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EVALUATION OF EFFICIENCY OF DIFFERENT PLANTS TO DEVELOP SILVER NANOPARTICLES

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Abstract

Nanotechnology involving nanoparticles is a remarkably important field of present time having various industrial and medicinal uses. Biological production of nanoparticles using plants or microorganisms being eco-friendly is the remotely adopted way to produce these particles. In present study plants used were black pepper (*Piper nigrum*), cloves (*Syzygium aromaticum*), red chili (*Capsicum annuum*), cinnamon (*Cinnamomum zeylanicum*), black cardamom (*Amomum subulatum*) and tea (*Camellia sinensis*) for development of nanoparticles using water as solvent. After being treated with silver nitrate, color of all plants extracts changed, indicating the formation of silver nanoparticles. The amount and efficiency of these nanoparticles was evaluated by testing their antibacterial activity against two bacterial strains named *Bordetella petrussis* (human pathogen) and *Xanthomonas axonopodis* (plant pathogen). It was found that silver nanoparticles showed larger inhibition zones for both bacterial species as compared to their relative pure plant extracts. Results also revealed that for bacterial specie *Bordetella petrussis*, the most activity was shown by silver nanoparticles of clove extract with an inhibition zone of 32mm. For the bacterial strain *Xanthomonas axonopodis* the largest inhibition zone of 23.4mm was formed by silver nanoparticles of black cardamom extract showing the most effective antibacterial activity. It was concluded that antibacterial drugs can be formulated from these nanoparticles with maximum efficiency.

Keywords: Nanotechnology, Silver nanoparticles, *Piper nigrum, Syzygium aromaticum, Camellia sinensis*

Introduction

Nanoparticles are very small probes that allow us to spy at the cellular machinery without introducing too much interference. Understanding of biological processes on the nanoscale level is a strong driving force behind development of nanotechnology (Salata, 2004). Nanoparticles are materials with overall dimensions in the nanoscale, i.e., under 100 nm. They have a number of properties that distinguish them from bulk materials simply by virtue of their size, such as chemical reactivity, energy absorption, and biological mobility. Nanoparticles are also referred to as "zero-dimensional" nanomaterials. This definition arises from the fact that all of their dimensions are in the nanoscale (Murthy, 2007).

In 21st century, nanotechnology has emerged as a rapidly growing field with numerous applications in science and technology. Contrary to alternative physical and chemical methods employing toxic chemicals which are unacceptable for medical applications, use of biological organisms such as microorganisms, plant extract, tissue-cultured plants or plant biomass could be used as an alternative for the production of nanoparticles in an eco-friendly manner. Physico-chemically synthesized metal nanoparticles have been used in cancer therapy, the targeted delivery of drugs, molecular imaging, wastewater treatment, catalysis, biosensor development, fuel elements, coatings, cosmetics and as antiseptics. It is significant to note that nanoparticles synthesized in plant extracts already have a functionalized surface that can contain (depending on reaction conditions) the organic ligands, proteins, polysaccharides, and polyatomic alcohols that are absent in nanoparticles synthesized using physical and chemical methods (Hegazy *et al.,* 2015; Elumalai *et al*., 2010).

The presence of these biological components promotes, as is known, an increase in the stability of the particles also facilitate subsequent attachment of functional molecules, such as antibodies or DNA, to nanoparticles. The medicinal applications of nanoparticles include fluorescent biological labels, drug and gene delivery, bio detection of pathogens, detection of proteins, probing of DNA structure, tissue engineering, tumor destruction via heating (hyperthermia), separation and purification of biological molecules and cells, MRI contrast enhancement, phagokinetic studies. Recent developments include tissue engineering, cancer therapy, multicolour optical coding for biological assays, manipulation of cells and biomolecules, protein detection (Salata, 2004; Sintubin *et al*., 2012).

