mordant black 17.

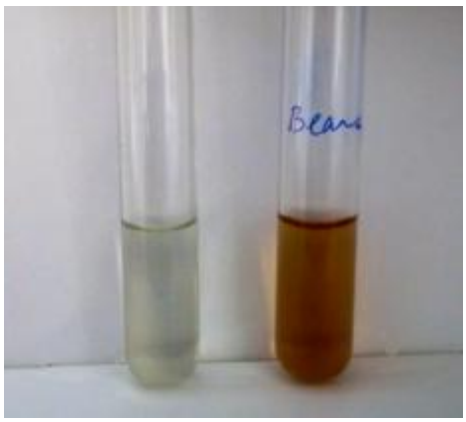
Results and discussion

It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. As the plant extracts of *Brassica oleracea capitata* and *Phaseolus vulgaris* were mixed in the aqueous solution of the silver ion complex, it started to change the colour from pale watery to yellowish brown due to reduction of silver ion which indicated formation of silver nanoparticle. This is due to the surface Plasmon resonance phenomenon. Surface Plasmon resonance is a physical process that can occur when plane polarized light hits a metal film under total reflection conditions. This has been further supported by Kasi Gopinath and et.al[7] in the Phytosynthesis of silver nanoparticles using Pterocarpus santalinus leaf extract and their antibacterial properties.

Fig 1: Colour change in the samples after addition of AgNO3



Brassica oleracea capitata

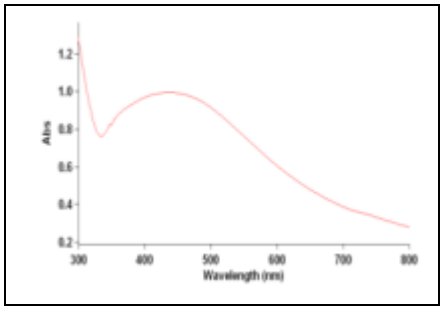


Phaseolus vulgaris

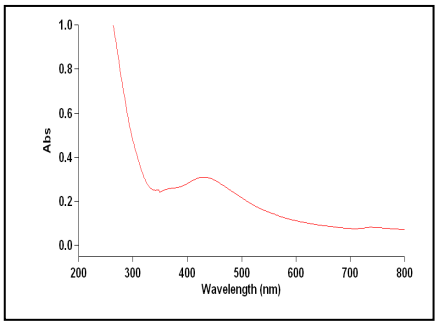
UV-VIS Spectra Analysis

UV-Vis absorption peak up for different size of nano particles is due to a physical process of Surface Plasmon resonance. This is one of the most widely used techniques for structural characterization of silver nanoparticles [8] .It is generally recognized that UV–Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions. The figure below shows the UV-Vis spectra recorded from the reaction medium.

Fig 2: The UV-Vis spectra of the samples.



Brassica oleracea capitata 438nm



Phaseolus vulgaris 430nm

The absorbance peak were observed at 438 and 430nm. Broadening of peak indicated that the particles are polydispersed.

Change In pH

The pH is one of the most important factors for nanoparticle formation. The shape and size of the nanoparticles are dependent on the pH of the solution. pH was The pH reading of the plant extracts was carried out at 30 min interval at different time interval has been obtained. The initial pH observed for the *Brassica oleracea capitata and Phaseolus vulgaris* was observed to be 5.75 and 5.6 respectively. It has been noticed that the bioreduction of silver was carried out in acidic pH as there was not much variation during the synthesis process. This has been illustrated in the table below.

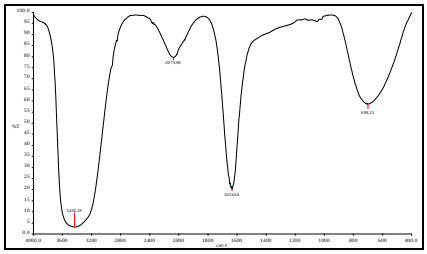
Table1: pH Reading Of The Plant Extracts At An Interval Of 30mins

Brassica oleracea capitata	*Phaseolus vulgaris*
5.75	5.62
5.79	5.94
5.8	5.97
5.71	5.96
5.78	5.96
5.82	5.96
5.39	5.98
5.54	6.17
5.57	6.21
5.59	5.7
5.6	5.65
5.61	5.63
5.59	5.63
5.62	5.62
5.64	5.62

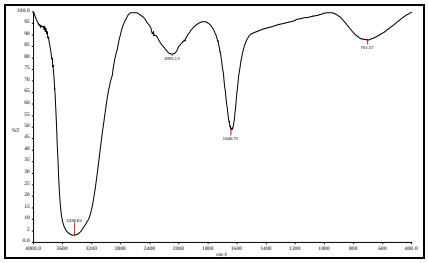
FTIR Analysis

FTIR analysis was used for the characterization of the extract and the resulting Nanoparticles. FTIR spectrum of Ag nanoparticles synthesized from the plant extracts have been shown in the figure below.

