A STUDY ON THE BIOSYNTHESIS, ANTIMICROBIAL ACTIVITY AND DNA DAMAGE OF SILVER NANOPARTICLES USING *CINNAMOMUM ZEYLANICUM*

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Abstract : The present study aims to synthesize Silver Nanoparticles using aqueous extract of powdered bark of *Cinnamomum zeylanicum*, elucidate its antimicrobial activity and its DNA Damage. The silver nanoparticle preparation was done using "Green Synthesis". The synthesis of Silver nanoparticles was confirmed by visual detection in which the colorless solution gets changed to a brown colored solution. Further the Characterization was done by UV- Visible Spectroscopy, Energy Dispersive X-ray (EDX), Scanning Electron Microscope (SEM) and FTIR analysis. The size of the silver nanoparticles was found to be 50 – 80 nm. The silver Nanoparticles were checked for their Antibacterial and Antifungal activity through microbiological techniques. The powdered cinnamon bark extract and the solution containing Silver Nanoparticles were tested for DNA damage through Agarose gel electrophoresis. The antibacterial and anti-fungal study showed clear zones of inhibition of microbial growth. The image projected through UV – Trans-illuminator proves that there is no DNA damage, thus indicating that biologically safe silver nanoparticles can be synthesized using *Cinnamomum zeylanicum*.

***Index Terms*:** *Cinnamomum zeylanicum*, Silver Nanoparticles, Green Synthesis, Characterization, Antimicrobial Activity, DNA damage.

I. INTRODUCTION

Nanotechnology is a newest and one of the most promising areas of research in modern medical science. Nanoparticles are usually a cluster of atoms ranging between 1-100 nm in size and exhibit new and improved properties based on size, distribution and morphology than larger particles of the bulk materials from which the nanoparticles are made (Pal *et al*., 2007). Silver is a non-toxic, safe inorganic antibacterial agent that is capable of killing about 650 types of diseased causing microorganisms (Jeong *et al*., 2005). There is an increasing interest in the silver nanoparticles on account of the antimicrobial properties they display (Choi *et al*., 2008). They are projected as future generation antimicrobial agents (Rai *et al*., 2009). Silver nanoparticles are being studied extensively, as they possess unique, electrical, optical as well as biological properties and are thus applied in catalysis, bio-sensing, imaging, drug delivery, nanodevice fabrication and in medicine (Jain *et al*., 2008). Synthesis of silver nanoparticles was extensively studied employing chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology (Natarajan *et al*., 2010). Synthesis of nanoparticles by physical and chemical methods may have considerable environmental effect, technically laborious and economically expensive (Gopinath *et al*., 2012). The biological methods using microorganisms and enzymes have been suggested as possible eco-friendly alternatives (Mohanpuria *et al*., 2008). Hence it is essential to develop environment friendly techniques for the synthesis of nanoparticles. The biological and green techniques for synthesis of nanoparticles are non-toxic, faster than other techniques and potentially eliminate the environmental issues. Green synthesis involves usage of plants and plant extracts successfully for synthesizing metal nanoparticles. Many reports are available on the biogenesis of silver nanoparticles using several plant extracts (Sulaiman *et al*., 2012). *Cinnamomum zeylanicum* is a small, tropical, evergreen tree most noted for its bark, which provides the world with the commonly known spice, cinnamon (Brierley, 1994). *C. zeylanicum* bark is widely used as a spice. It is principally employed in cooking as a condiment and flavouring material. In folk medicine, it acts like other volatile oils and was once used as a cure for colds. It has also been used to treat diarrhea and other problems of the digestive system. *C. zeylanicum* bark is high in antioxidant activity (Priyanga *et al*., 2013). *C. zeylanicum* bark has been reported to have remarkable pharmacological effects in the treatment of type II diabetis and insulin resistance. (Verspohl *et al*., 2005). The present study thus aims to synthesize silver nanoparticles using the commercially and abundantly available *C. zeylanicum* bark, and characterization of the synthesized nanoparticles utilizing UV- Visible Spectroscopy, Energy Dispersive X-ray (EDX), Scanning Electron Microscope (SEM) and Fourier transform infrared spectroscopy (FTIR) analysis. Besides this, the DNA damage and the antimicorobial activity against representatives of human pathogenic microorganisms are also to be investigated.

