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Synthesis of silver nanoparticles from *in vitro* derived plants and callus cultures of *Costus speciosus* (Koen.); Assessment of antibacterial activity

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This study demonstrates for the first time the efficiencies of *in vitro* derived plants and callus cultures of *Costus speciosus* extract in the rapid biosynthesis of stable silver nanoparticles. Synthesis of silver nanoparticles may be influenced directly or indirectly by phytochemicals in plants such as phenolics, flavonoids, and diosgenin compounds. This study also highlights a cost effective and environment friendly technique for green synthesis of silver nanoparticles. These silver nanoparticles were found to be highly toxic against different multi drug resistant clinical samples such as gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and the gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae*. This also provides evidence for developing large scale commercial production of value-added products for biomedical / nanotechnology based industries, which is an important step in the field of application of nanotechnology.

Key words: *Costus speciosus*, Karnataka, silver, nanotechnology

Synthesis of silver nanoparticles using a plant system is one of the useful technologies, which has many practical applications in the modern medicine (Malabadi *et al.* 2012: Xia *et al.* 2010; Zhang *et al.* 2008). Silver ion and silver-based compounds are highly toxic to microorganisms showing a strong biocidal effect against microbial species because these are highly reactive species with a large surface area (Malabadi *et al.* 2012; Ghosh *et al.* 2012). Silver nanoparticles produced using microbes and plant extracts are known to exhibit potent antimicrobial activity (Ghosh *et al*. 2012). Nanoparticles exhibits important role in several aspects such as drug delivery, diagnosis, antimicrobial activities and tissue engineering (Malabadi *et al.* 2012; Sharma *et al*. 2007; Song and Kim, 2009; Gardea-Torresdey *et al*. 2002, 2003). Plant derived silver nanoparticles have many advantages over traditional chemical and physical

methods in terms of cost effective, large scale production, easily affordable, no need of costly equipment and infrastructure, and it is not necessary to use toxic chemicals (Malabadi *et al.* 2012; Xia *et al.* 2010; Zhang *et al.* 2008; Song and Kim, 2009; Gardea-Torresdey *et al.* 2002, 2003; Shankar *et al*. 2003, 2004a, 2004b; Nabikhan *et al.* 2010; Geethalakshmi and Sarada, 2010; Farooqui *et al.* 2010; Elumalai *et al.* 2010; Khandelwal *et al*. 2010; Saxena *et al.* 2010; Sharma *et al*. 2007). The use of plant materials for the synthesis of nanoparticles could be more advantageous, because it does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell culture (Malabadi *et al.* 2012). Plant extracts are suitably scaled up for large scale biosynthesis of silver nanoparticles in a controlled manner according to their shape, size and sensitivity (Malabadi *et al.* 2012; Gnanadesigan *et al.* 2011; Shankar *et al.* 2003; Geethalakshmi and Sarada, 2010; Mukunthan *et al.* 2011; Vankar and Shukla, 2012; Ghosh *et al.* 2012). The use of plants for the synthesis of silver nanoparticles is a very low cost method, and also generates good quality and quantity of nanoparticles within few hours for the various medical applications (Shankar *et al.* 2003, 2004a, 2004b; Nabikhan *et al.* 2010; Malabadi *et al.* 2012). Several plant species have been used for the synthesis of silver nanoparticles including tissue culture derived callus of *Carica papya* (Nabikhan *et al.* 2010). Traditionally silver has been known to have a disinfecting effect and this property of silver has been exploited for the pharmaceutical applications. Several salts of silver and their derivatives are commercially manufactured as antimicrobial agents (Malabadi *et al.* 2012; Savithramma *et al.* 2011a, 2011b). Furthermore, very low concentrations of silver are safe for human cells, but lethal for microorganisms (Malabadi *et al.* 2012; Prabhu *et al.* 2010; Savithramma *et al.* 2011a, 2011b). It was also reported that silver nanoparticles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic microorganisms at low concentrations without any side effects (Savithramma *et al.* 2011a, 2011b; Malabadi *et al.* 2012).