Fig 3: FTIR spectrum of Ag nanoparticles synthesized from the Phaseolus vulgaris extracts, before and after reduction of Ag ions.



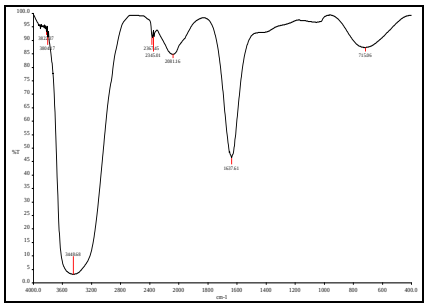
Phaseolus vulgaris (control)



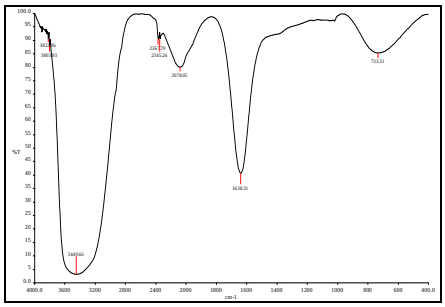
Phaseolus vulgaris (sample)

FTIR absorption spectra of sample *Phaseolus vulgaris* before and after reduction of Ag ions are shown. The band at 3436.19 cm^{-1} and 3436.82 cm^{-1} and corresponds to O-H stretching H-bonded alcohols and phenols displaying the characteristic absorption of hydroxyl groups. The peak at 2073.98 cm^{-1} and 2092.13 cm^{-1} corresponds to aromatic C-O stretching bonds of polyol group. The band at 1634.04 cm^{-1} and 1640.75 cm^{-1} is assigned to CH out of plane bending vibrations substituted ethylene systems –CH=CH (cis).The peak at 698.23 cm^{-1} and 701.5 cm^{-1} corresponds to C-H bend of alkynes. These characters are the typical absorption of amide I compounds, rising due to carbonyl stretching in proteins and aliphatic amine groups.

Fig 4: FTIR spectrum of Ag nanoparticles synthesized from the *Brassica oleracea capitata* extracts, before and after reduction of Ag ions.



Brassica oleracea capitata (control)



Brassica oleracea capitata (sample)

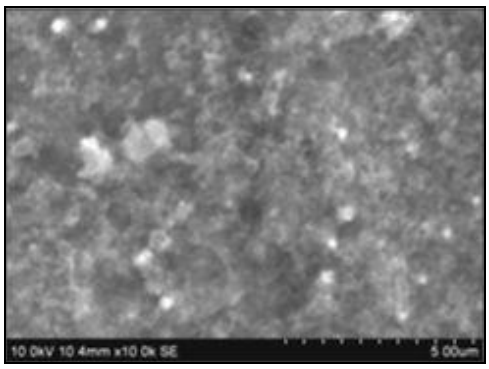
FTIR absorption spectra of sample *Brassica oleracea capitata* before and after reduction of Ag ions are shown. The representative spectra of nanoparticles obtained, manifests absorption peaks located at about 3822.37cm^{-1}, 3804.17cm^{-1} and 3822.06cm^{-1}, 3803.93cm^{-1} corresponds to –NH stretching bonds of amines. The band at 3448.68cm^{-1} and 3449.66cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peaks at 2367.45 and 2345.01 correspond to C-N stretching bonds of nitriles. The peak at 2081.16cm^{-1} and 2078.05cm^{-1} corresponds to aromatic –CH stretching bonds. The band at 1637.61cm^{-1} corresponds to N-H bend primary amines corresponding to amide I arising due to carbonyl stretching in proteins. The peak at 715.06cm^{-1} and 733.31cm^{-1} corresponds to C-H bend of alkynes. The corresponding values have confirmed the fact that the carbonyl group form amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium.