II. MATERIALS AND METHODS

2.1. CHEMICALS

Synthesis of AgNPs was done with Silver Nitrate ($AgNO_3$) purchased from Sigma Aldrich. Nutrient agar and Potassium Dextrose were purchased from Himedia which were used for microbial plating. Agarose gel, TAE buffer, Ethylene bromide from Himedia were used for Gel electrophoresis.

2.2. PREPARATION OF PLANT EXTRACT

Cinnamomum zeylanicum bark was collected from the cinnamon fields of Spices board of India, Calicut, Kerala. It was washed to remove any impurities and dried under sunlight for a week to completely remove the moisture. The bark was cut into small pieces and powdered in a mixer and then sieved using a 20-mesh sieve to get uniform size range. The powdered cinnamon was analyzed through gas chromatography to find out the components. For the production of the extract the powder was mixed (in grams) with double distilled water (in ml) in the ratio 1:14. The mixed solution was kept in the water bath maintained at 90ºC for 1 hour. The filtrate was obtained by filtering with muslin cloth. The filtrate was centrifuged for 10 min at 4600 rpm for getting a clear supernatant. The clear supernatant was stored for further use.

2.3. SYNTHESIS OF SILVER NANO PARTICLES

Silver nanoparticle preparation was done using the green synthesis method. 2mM solution and 1mM solutions of $AgNO_3$ were prepared using distilled water and $AgNo_3$ was purchased from Sigma Aldrich. 5ml of plant extract were added to 10ml of 2mM and 1mM solutions. The solutions were kept in darkness for 24 hours. Then the samples were studied for identification and characterization of nanoparticles using UV- Visible Spectroscopy, Energy Dispersive X-ray (EDX), Scanning Electron Microscope (SEM) and Fourier transform infrared spectroscopy (FTIR) analysis.

2.4. MICROBIAL PLATING

Microbial plating was done for bacteria such as *E.coli, Klebsiella pneumoniae, Staphylococcus aureus* and the fungi *Aspergillus niger*. Nutrient agar medium and Potato dextrose medium were used for bacteria and fungi respectively. The medium and the equipments were sterilized for 121ºC for 15 minutes and plating was done with well diffusion method. 100µl of cultures of bacteria were spread on petri plates containing nutrient agar and fungi were spread on Potato Dextrose Medium. Wells were punched in the medium with sterile cork borer. 50µl of samples along with standard ofloxacin discs were inoculated into the wells and the plates were incubated at 37ºc overnight (Manjunath *et al*., 2014). Petri plate inoculated with fungi kept at room temperature.

2.5. DNA DAMAGE STUDY

This study was carried out through agarose gel electrophoresis. The wells were filled with marker DNA, plant extract and Nano particles and were run at 50 v. After running the gel, the DNA damage was studied using UV Transilluminator. The purpose of this study was to check if the synthesized product can be used on animals.

III. RESULTS

3.1. Gas Chromatography result on analysis of powdered Cinnamon bark.

Cinnamon bark powder was analyzed using gas chromatography to find out the components (Table 1). Equipment used was THERMO GC - TRACE ULTRA VER: 5.0,THERMO MS DSQ II and the column was MS CAPILLARY STANDARD NON - POLAR COLUMN. Carrier gas was He with a flow rate of 1.0ml/min.

