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### ANALYSIS OF ANTIMICROBIAL ACTIVITY SILVER NANOPARTICLE FROM THE BRASSICACEA FAMILY VEGETABLES

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#### ABSTRACT

Extract of Brassicacea family vegetables is used as reducing agent for the environmentally friendly synthesis of silver nanoparticles at room temperature. The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, and non toxic in most cases. The nanoparticles were characterized using UV-visible spectroscopy, Fourier transform infrared spectroscopy, Scanning Electron Microscope and energy dispersive X-ray analysis. The antimicrobial activity of the silver nanoparticles was performed by a disc diffusion method. The synthesized silver nanoparticles exhibited antimicrobial activity against pathogenic bacteria such as Klebsiella pneumonia, Bacillus Subtilis, Staph.aureus and E.coli and also for the fungals Candida albicans and Aspergillus.

**KEYWORDS:** Silver nanoparticles, Brassicacea family, Green synthesis, Antibacterial.

#### INTRODUCTION

Nanoscience is the science of objects with typical sizes of 1-100 nm. If matter is divided into such small objects the mechanical, electric, optical, and magnetic properties can change. Interfaces rather than bulk properties dominate... Nanoscience and Nanotechnology are inter-disciplinary, crossing boundaries between physics, chemistry, chemical, electric and mechanical engineering. Miniaturization of electronic devices to sizes of the elementary units below 1  $\mu\text{m}$  has revolutionized our daily life. New technologies were required to enter the nanoscale because many of the traditional techniques do not work at the nanoscale. The relation between nanoscience and technology is like a symbiosis. Scientific discoveries lead to new technologies. The technology enables new fundamental insights. Two new technologies which enabled the progress of nanoscience are scanning tunneling and scanning force microscopy. They allow to image and manipulate objects on surfaces with sufficient precision even in ambient conditions or in liquids [1]. Among them silver nanoparticles possess unique electrical, optical as well as biological properties and are thus applied in catalysis, bio sensing, imaging, drug delivery, nano device fabrication and in medicine [2]. In the past few years, there has been an increasing interest in silver nanoparticles on account of the antimicrobial properties that they display [3]. They are even being projected as future generation antimicrobial agents [4]. Use of plants to synthesize the silver nanoparticle will provide an enhanced platform and it is free from toxic chemicals and provides natural capping agents [5]. Moreover, use of plant extracts also reduces the cost of microorganism's isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms [6].

*Table 1. Classification of Plants*

Common name	Latin name	
	Genus	species
Cauliflower	<i>Brassica</i>	<i>oleracea</i>

Cabbage	<i>Brassica</i>	<i>Oleracea var capitata</i>
Radish	<i>Raphanus</i>	<i>sativus</i>

The present study focused on the synthesis of silver nanoparticles using the Brassicaceae family vegetables and the antibacterial and antifungal activity was carried out using this synthesized vegetable extract i.e. silver nanoparticle. Brassicaceae family vegetables i.e. cauliflower, cabbage and radish were selected for the present work.

Botanical name for Cauliflower(A) is *Brassica oleracea*. It is packed with essential nutrients. Its compact flower heads hold numerous health benefiting phyto-nutrients such as vitamins, indole-3-carbinol, sulforaphane etc., that help prevent overweight, diabetes and offer protection from prostate, ovarian, and cervical cancers. Cauliflower contains several anti-cancer phyto-chemicals like sulforaphane and plant sterols such as indole-3-carbinol, which appears to function as an anti-estrogen agent. Vitamin-C is a proven antioxidant that helps fight against harmful free radicals, boosts immunity, and prevents infections and cancers. It contains good amounts of many vital B-complex groups of vitamins such as folates, pantothenic acid (vitamin B5), pyridoxine (vitamin B6) and thiamin (vitamin B1), niacin (B3) as well as vitamin K. These vitamins is essential in the sense that body requires them from external sources to replenish and required for fat, protein and carbohydrate metabolism[8].