Electron Microscopic Study

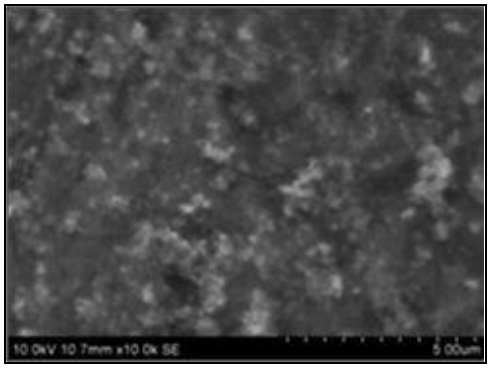
SEM and TEM characterization

Scanning electron microscopy (SEM) image shows the morphological character of the synthesized silver nanoparticles. The studies of silver powder were carried out by means of a scanning electron Microscope from the Madras Veterinary College, Veppery, Chennai. The SEM analysis showed the particle size between 25-50nm as well the cubic structure of the nanoparticles.

Fig 5: Image of Scanning Electron Microscopy



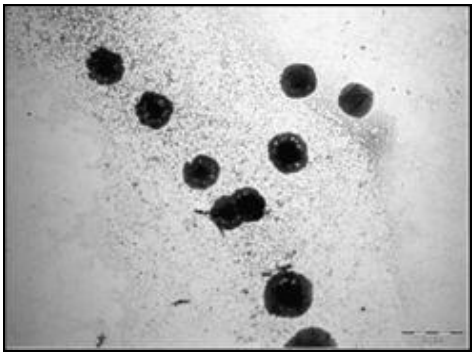
Brassica oleracea capitata



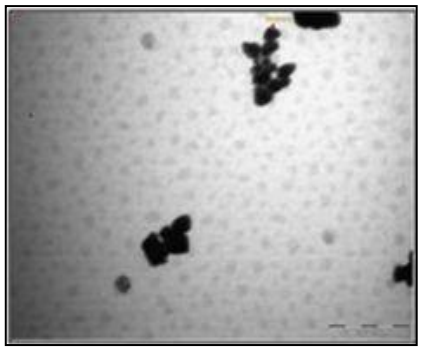
Phaseolus vulgaris

The TEM image of the synthesized silver nano particles shows they are spherical shape with a smooth surface morphology. The diameter of the nano particles is found to be approximately 16 nm and analysed that the produced nano particles are more or less uniform in size and shape.

Fig 6: Image of Transmission Electron Microscopy



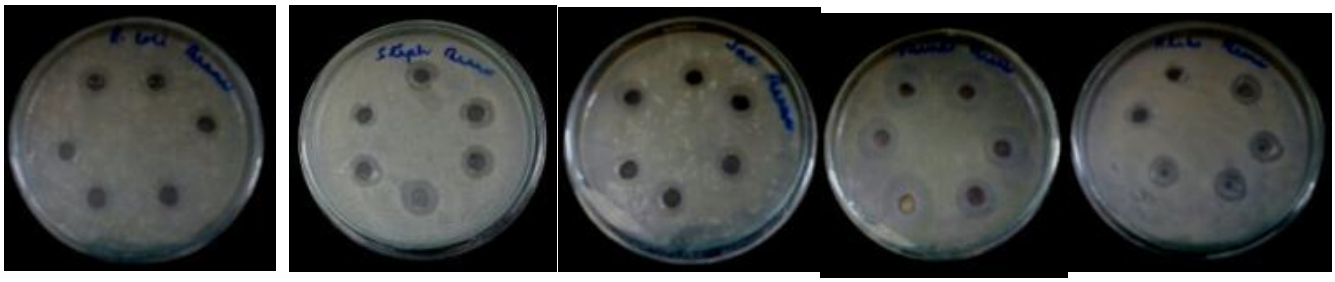
Brassica oleracea capitata



Phaseolus vulgaris

Anti-Microbial Activity Of Silver Nanoparticles (Ag-Nps) Against Pathogenic Bacteria

The antibacterial activity of silver nanoparticles was investigated against various pathogenic bacteria of Gram positive (*S. aureus,*) and Gram negative strains (*E. coli, S. typhi, Pseudomonas aeruginosa, Klebsiella pneumonia,*) using well diffusion technique.