Costus speciosus (Koen.) Sm (family Zingiberaceae) commonly known as "*Spiral ginger*" is a rhizomatous perennial herb with pinkish white flowers in reddish bracts. It is distributed below 1500 m altitude in tropical forests throughout India (Malabadi, 2002, 2005; Malabadi *et al*. 2005). The plant serves as an ornamental and the rhizome serves as a source of an antihelmintic compounds and an alternative source of diosgenin (Chopra *et al.* 1956; Roy and Pal, 1991; Chaturvedi *et al.* 1984; Malabadi, 2002, 2005; Malabadi *et al*. 2004, 2005). It is also used locally for diabetes and jaundice. The root extract act as an astringent, aphrodisiac, depurative, purgative and is useful in catarrhal fever, coughs, skin diseases and snake bites (Rastogi and Mehrotra, 1991, Khanna *et al.* 1977, Rathore and Khanna, 1978; Malabadi, 2002, 2005; Malabadi *et al*. 2004, 2005). The plant is conventionally propagated by vegetative techniques using rhizome and sucker segments and through seeds. *In vitro* methods of propagation have been successfully developed for *Costus speciosus* to meet the current demand as herbal medicine (Chopra *et al.* 1956; Roy and Pal, 1991; Chaturvedi *et al.* 1984; Malabadi, 2002, 2005; Malabadi *et al*. 2004, 2005). The present study was conducted to investigate antibacterial activity of silver nanoparticles synthesized from *in vitro* derived plants and callus extracts of *Costus speciosus* (Koen.) by preliminary bioassay screening. Antibacterial activity was evaluated by using the disc-diffusion assay, and minimal inhibitory concentration (MIC), values were determined by using the microdilution assay. The extracts were tested against the gram-positive

bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and the gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae*.

Materials and methods

Source of plant material and establishment of *in vitro* derived plants

The establishment of *in vitro* derived plants and callus initiations were done according to our previous published protocol (Malabadi *et al.* 2005). Rhizomes of *Costus speciosus* (Koen.) Sm collected from the Western Ghat Forest near Belgaum - Panaji Highway road, Karnataka, India were washed thoroughly in sodium hypochlorite solution (commercial bleach 5%) with few drops of Tween – 20 (Sigma) for 10 min, and then under sterile distilled water for three times (Malabadi *et al.* 2005). Whole rhizomes were surface decontaminated sequentially with 70% ethanol (5 min) and 0.1% $HgCl_2$ (2 min) before rinsing thoroughly with sterilized distilled water (Malabadi *et al.* 2005). Transverse- thin sections of approximately 1-5 mm thick were cut from rhizomes and these thin sections were cultured on a Gamborg - B_5 basal medium (Gamborg *et al.* 1968), with 30 g l^{-1} sucrose (Analar grade), 7 g l^{-1} agar (Hi-media, Bombay), 0.2 g l^{-1} p-aminobenzoic acid, 0.1 g l^{-1} Biotin, 0.1 g l^{-1} Folic acid, 0.1 g l^{-1} meso-inositol, 0.5 g l^{-1} L-glutamine, , 0.5 g l^{-1} casein hydrosylate and supplemented with 5 µg l^{-1} Triacontanol (TRIA) without any other growth hormones (Malabadi *et al.* 2005). The cultures were raised in 25 mm X 145 mm glass culture tubes (Borosil) containing 15 ml of the medium under cool white fluorescent light (100 µmol m^{-2} s^{-1}) at 25 ± 2^0 C with a relative humidity of 55 - 60 % (Malabadi *et al.* 2005). The nutrient medium without TRIA served as control. The pH of the media was adjusted to 5.8 with NaOH or HCl before agar was added. The media were then sterilized by autoclaving at 121^0C and 1.04 kg cm^{-2} for 15 min. TRIA, meso-inositol, Biotin, p-aminobenzoic acid, L-glutamine and Folic-acid were filter sterilized and added to the media after it had cooled to below 50^0 C (Malabadi *et al.* 2005).

The cultures were maintained for 6 to 8 week for the shoot bud initiation under cool white fluorescent light (100 µmol m^{-2} s^{-1}) at 25± 2^0 C with a relative humidity of 55 - 60 %. The freshly initiated shoot buds were again subcultured for another 2 week on modified Gamborg – B_5 basal medium (Gamborg *et al.* 1968) containing 5 µg l^{-1} TRIA (Malabadi *et al.* 2005). The well-developed shoots with 2 to 3 leaves were transferred onto B_5 basal medium supplemented with2 µg l^{-1} TRIA for rooting. The shoots with well developed roots on 2 µg l^{-1} TRIA supplemented basal medium were hardened and transferred to soil (Malabadi *et al.* 2005). Furthermore, the proliferation of callus was also found within 4 weeks and it was harvested. The rhizome and callus of these *in vitro* derived plants were used for the synthesis of silver nanoparticles for the assessment of antibacterial activity.