Fig1. Image of Cauliflower

Botanical name for Cabbage(B) is *Brassica oleracea var capitata*. Cabbage is a hardy vegetable that grows especially well in fertile soils. There are various shades of green available, as well as red or purple types. Head shape varies from the standard round to flattened or pointed. Most varieties have smooth leaves, but the Savoy types have crinkly textured leaves. The vegetable is a storehouse of phyto-chemicals like *thiocyanates*, *indole-3-carbinol*, *lutein*, *zeaxanthin*, *sulforaphane*, and *isothiocyanates*. These compounds are powerful antioxidants and known to help protect against breast, colon, and prostate cancers and help reduce LDL or "bad cholesterol" levels in the blood. And also it is an excellent source of natural antioxidant, vitamin C[7].



Fig.2. Image of Cabbage

Botanical name for Radish(C) is *Raphanus sativus*. Radish is one of the nutritious root vegetables featured in both raw salads as well as in main recipes. This widely used root vegetable belongs to this Brassica family. The sharp, pungent flavor of radish comes from "isothiocyanate" compound in them, varying from mild in case of white-icicles to very hot in red globe and other pigmented varieties. Tender top greens of radish are also eaten as leafy-greens in some parts of the world.[9]. Radish, like other cruciferous and Brassica family vegetables, contains *isothiocyanate* anti-oxidant compound called sulforaphane. Studies suggest that sulforaphane has proven role against prostate, breast, colon and ovarian cancers by virtue of its cancer-cell growth inhibition, and cyto-toxic effects on cancer cells[10]. They are a very good source of anti-oxidants, electrolytes, minerals, vitamins and dietary fiber.

They contain many phytochemicals like *indoles* which are detoxifying agents and zeaxanthin, lutein and beta carotene, which are flavonoid antioxidants.



*Fig.3. Image of Radish*

## MATERIALS AND METHODS

### PREPARATION OF VEGETABLE EXTRACT

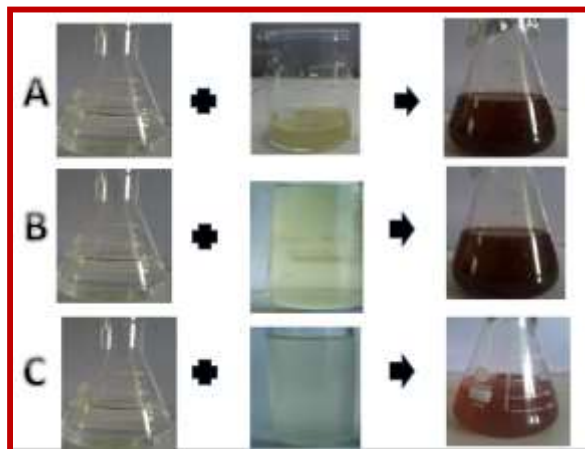
Cauliflower, Cabbage and Radishes were procured from the local market. Aqueous extract of these vegetables were prepared using 25 gm. It was washed thoroughly in deionised water, dried, chopped into small pieces and were boiled in 150 ml of deionized water for 5-10 minutes. The extract was filtered through Whatmann No.1 filter paper and used for further research. This work was done in the room temperature



*Fig.4. Chopped vegetables*

### SYNTHESIS OF SILVER NANOPARTICLES

1mM aqueous solution of Silver nitrate ( $\text{AgNO}_3$ ) was prepared and used for the synthesis of silver nanoparticles. 10 ml of the vegetable extract was added into 90 ml of aqueous solution of 1 mM Silver nitrate and incubated 15 minutes at room temperature. After 15 minutes, the colour of the solution changed from colourless to dark brown indicating the formation of silver nanoparticles.



*Fig.5. Synthesis of silver nanoparticles using the vegetables A, B and C*

**CHARACTERIZATION OF SILVER NANOPARTICLES**

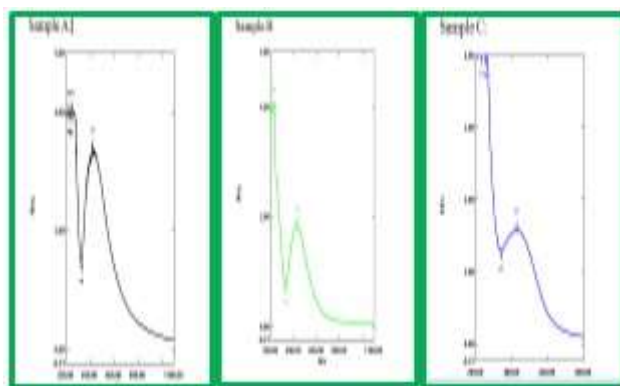
The characterization of silver nanoparticles was carried out by different techniques such as UV-Vis, FTIR, SEM and EDAX analysis.