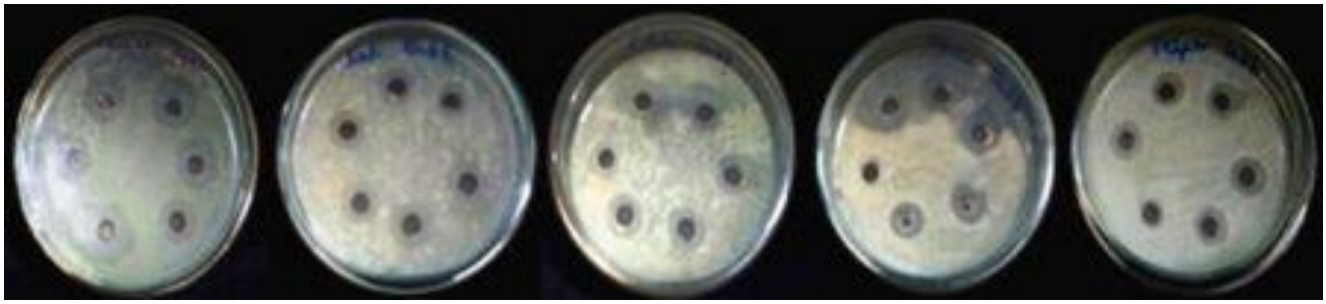
A ***B*** ***C*** ***D*** ***E***

Fig 7:Anti-microbial activity (mm) of silver nanoparticles synthesized from Phaseolus vulgaris against pathogenic strains of:
[A] E. coli [B] S. aureus [C] S. typhi [D]Pseudomonas aeruginosa [E]Klebsiella pneumonia

The diameter of inhibition zones around each well with AgNPs is represented in table.

Table 2: Zone of inhibition (mm) of silver nanoparticles synthesized from *Phaseolus vulgaris* against pathogenic bacterial strains

	5 µl	10 µl	20 µl	30 µl	40 µl	50 µl	100 µl
E. coli	-	-	10	11	14	12	12
P.aeruginosa	-	15	18	19	20	19	20
K.pneumoniae	-	-	12	12	14	14	13
S. aureus	-	-	14	15	13	12	13
S. typhi	-	12	12	14	15	13	14



A ***B*** ***C*** ***D*** ***E***

Fig 8: Anti-microbial activity (mm) of silver nanoparticles synthesized from *Brassica oleracea capitata* against pathogenic strains of*:*

[A] Pseudomonas aeruginosa [B] S. typhi [C] E.coli [D]Klebsiella pneumonia

[E] S. aureus

Table 3: Zone of inhibition (mm) of silver nanoparticles synthesized from *Brassica oleracea capitata* against pathogenic bacterial strains

	5 µl	10 µl	20 µl	30 µl	40 µl	50 µl	100 µl
E. coli	-	15	14	15	18	21	23
P.aeruginosa	-	15	18	21	20	18	22
K.pneumoniae	-	-	13	15	17	17	20
S. aureus	-	-	15	14	18	14	13
S. typhi	-	12	14	15	16	15	15

The diameter of inhibition zones around each well with AgNPs is represented in table. The minimal bactericidal concentration values of Ag-NPs against the pathogenic strains were observed in the range of 30 µl/5ml or 1.08 µg/1ml. However, against the pathogenic strain of *Pseudomonas aeruginosa*, synthesized AgNPs have showed an effective inhibition zone in the concentration of 10 µl/5ml or 0.36µg/1ml.These results suggest that Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems.

Minimum inhibitory concentration (MIC)

The **minimum inhibitory concentration** (MIC) was read after 24 h of incubation at 37°C. The MIC was determined as the lowest concentration that inhibited the visible growth of the used bacterium. The absorbance values for both blank and the test tubes were measured at 600nm.The MIC values of Ag-NPs against the bacterial strains were observed in the range of 20-30 µl, indicating very well bacteriostatic activity of the antibacterial agents. Against bacterial strains of Pseudomonas aeruginosa, S. Aureus and S. typhi, the MIC value was found to be 20 µl/5ml (v/v),or 0.72 µg/1ml (w/v) which was followed by the strains of E.coli and Klebsiella pneumonia in the range of 30µl/5ml or 1.08 µg/1ml(w/v).