Table 1: Components present in the cinnamon bark powder extract analyzed through gas chromatography

No.	Name of the compound	Mol. Formula	MW	Compound Nature
1	ç-Terpinene	C10H16	136	Alkaloids
2	Cinnamaldehyde	C9H8O	132	Phenyl group
3	Phenol, 2-methoxy-4-(2-propenyl)-, acetate (CAS)	C12H14O3	206	Alkaloids
4	Cinnamaldehyde dimethylacetal	C11H14O2	178	Aldehydes
5	Cis-2-Methoxycinnamic acid	C10H10O3	178	Carbonyl compunds
6	Caryophyllene	C15H24	204	Essential oils
7	Ortho methoxy Cinnamic aldehyde	C10H10O2	162	Bio active compound
8	Tetradecanal	C14H28O	212	Myristic acid
9	9-Octadecena	C18H34O	266	Aldehyde
10	Trans-Z-à-Bisabolene epoxide	C15H24O	220	Sesquiterpene oxide
11	Campesterol	C28H48O	400	Steroid

3.2. CHARACTERIZATION OF NANO PARTICLES

3.2.1. Color Change

For the Green synthesis of Silver nanoparticles by *Cinnamomum zeylanicum,* plant extracts were carried by adding 5ml of plant extract to 10ml of 2mM and 1mM $AgNo_3$ Solution. A simple procedure was adopted to synthesize Silver nanoparticles from cinnamon bark extract. On mixing the plant extract of *Cinnamomum zeylanicum with* silver nitrate solution (2mM and 1mM), the color of the reaction mixture started to change from colorless to dark brown color indicating the generation of silver nanoparticles, due to the reduction of silver metal ions Ag^+ in to silver nanoparticles via the active molecules present in the *Cinnamomum zeylanicum* bark extracts. Changing in color after the reduction of Ag^+ to silver nanoparticles is shown in (Fig.1).

Figure 1: Appearance of dark brown color in solutions of 1mM and 2mM $AgNO_3$ indicating the synthesis of Silver Nanoparticles

3.2.2. UV – VISIBLE SPECTROSCOPY

The formation of Silver nanoparticles was identified by scanning the solution containing Silver nanoparticles at the wave length ranging from 400 – 700nm using Shimadzu UV – 1601 spectrophotometer. The maximum absorptions were obtained at a wave length of 320 and 350 nm in case of 1mM solution (Fig. 2) and 360 nm and 480 nm in case of 2mM solution (Fig. 3). The SPR bands centered between 350 and 480 nm, confirms the formation of AgNPs in the solution. The appearance of the peak is due to the size dependent quantum mechanical phenomenon called Surface Plasmon Resonance (SPR). This effect become influential when the De – Brogile wavelength of the valence electrons becomes equal to or less than the size of the particle (less than 50nm).