Synthesis of silver nanoparticles

The callus and rhizome material of *Costus speciosus* were oven dried at 50^o C and ground to make a fine powder. Further, 25 grams of powdered callus and rhizome were taken separately in two 250ml beaker containing 100ml of sterile distilled water. Mixture was boiled for 10 min at 100^o C. The callus and rhizome extracts were collected separately in two beakers by a standard filtration (Whatman filer paper) method. 1mM $AgNO_3$ (silver nitrate) solution was prepared and stored in amber colour bottle. 10ml of callus and rhizome extracts were taken in beaker separately and 50ml of 1mM $AgNO_3$ solution was added to the beaker drop wise with constant stirring at 50-60^o C and colour change was observed (Malabadi *et al.* 2012; Linga Rao and Savithramma, 2012). The colour change was checked periodically and the beakers were incubated at room

temperature for 40 hours (Malabadi *et al.* 2012; Linga Rao and Savithramma, 2012). The color change of the callus and rhizome extracts from yellow to brown indicated the presence and synthesis of silver nanoparticles. The extract contents were then centrifuged at 10,000 rpm for 20 min (Malabadi *et al.* 2012; Linga Rao and Savithramma, 2012). The supernatants were used for the spectrometric UV analysis and for the evaluation of antibacterial activity (Malabadi *et al.* 2012; Linga Rao and Savithramma, 2012). The spectrometric analyzed results highlighted the presence and reduction of silver ions in the tested samples. The reduction of silver ions was monitored by measuring the absorbance of the reaction mixture in a range of wavelength from 300 to 600 nm using spectrocolorimeter to find the absorbance peak (Malabadi *et al.* 2012).

Stabilization of silver nanoparticles

The protocol for the stabilization of silver nanoparticles has been adopted from previous published papers (Gardea-Torresdey *et al*. 2002, 2003; Shankar *et al*. 2003, 2004a, 2004b; Sharma *et al*. 2007) with slight modifications in our experiments. In this method, 0.9 g of polyvinyl alcohol (PVA) was dissolved in 100ml sterile distilled water at 100^{o} C by vigorously stirring to form homogenous solution (Nabikhan *et al.* 2010). An aqueous solution of 20 ml of silver nanoparticles synthesized by callus and rhizome extracts was added with PVA solution (Nabikhan *et al.* 2010). This solution was then allowed to stir in a flask for about 10 min, and then the solution was purged with nitrogen. A fresh solution of $5x10^{-3}$ M was prepared and introduced drop by drop into PVA-$AgNO_3$ solution (Nabikhan *et al.* 2010). The solution was then stirred for 15min under inert atmosphere at room temperature of 28^{o} C. Silver nanoparticles were also similarly prepared in the absence of PVA. The color intensity at 420 nm was measured separately for callus and rhizome extracts stabilized with and without PVA (Nabikhan *et al.* 2010).

Screening of antibacterial activity

The test pathogenic organisms used in the investigations of antibacterial activity were *Bacillus subtilis* and *Staphylococcus aureus*, and the gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* (Malabadi, 2005; Malabadi *et al*. 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012). Tenfold serial dilution of overnight MH broth cultures were prepared and 100 µl of each dilution were spread onto MH agar plates using a glass spreader (Malabadi, 2005; Malabadi *et al.* 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012). The plates were incubated overnight at 37^{o} C and colonies were counted using a colony counter. Following the assumption that each living bacterial cell will grow into a separate colony on the plate, the number of cells present per milliliter of the original overnight cultures was calculated (Malabadi, 2005; Malabadi *et al*. 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012). The optical density (OD) at 600 nm for each dilution was determined using spectrophotometer, and used to indicate numbers of bacterial cells in cultures for the antibacterial screening and MIC determination (Malabadi, 2005; Malabadi *et al*. 2012).