**RESULTS AND DISCUSSION****SYNTHESIS OF SILVER NANOPARTICLES**

The colour change was noted by virtual observation in fresh tomato pomace extract incubated with aqueous solution of silver nitrate. It started to change colour from colourless to light brown and then brownish red colour due to the reduction of  $Ag^+$  ions, this exhibit the formation of silver nanoparticles. In the fig A represents the Initial, B & C indicates that the reaction mixture after 15 minutes and one hour of incubation. The intensity of colour increases with increase in time and after one hour there is no significant change in colour was observed due to the completion of reaction.

**UV-VIS SPECTROSCOPY**

Ultraviolet (UV)-visible spectrometers are used to analyze the interactions between radiation and matter in the UV-visible region of the electromagnetic spectrum. The principle involved is the measurable absorption of energy by chemical compounds when certain electronic transitions are excited between their molecular orbitals. It is used for characterizing the various metal nanoparticles in the size range of 2 to 100 nm. Spectrophotometric absorption measurements in the wavelength ranges of 400-450 nm are used in characterizing the silver nanoparticles. The silver nanoparticle sample were subjected to optical measurements, which were carried out by using a UV-Vis spectrophotometer (Shimadzu)



*Fig.6. . UV-Vis spectra of the formed silver nanoparticles using the vegetables A,B & C*

Absorption spectra of silver nanoparticles formed in the reaction media had shown absorbance peaks within the range 410-430 nm. i.e. 420nm for sample A, 428nm for sample B and 426 nm for sample C. The spectra are shown in the Fig.6.

**FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)**

FTIR spectrometers (Fourier Transform Infrared Spectrometer) are widely used in organic synthesis, polymer science, petrochemical engineering, pharmaceutical industry and food analysis. In addition, since FTIR spectrometers can be hyphenated to chromatography, the mechanism of chemical reactions and the detection of unstable substances can be investigated with such instruments. FTIR spectroscopy is useful for characterizing the surface chemistry. Organic functional groups attached to the surface of nanoparticles and the other surface chemical residues are detected using FTIR. It is particularly useful for identification of organic molecular groups and compounds due to the range of functional groups, side chains and cross-links involved, all of which will have characteristic vibrational frequencies in the infra-red range. In FTIR analysis the samples were recorded in the range of 400-4000 $cm^{-1}$ . In all three silver nanoparticles solutions the prominent bands of absorbance were observed at around 3430, 1637, 1402, 1110 and 685  $cm^{-1}$ . The observed peaks denote that the nanoparticle contains the functional group of hydroxyl, carboxylic acids, aromatic rings, alkane and amides groups.

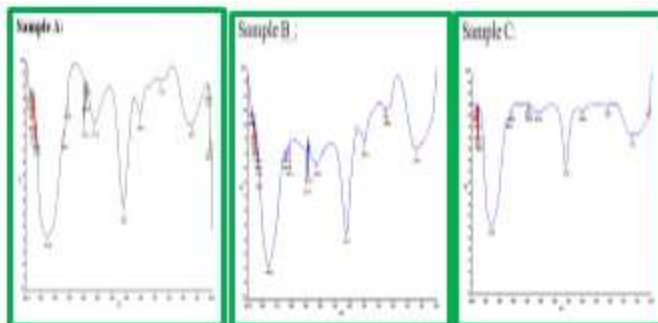


Fig.7. FTIR spectrum of silver nanoparticles synthesized using the vegetables A,B & C

### SCANNING ELECTRON MICROSCOPY

Electron microscopy is commonly used method of characterization. It is used for morphological characterization at the nanometer to micrometer scale. Sample of SEM was prepared by placing the drop of silver nanoparticle suspension over carbon coated grid then it was dried, examined and photographed in SEM Instrumentation lab, Gandhigram Rural University, Gandhigram, South India. The flake shaped nanoparticle was produced in SEM for sample A. Similarly we got spherical shaped nanoparticle for sample B and multigonal shaped nanoparticle for sample C.