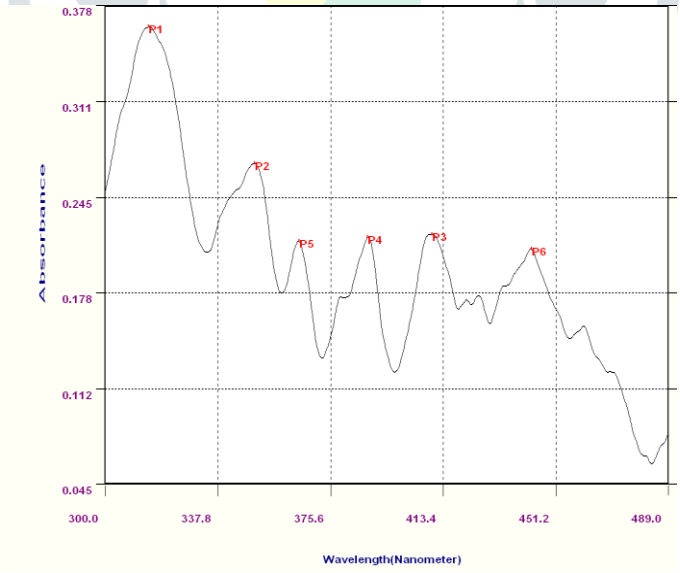


Figure 2: UV - Vis spectrum of 1mM Silver Nanoparticles

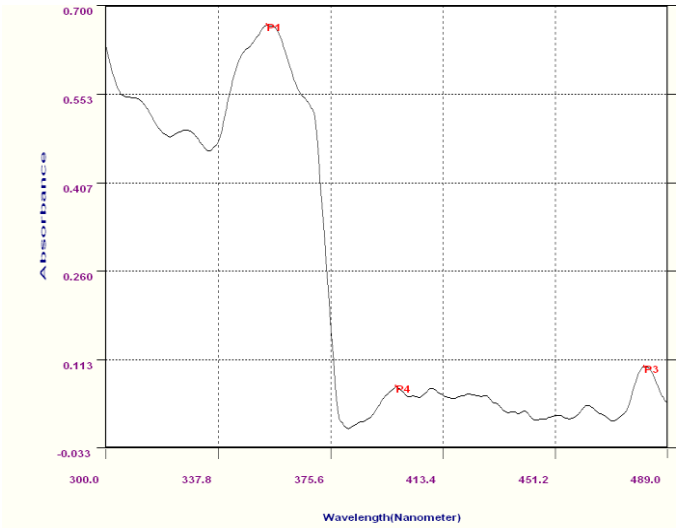
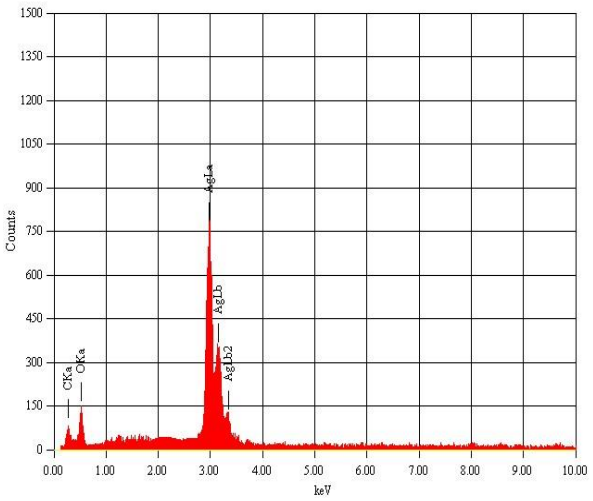


Figure 3: UV – Vis spectrum of 2mM Silver Nanoparticles

3.2.3. EDX AND SCANNING ELECTRON MICROSCOPY ANALYSIS

Energy-dispersive X-ray (EDX) analysis was carried out using JEOL JEM 2100 high resolution transmission electron microscope, to confirm the presence of Silver in the particles, as well as, to detect other elementary compositions of the particles. Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, the extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minute. The synthesis of Silver Nanoparticles using cinnamon extract was confirmed by the characteristic peak obtained in the EDX image (Fig 4) and the structural view under scanning electron microscope (Fig. 5)



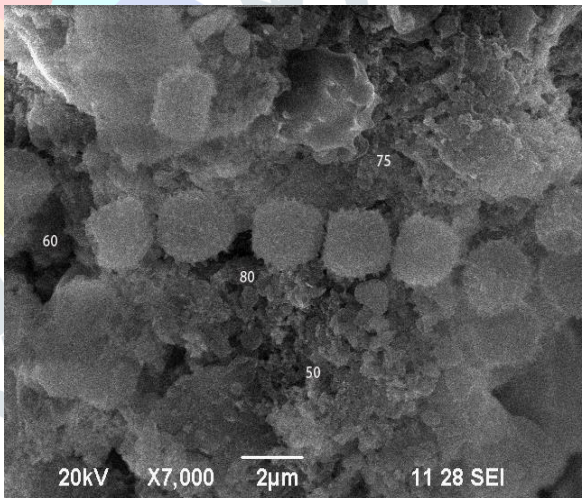


Figure 4: EDX image with four dominant peaks

Figure 5: SEM image showing AgNPs in the range of 50 – 80 nm

3.2.4. FTIR ANALYSIS

The FTIR investigations were carried out with a Scimitar Series FTS 2000 Digilab spectrophotometer in the range of middle infrared of 4000-400 cm–1. 0.0007 g sample was pressed with 0.2000g of KBr for IR spectroscopy Shimadzu, Japan. The number of scans 16 and the resolution of 4 cm-1 characterized these measurements. The peaks show the presence of Silver nanoparticles. Absorbance bands are observed in the region of 1200 -1800 cm^{-1}. The FTIR spectroscopic study has confirmed that the carbonyl group of amino acid residue and peptides of proteins of plant extract has strong ability to bind metal, and most possibly might have formed a layer on the Silver Nanoparticles. (Fig. 6)