The antibacterial activity was done by disc-diffusion assay method (Malabadi, 2005; Malabadi *et al*. 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012). Filter paper discs (Whatman No 3 and 6 mm in diameter) were sterilized by autoclaving. In this method, 100 µl of silver nanoparticles prepared from callus and rhizome extracts was mixed in 1 ml distilled water and then applied to sterile paper discs

of 5mm diameter (Whatman Filter papers)(Malabadi *et al.* 2012). These discs were air-dried under sterile conditions. Similarily 100µl nanocomposite of silver nanoparticle and PVA prepared from callus and rhizome extracts was mixed in 1ml of distilled water and applied to sterile paper disc. These discs were then placed on Muller Hinton Agar swabbed with clinical strains of bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*, and the gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* at a concentration of 10^6 bacteria/ml for bacteria (Malabadi, 2005; Malabadi *et al.* 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012). The plates were incubated at 37° C for overnight. The zone of inhibition was measured in millimeter after 24 h of incubation and recorded. Each extracts of callus and rhizome were tested in quadruplicate (four discs per plate), with a silver sulphadiazine (1mg ml^{-1}) disc as a reference or positive control (Malabadi, 2005; Malabadi *et al*. 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi *et al.* 2012). The ratio between the diameter of the inhibition zones (mm) produced by callus and rhizome extracts and the inhibition zone around the disc with silver sulphadiazine (mm) was used to express antibacterial activity. The activity of Silver Sulphadiazine was included in this equation to adjust for plate-to-plate variations in the sensitivity of a particular bacterial strain (Malabadi, 2005).

The antibiotic silver sulphadiazine was included as standard in each assay. Extract-free solution was used as blank control (Malabadi, 2005; Malabadi *et al*. 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008). The microplates were incubated overnight at 37° C. As an indicator of bacterial growth, 40µl p-iodonitrotetrazolium violet (INT) (Sigma) dissolved in water were added to the microplate wells and incubated at 37° C for 30 min (Eloff, 1998a, 1998b). MIC values were recorded as the lowest concentration of extract that completely inhibited bacterial growth (Malabadi, 2005; Malabadi *et al.* 2005, 2007, 2010; Malabadi and Vijayakumar, 2005, 2007, 2008). Since the colorless tetrazolium salt is reduced to a red colored product by biologically active organisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with INT (Eloff, 1998a, 1998b).

Results and Discussion

The antibacterial activity of silver nanoparticles against tested clinical samples has been presented in the table-1. In our present study, silver nanoparticles synthesized from *in vitro* derived plant extracts of *C. speciosus* showed antibacterial activity against clinical samples of the gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and the gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae*. Antibacterial activity determined using the disc diffusion method confirmed that silver nanoparticles resulted in a greater bactericidal effect on the test pathogens than either of the antibacterial agents used alone. Furthermore, the addition of polyvinyl alcohol during stabilization of silver nanoparticles has a profound effect on the MIC values. Polyvinyl alcohol stabilized silver nanoparticles showed the highest toxicity as compared to unstabilized silver nanoparticles. Further rhizome extracts showed the highest toxicity against callus extracts (Table-1). In case of *D. bulbifera*, diosgenin is reported to be a predominant saponin which might contribute to the surfactant properties of *D. bulbifera* tuber extract (Ghosh *et al.* 2012). Plant surfactants are widely used in the synthesis of silver nanoparticles. This also confirms the present study of *Costus specious* which is the source of diosgenin. The main biomolecules responsible for nanoparticle synthesis were polyphenols or flavonoids (Ghosh *et al.* 2012).

In another recent study, the synthesis of silver nanoparticles using whole plant extracts of *Clitoria ternatea* has been reported (Malabadi *et al.* 2012). Antibacterial activity of silver nanoparticles was assessed by using disc diffusion method against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*, since *Bacillus* species and *S. aureus* strains may cause diarrhoea and an enteropathogenic form of *E. coli*, and *Klebsiella* species may cause food poising (Malabadi *et al.* 2012). The results of this study also clearly indicated that silver nanoparticles synthesized from plant extracts of *Clitoria ternatea* has many pharmaceutical applications for the control of deadly pathogens (Malabadi *et al.* 2012). The significant and higher antibacterial activity of *Clitoria ternatea* are probably due to the presence of flavonoids in the plant (Malabadi *et al.* 2005; Malabadi *et al.* 2012). On the other hand antibacterial activity might be due to the presence of a phenol glycoside 3-5-7-4 tetra-hydroxy- flavone-3- rhamnoglycoside an alkaloid called *clitorin* (MP 235^{o} C) (Malabadi *et al.* 2012).