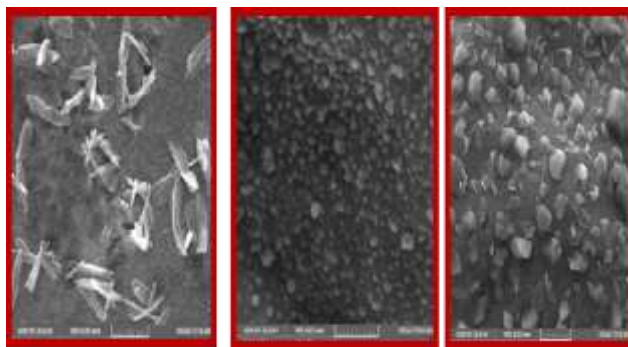


Fig.8. SEM images of the synthesized vegetables A,B and C

### ENERGY DISPERSIVE X-RAY ANALYSIS

The EDAX analysis obtained in the present study also confirmed the presence of silver nanoparticles synthesized from Cauliflower, Cabbage and radish extract. Metallic silver nanoparticles generally show typical optical absorption peak approximately at 3 Kev due to surface Plasmon resonance. The fig.9 clearly showed the presence of silver nanoparticles.

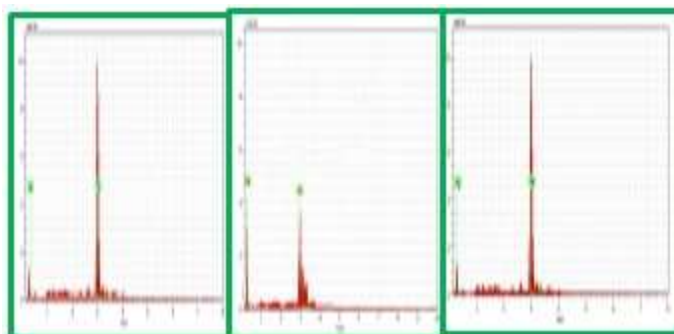


Fig.9. EDAX spectrum of the vegetables A,B & C

### ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES (AGAR WELL DIFFUSION ASSAY)

Agar well diffusion assay was used to evaluate the antibacterial activity of silver nanoparticle against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. The results indicated that silver

nanoparticles synthesized from these vegetables showed effective antibacterial activity both in Gram negative and Gram positive bacteria which is compared with amikacin. Among the four *Bacillus Subtilis* have the maximum inhibition. Its zone of inhibition was 16mm. The four images showed the bacterial activity of four bacteria species A-*Klebsiella pneumoniae*, B-*Bacillus subtilis*, C-*Staphylococcus aureus* and D-*Escherichia coli*. In each image A and B indicated the activity by our sample A and sample B. Ampicilin was taken as the positive control for the measurement of zone of inhibition (in mm).

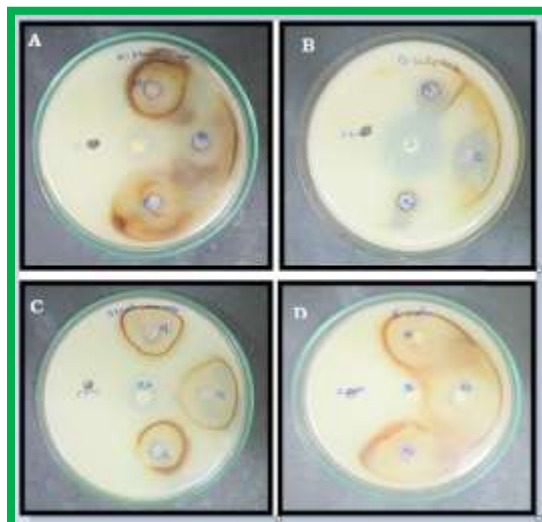


Fig.10. Bacterial activities of synthesized Ag nanoparticles against four bacteria species

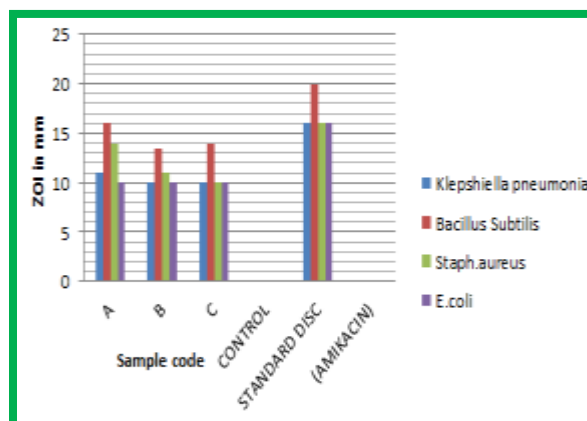


Fig.11.Graphical Representation for antibacterial activity

Table2: The antibacterial activity of silver nanoparticles synthesized using the vegetables A,B & C