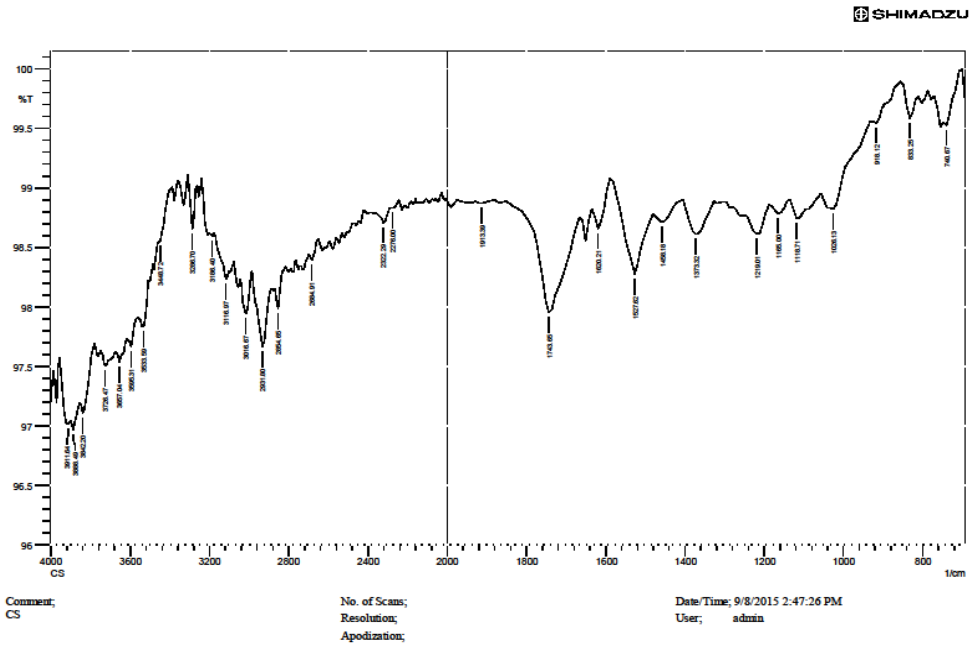


Figure 6: FTIR analysis of the solution containing Silver Nanoparticles

3.2.5. ANTI BACTERIAL AND ANTI FUNGAL ACTIVITY

The microbial plating was done after all the sterilizing techniques using well diffusion method. The bacteria used were *Klebsiella pneumoniae, E.coli, Staphylococcus aureus* and Fungi was *Aspergillus niger*. Bacterial plates were incubated and Fungi plate was maintained at room temperature. Efficient growth of bacteria with clear zones of inhibition of growth around the wells of nanoparticles was observed. The nanoparticles were efficient than the standard antimicrobial disc (Table 2) (Fig. 7 to Fig. 10)

Table 2: Measurements of zone of inhibition of microbial growth in the petri plates (in mM)

Microorganism	1mM$AgNO_3$	2mM$AgNO_3$	1mMAgNPs	2mMAgNPs
S. aureus	2	1.5	2	5
K. pneumoniae	1	1	3	5
E.coli	2	3	3	5
A. niger	2	2	3	3
Disc	Nil			

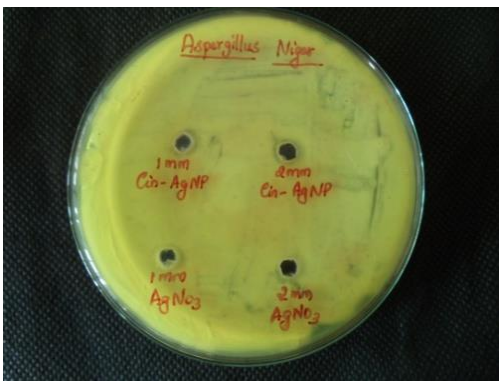


Figure 7: Antifungal activity of AgNPs (*A.niger*)