Table 1. Antibacterial activity of *in vitro* derived plants, and callus cultures of *Costus speciosus* on the clinical samples by disc diffusion method

Clinical samples	Diameter of inhibition zone (mm)			
	Plant extracts	**Silver nanoparticles**	**Silver nitrate $AgNO_3$**	**Methanol extracts**
Bacillus subtilis	**Rhizome**	**Rhizome**		
	+PVA	18		
	-PVA	11	11	5
	Callus	**Callus**		
	+PVA	19		
	-PVA	09		
Staphylococcus aureus	**Rhizome**	**Rhizome**		
	+PVA	16		
	-PVA	08		
	Callus	**Callus**	13	6
	+PVA	15		
	-PVA	06		
Escherichia coli	**Rhizome**	**Rhizome**		
	+PVA	21		
	-PVA	12	11	5
	Callus	**Callus**		
	+PVA	20		
	-PVA	14		
Klebsiella pneumoniae	**Rhizome**	**Rhizome**		
	+PVA	23		
	-PVA	13	16	9
	Callus	**Callus**		
	+PVA	17		
	-PVA	13		

+PVA= Addition of polyvinyl alcohol for stabilization of silver nanoparticles
-PVA= without polyvinyl alcohol

The antimicrobial activity in terms of inhibition zone significantly varied with test microbes and the type of the extracts. This differential antimicrobial activity of silver nanoparticles can be attributed to their differential sizes and shape: the antimicrobial activity increases with decreasing size of the silver nanoparticles. This can be attributed to the fact that silver at low concentrations does not enter the fungus cells, but it is adsorbed onto the bacterial surface just as silver tends to adsorb to other surfaces, thus silver ions immobilize dehydrogenation because respiration occurs across the cell membrane in bacteria rather than across the mitochondrial membrane as in eukaryotic cells of fungi (Nabikan *et al.* 2010). Ghosh *et al*. (2012) reported successful synthesis of silver nanoparticles using *D. bulbifera* tuber extract followed by an estimation of its synergistic potential for enhancement of the antibacterial activity of broad spectrum antimicrobial agents. In another development, preparation of silver nano-particles have been reported using aqueous extract of lemon leaves (*Citrus limon*) which acts as reducing agent and encapsulating cage for the silver nanoparticles (Vankar and Shukla, 2012). These silver nanoparticles have been used for durable textile finish on cotton and silk fabrics. Remarkable antifungal activity has been observed in the treated fabrics (Vankar and Shukla, 2012). The antimicrobial activity of silver nanoparticles derived from lemon leaves showed remarkable enhancement in activity due to synergistic effect of silver and essential oil components of lemon leaves (Vankar and Shukla, 2012). This investigation demonstrated the extracellular synthesis of highly stable silver nanoparticles by biotransformation using the extracts of lemon leaves by controlled reduction of the Ag+ ion to Ag^{O} (Vankar and Shukla, 2012). Further the silver nanaparticles were used for antifungal treatment of fabrics which was tested by antifungal activity assessment of textile material by agar diffusion method against *Fusarium oxysporum* and *Alternaria brassicicola* (Vankar and Shukla, 2012). *Aloe vera* plants have been exploited for the synthesis of silver nanoparticles and gold nanotriangles (Chandran *et al.* 2006). Further tamrind leaf extract have been utilized for the synthesis of gold nanotriangles and studies their potential application in vapor sensing (Ankamwar *et al*. 2005). The mechanism for the antimicrobial action of silver ions is not properly understood; however, the effect of silver ions on microbe can be observed by the structural and morphological changes (Vankar and Shukla, 2012). The silver nanoparticles show efficient antimicrobial activity compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. Therefore, silver is ideally suited for effective control of germs, molds and fungus. Its benefit over the use of antibiotics can be used as a powerful strategy to combat the increasing spread of multidrug resistance resulting from broad use of antibiotics. Therefore, clinical efficiency of antibiotics has been compromised (Ghosh *et al*. 2012).