The plants understudy for their potential of producing nanoparticles belong to different classes and families, which are black pepper (*Piper nigrum*), cloves (*Syzygium aromaticum*), red chili (*Capsicum annuum*), cinnamon (*Cinnamomum zeylanicum*), black cardamom (*Amomum subulatum)* and tea (*Camellia sinensis*).

Black pepper (*P. nigrum*) has a limited usage in medicine as a carminative and as a stimulant of gastric secretions (Garg, 2012). Cloves (*S. aromaticum*) are used for dental emergencies. Cloves are used as a carminative, to increase hydrochloric acid in stomach and to improve peristalsis (Subhankari & Nayak, 2013). *C. annum* fruits are an excellent source of natural, micronutrient antioxidants (vitamins C and E and carotenoids) which appear to be critically important in preventing or reducing chronic and age-related diseases (Palevitch & Craker, 1996). Cinnamon is being used traditionally for the treatment of inflammation, cough, toothache, antiseptics expectorant, and some fungal infection like candidaisis (Rastogi & Mehrotra, 2002). Black cardamom (*A. subulatum*) has pharmacognostic properties such as analgesic, antimicrobial, anti-inflammatory, antioxidant, antiulcer, cardiac stimulant, carminative, diuretic and antiulcer (Bisht *et al*., 2011). Tea have polyphenols which can decrease the risk factor of specific type of cancers by inducing phase I and phase II metabolic enzymes that

increase the formation and excretion of detoxified metabolites of carcinogens.

A comparative study is conducted in order to understand and distinguish this ability of different plants to synthesize nanoparticles. These biosynthesized nanoparticles are then being studied for their antibacterial activity.

Materials and Methods

The plant materials i.e., powdered form of black pepper, cloves, red chili, cinnamon, black cardamom and tea were purchased from the local market. Finely powdered plant materials was weighed accurately each time for each plant material and measured quantity of water (100mL) was added in beaker. Each mixture was allowed to macerate on hot plate for 6 hours in the solvent to get the extract. The extract was filtered with whatmann No. 1 filter paper. The filtered extract was then separated into five concentrations of 5mL, 10mL, 15mL, 20mL and 25mL separately. To make the reagent, 0.68 grams of silver nitrate ($AgNO_3$) was dissolved in 100mL of water and 10mL of this reagent was added into each concentration of plant extract and development of nanoparticles was observed over a period of 2 hours by noticing the change in color. Pictures were taken every hour to make the record of the change. After 2 hours the solutions were taken in eppendorfs to centrifuge. Solutions were centrifuged for 10 minutes at 10000 rpm. This was done for the purpose to separate nanoparticles formed in the form of pallets, which were then stored in refrigerator for further use.

Different techniques (UV Visible Spectroscopy, scanning electron microscopy and energy dispersive X ray spectroscopy) were used for characterization of NPs. UV analysis was done for each system to check reduction of silver ions. This technique was used as a tool to determine the formations of nanoparticles. Formation of nanoparticles was monitored at regular intervals by taking its spectrum between 300-700 nm by using spectrophotometer.

Scanning electron microscopy was used to determine the surface morphology of developed nanoparticles. Prepared purified samples of nanoparticles were subject to SEM for analysis. Crystalline nature and composition of NPs was evaluated by EDX analysis. Same instrument was used for SEM and EDX analysis.

The nanoparticles formed were tested to determine their antibacterial activity. Agar well diffusion method was used to evaluate the antibacterial activity against two bacterial species *Bordetella perlutris* (Human pathogen) and *Xanthomonas axonopodis* (plant pathogen) (Salar *et al*., 2015). Different concentrations of NPs were used to determine their antibacterial activity. Plant extract was used as positive control and $AgNO_3$ was used as negative control. Zone of inhibition were measured in millimeter after 24 hours at 37 ^oC. The data thus generated was analyzed through one way analysis of variance (ANOVA), and statistical computer software 'COSTAT' was used for this purpose. Duncan's Multiple Range test was applied as post hoc test to find the significance at 5% level of significance.