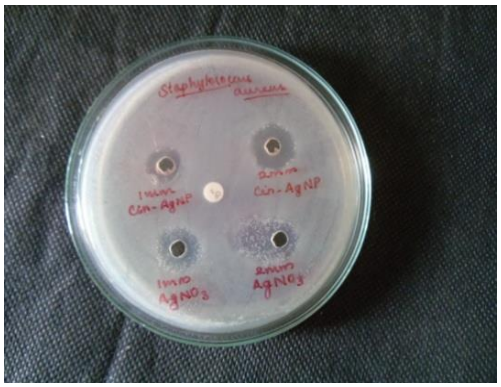


Figure 8: Antibacterial activity of AgNPs(*S.aureus*)

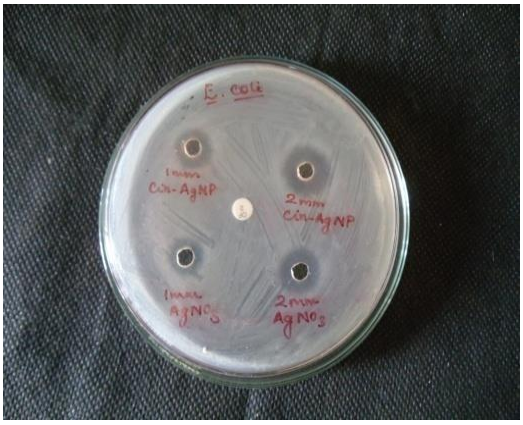


Figure 9: Antibacterial activity of AgNPs (*E.coli*)

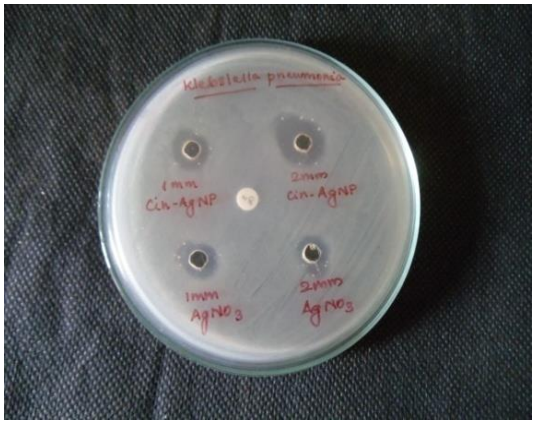


Figure 10: Antibacterial activity of AgNPs (*K.Pneumoniae*)

3.3. DNA DAMAGE STUDY

DNA damage study is undertaken to find out the toxicity of synthesized Silver Nanoparticles. All metals are known to have toxic effects on living cells. But in this research biologically synthesized silver nanoparticles do not show any damaging effect on the DNA. Therefore this can be used for various purposes on animals. The photograph (Fig. 11) shows clear orange color bands representing the presence of DNA and the comparison was done between marker DNA, plant extract and biocompatible nanoparticles.

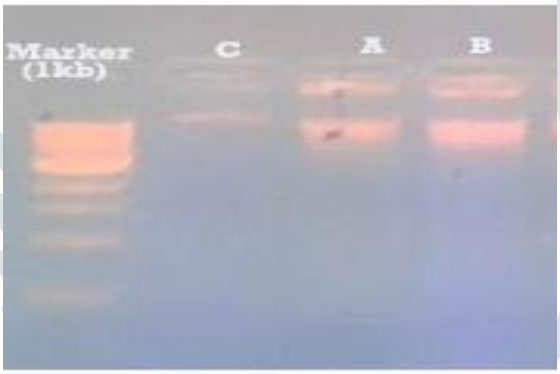


Fig 11: Photograph showing the illumination of the gel in UV –Transilluminator
C – Control, A- Plant extract, B – AgNPs