Decolourization of the Dye by Silver Nanoparticles

After 24hr interval samples were withdrawn and analyzed spectrophotometrically using UV-Visible spectrophotometer at 400-560nm.The absorbance readings of the dyes have been illustrated in the following table. A significant decolourization rate was observed for the dye Congo red while Mordant black 17 comparatively, showed negative results. The % reduction of the dyes is calculated using the formula,

$$\%red = \frac{O.D.\ of\ control - O.D.of\ test}{O.D.of\ control} \times 100$$

Table 4:% Reduction of the dyes *by* synthesized *Ag-Nps*

Ag-Nps	Conc.of AgNPs (µl)	% Reduction of Dye	
		CONGO RED	MORDANT BLACK
Ag-Nps synthesized from Cabbage	25	63.83	11.19
	10	57.73	1.57
Ag-Nps synthesized from Beans	25	51.68	1.13
	10	50.31	5.18

A significant decolourization rate of 50-60% of Congo Red dye by synthesized silver nanoparticles was observed within 24 hours of incubation. A slower rate of decolourization (1-10%) was however observed for Mordant black 17 which can be attributed to the higher molecular weight or structural complexity of the azodyes.

Discussion

Nano-science is the study of phenomena and manipulation of materials at atomic molecular and

macromolecular scales.The development of easy, reliable and eco-friendly methods in the synthesis and application of nanoparticles are very promising and is beneficial for mankind[9] .The plant extract promotes the formation of silver nanoparticles at room temperature with a fast kinetics and makes it more efficient as a biosynthetic pathway. Silver nanoparticles exhibited yellowish brown color in aqueous solution due to the surface plasmon resonance phenomenon[10]. The same has been reported by Dubey *et al*. where silver nanoparticles were synthesized using the *E. hybrida* at 3 h of incubation and reported the flavanoid and terpenoid constituents of the leaf extract which might be due to the surface active molecules stabilizing the nanoparticles[11]**.** Similarly, in the present study silver nanoparticles were synthesized using the extracts of *Brassica oleracea capitata* and *Phaseolus vulgaris*. The reduction of the metal ions through these extracts lead to the formation of silver nanoparticles of fairly well-defined dimensions.

The synthesized silver nanoparticles were further evaluated through UV-vis spectroscopy, Fourier transform infrared (FTIR) spectroscopy measurements, transmission electron microscopy measurements (TEM) & scanning electron microscopy measurements (SEM) have been analysed. The spectrophotometric studies at a wavelength range of 350–800 nm showed The absorbance peak at 438nm and 430nm. Broadening of peak indicated that the particles are polydispersed. UV-Vis spectroscopy is well known to investigate shape and size controlled of nanoparticles. The absorption peak up for different size of nano particles is due to a physical process of Surface Plasmon resonance. This has been further supported by Amal Kumar Mondal and et.al. in the *Synthesis of Ecofriendly Silver Nanoparticle from Plant Latex* [12]

In this study, the application of silver nanoparticles as an antimicrobial agent was evaluated. The results of this study clearly demonstrated that the colloidal silver nanoparticles inhibited the growth and multiplication of the tested bacterial strains such as S. aureus, E. coli, S. typhi, Pseudomonas aeruginosa and Klebsiella pneumonia.This has also been reported by Aditi P. Kulkarni and et.al in the Plant mediated synthesis of silver nanoparticles using the extracts of Anthoceros (Bryophyta- Anthocerotae). The antibacterial activity of silver nanoparticles is reported to a large extent. The silver nanoparticles obtained from Anthoceros showed antibacterial activity against four strains of laboratory pathogens viz. Escherichia coli, Klebsiella pneumoniae,

Pseudomonas aeruginosa and Bacillus subtilis and thus can be used in various fields such as paint industry, pharmaceutical industry and so on[13].

The present study also revealed the ability of silver nanoparticles to decolourize the dye Congo red. A slower rate of decolourization (1-10%) was however observed for Mordant black 17 which can be attributed to the higher molecular weight or structural complexity of the azodyes. These preliminary results suggest that silver nanoparticles can be used for treatment of textile effluents[14]. The development of such particles may be considered a breakthrough in the field for the efficient clean up of the dyes on large scale process since they are easy to synthesize on large scale and are cost effective.

In conclusion, it has been demonstrated that the extract of *Brassica oleracea capitata* and *Phaseolus vulgaris* are capable of producing silver nanoparticles and are quite stable in solution. This green chemistry approach toward the synthesis of silver nanoparticles appears to be cost efficient alternative to conventional physical and chemical methods of synthesis and would be suitable for developing a biological process for large-scale production. Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents and can be used in bactericidal, wound healing and other medical and electronic applications.

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