Sample Code	Klepshiella pneumonia	Bacillus Subtilis	Staph.aureus	E.coli
A	R	16	14	10
B	R	16	R	10
C	R	14	10	10
CONTROL	R	R	R	R
STANDARD DISC (AMIKACIN)	16MM	20	16	16

**ANTI-FUNGAL ACTIVITY**

The activity of synthesized silver nanoparticles on various fungal strains was assayed by agar well methods. The fungal strains used in the present study were *Candida albicans*, *Aspergillus*. Agar plates were prepared, sterilized

and solidified, after solidification fungal cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10mg/ml) and placed in the agar plate and kept for incubation for 24 hrs. After 24 hrs days zone of inhibition was measured. The Anti-fungal activity of biogenic Silver nanoparticles was evaluated by using standard Zone of Inhibition (ZOI) microbiology assay. The nanoparticles showed inhibition zone against two fungal that is *Candida albicans* and *Aspergillus*. (Table: 3 and Fig: 12)

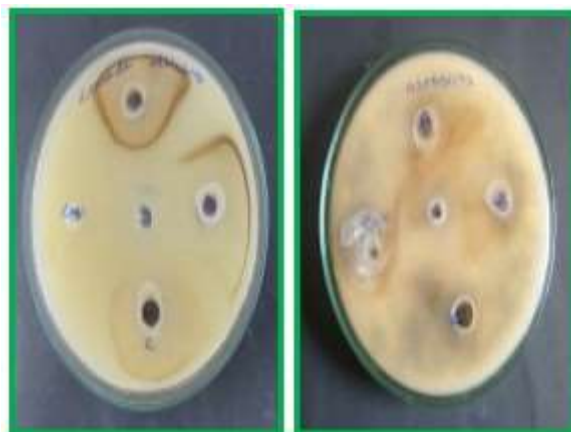


Fig.12. 3Antifungal activity of Ag nanoparticles against *Candida albicans* and *Aspergillus*

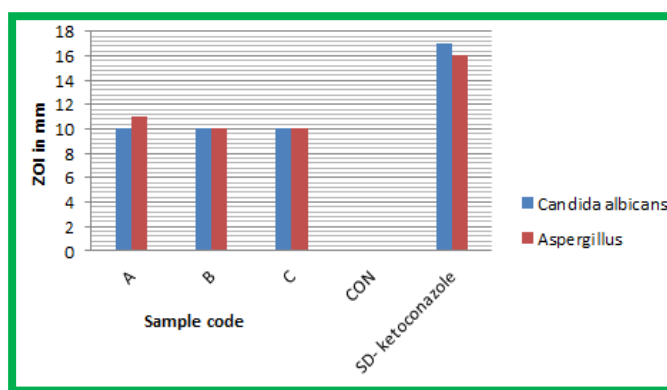


Fig.13. Graphical Representation for Antifungal activity.

Table: 3Antifungal activity of Ag nanoparticles against *Candida albicans* and *Aspergillus*.

Sample code	<i>Candida albicans</i>	<i>Aspergillus</i>
A	10	11
B	10	10
C	10	10
CON	R	R
SD- ketoconazole	17	16

The sample A exhibited potential of antifungal Maximum ZOI was found to be 11 mm for *Aspergillus*. Sample B and C exhibited potential antifungal Maximum ZOI was found to be 10 mm for both *Candida albicans* and *Aspergillus*. Among these three results, the best results obtained from sample A for *Aspergillus* fungal (Fig.13).

### CONCLUSION

The silver nanoparticles were synthesized using the *Brassicacea* family vegetables such of Cauliflower, Cabbage and Radish. This silver nanoparticles were initially confirmed by UV-Vis spectroscopy. The peaks got from this UV-Vis spectrum was confirm silver nanoparticle range, that is between 410-430nm. And further it was confirmed by SEM and EDAX. There were three different shaped nanoparticle got for the three vegetables. In EDAX we got the evidence

of silver peak at 3Kev. Then the antibacterial and antifungal activity was carried out for the silver nanoparticles and the results were analyzed.

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