IV. DISCUSSION

The extract was already pale and the color of the solution was immediately intensified by the addition of the AgNO3 Solution. The color intensity increased with time. After 2 hours the color intensity was even higher and giving darker looks after 8 hours. The intensity of the color increased on increasing the time of the reaction (Sathishkumar *et al*., 2009). *Cinnamomum zeylanicum* bark is rich in terpenoids, including linanool, eugenol and methyl chavicol (Jayaprakasha *et al*., 2002). In addition, some protein is also present in the bark. Terpenoids are believed to play an important role in silver nanoparticle biosynthesis through the reduction of silver (Ag) ions (Shankar *et al*., 2003). This result is in agreement with the study of Raut *et al.*, (2009), in which they reported that reduction of silver ion into silver nanoparticles during exposure of plant extracts could be followed by color change due to excitation of surface plasmon vibrations in silver nanoparticle.

Shankar *et al.*, (2003) reported the possibility of terpenoids from Geranium leaf in the synthesis of nano-sized silver particles. Polyols such as terpenoids, flavones and polysaccharides in the *Cinnamomum zeylanicum* leaf were reported to be the main cause of the bioreduction of silver ions and proteins are reported to bind to the nanoparticles either through free amine groups or cysteine residues in the proteins (Gole *et al*., 2001). A similar mechanism may have operated in the present case where the proteins extracted from the *C. Zeylanicum* bark capped the Ag nanoparticles, thereby stabilizing them. To summarize these results, the water-soluble fractions comprised of complex polyols (Schoene *et al*., 2005) in the biomass were believed to have

played a major role in the bioreduction of Ag ions. Optical properties and color exhibited by silver nanoparticles are due to surface Plasmon resonance (Sulaiman *et al*., 2013).

The synthesis of Silver Nano particles was effectively done using *Cinnamomum zeylanicum* by green synthesis method**.** The Silver Nanoparticles synthesized with cinnamon was efficient in proving its anti-microbial and antifungal activity. Three different bacteria were used for the study. The Silver Nanoparticles were more effective against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *E.coli* (Table 2). Also petri plates inoculated with bacteria were treated with 1mM and 2mM solution of Silver Nanoparticles. In the studies for characterization and identification, 2mM solution of plant extract with $AgNO_3$ seems to show effective synthesis of Silver Nanoparticles and clear zones of inhibition of growth were obtained in case of 2mM solution of Silver Nanoparticles. DNA damage studies also shows that the biologically synthesized Silver Nanoparticles can be used effectively in the field of medicine, since there is no damage caused to the sample DNA.

Several main mechanisms underlie the biocidal properties of silver nanoparticles against microorganisms. First, silver nanoparticles attach to the negatively charge cell surface, alter the physical and chemical properties of the cell membranes and the cell wall and disturb important functions such as permeability, osmoregulation, electron transport and respiration (Nel *et al*., 2009). Second, silver nanoparticles can cause further damage bacterial cells by penetrating the cell, where they interact with DNA, proteins and other phosphorus and sulphur containing cell constituents (Marambio Jones *et al*., 2010). Third, silver nanoparticles release silver ions, generating an amplified biocidal effect, which is size and dose dependant (Marambio Jones *et al*., 2010). Silver nanoparticles showed more bactericidal activity compared with the silver salt. The high bactericidal activity of silver nanoparticles is due to their extremely large surface area, which provides better contact with microorganisms. Moreover, silver nanoparticles act as reservoirs for the Ag^4 bactericidal agent.

V. CONCLUSION

The research work proves that the *Cinnamomum zeylanicum* supports the synthesis of Silver Nanoparticles and also reduces its toxicity. Silver found to be effective among different types of bacteria. Silver nanoparticles have been extensively reviewed and it seen that Silver Nanoparticles are non-toxic. When compared, the Silver Nanoparticles synthesized using 2mM solution were more effective. Also, the DNA damage studies shows that the plant extract as well as the Silver Nanoparticles do not cause damage to DNA, thus ensuring a scope of using the product in the field of medicine.

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