The antimicrobial activity of silver nanoparticles from lemon leaves showed enhancement in activity due to synergistic effect of silver and essential oil components of lemon leaves (Vankar and Shukla, 2012). Silver is inherently anti-microbial and antibacterial substance. Silver has been widely utilized for thousands of years in human history. Its applications include jewels, utensils, currency, dental alloy, photography and explosives. Among the silvers many applications, its disinfectant property is being exploited for hygienic and medicinal purposes, such as treatment of mental illness, nicotine addiction and infectious diseases like syphilis and

gonorrhea (Mukunthan *et al.* 2011). It is also suggested that when DNA molecules are in relaxed state the replication of DNA can be effectively conducted. But when DNA is in condensed form it loses its replication ability hence, when the silver ions penetrate inside the microbial cell the DNA molecules turns into condensed form and loses its replication ability leading to cell death (Vankar and Shukla, 2012). In another report, it has been stated that heavy metals react with proteins by getting attached with thiol group and the proteins get inactivated (Vankar and Shukla, 2012). Further silver at low concentrations does not enter cells, but it is adsorbed onto the bacterial surface just as silver tends to adsorb to other surfaces. Thus, silver ions resist dehydrogenation because respiration occurs across the cell membrane in bacteria rather than across the mitochondrial membrane as in eukaryotic cells. Therefore, silver nanoparticles and antibiotics kill the bacteria with different mechanisms. Thus, synergistic effect can act as a powerful tool against resistant microorganisms. A bonding reaction between antibiotics and silver nanoparticles by chelation ultimately increases the concentration of antimicrobial agents at specific points on the cell membrane. This may be attributed due to the selective approach of silver nanoparticles towards the cell membrane that consists of phospholipids and glycoprotein (Ghosh *et al.* 2012). Therefore, silver nanoparticles facilitate the transport of antibiotics to the cell surface acting as a drug carrier. As the silver nanoparticles bind to the sulfur-containing proteins of the bacterial cell membrane, the permeability of the membrane increases, facilitating enhanced infilteration of the antibiotics in the cell (Ghosh *et al.* 2012). Recently it was also shown that silver nanoparticles are composed of silver (0) atoms. Silver nanoparticles are larger in size than a silver ion, which makes them react with more molecules, leading to more antimicrobial activity (Ghosh *et al.* 2012; Mukunthan *et al.* 2011).

In another report, it was found that major mechanism through which silver nanoparticles manifest antibacterial properties was either by anchoring or penetrating the bacterial cell wall, and modulating cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues (Mukunthan *et al.* 2011). By incorporating silver of nanoscales into textiles, the manufacturers can make materials that use small amount of silver to kill the microbes present on the surface of the clothing material, thus can be treated with silver nanoparticles to help prevent spoilage rising from microbial growth in damp areas (Vankar and Shukla, 2012). Polyphenols like tannic acids are the plant-derived compounds, which are efficient reducing agent in the synthesis of silver nanoparticles. It is also well known fact that proteins can bind to silver nanoparticles through either free amino groups or cysteine residues in the proteins and the surface bound proteins stabilize the silver nanoparticles during synthesis. However, silver ions or salts have only limited usefulness as antimicrobial agents for several reasons: Interfering effects of salts and discontinuous release of inadequate concentration of silver ions from the metal. In contrast, these kinds of limitations can be overcome using silver nanoparticles as these are highly reactive species because of larger surface area (Nabikhan *et al*. 2010). Moreover, silver nanoparticles have well developed surface chemistry and chemical stability, and are of appropriate size. They are able to maintain a constant shape and size in solution. Thus silver nanoparticles are a good choice as inorganic nanomaterials in combination with various classes of antibiotics for use against pathogenic microorganisms. Shanker *et al*. (2003) demonstrated the rapid synthesis of stable

silver nanoparticles in high concentration using proteins/enzymes extracted from *P. graveolens* leaf. The reduction of metal ions and stabilization of the silver nanoparticles is believed to occur by an enzymatic process (Shankar *et al*. 2003). Therefore, synthesis of silver nanoparticles from plant is evolved into an important branch of nanotechnology. The approach appears to be cost effective alternative to conventional methods of assembling silver nanoparticles.

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