Results

In present study, five concentrations of each plant extract were taken with 10 mL of $AgNO_3$ and were observed for the color change to indicate nanoparticles formation. The experiment was setup for 2 hours, to allow silver nitrate to react with plant extract to form nanoparticles. It resulted in color change from dark brown to blackish brown in the different concentrations of Plant extracts which indicated the formation of silver nanoparticles (Fig 1(a-f).

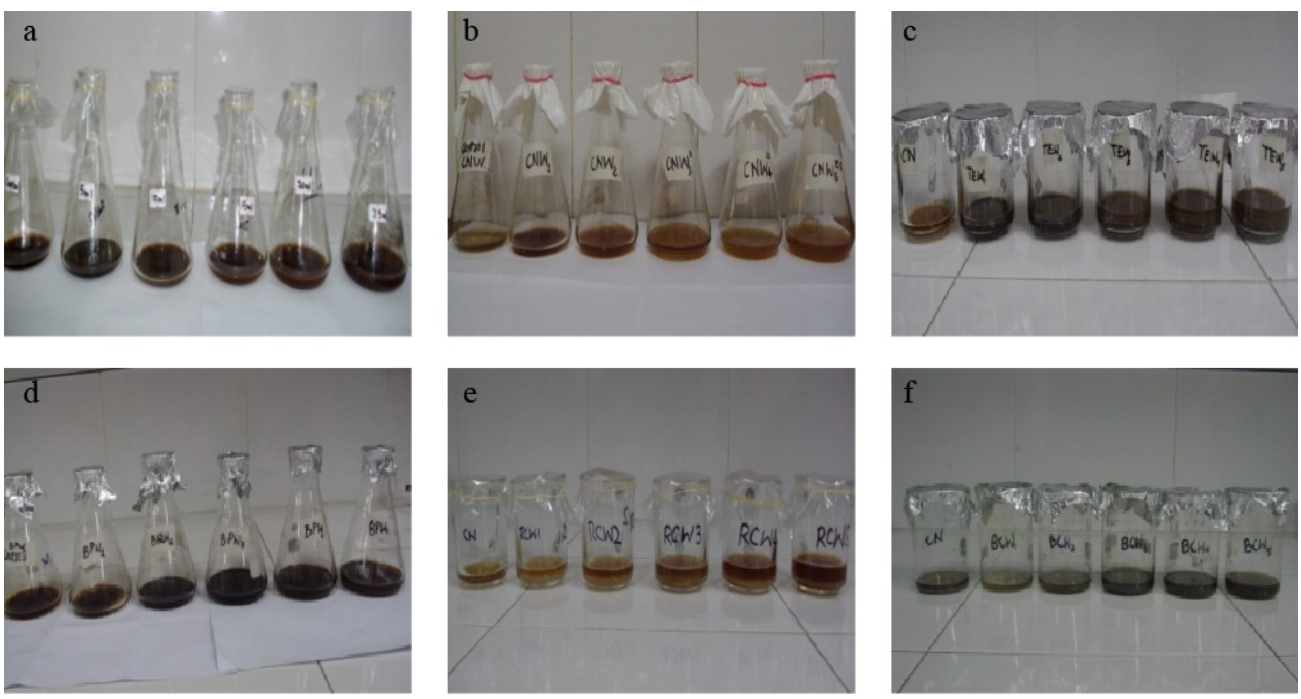

Fig. 1. After 2 hours of Reaction of $AgNO_3$ and Plant extract a) clove extracts; b) cinnamon extracts; c) tea extracts; d) black pepper extracts; e) red chili extracts; black cardamom extracts

UV-Visible spectrophotometeric analysis also showed a shift of λ_{max} from higher wavelength to lower wavelength. The sharp bands of silver nanoparticles were observed around 400 nm - 500 nm in all the synthesized nanoparticles (Fig. 2). Dhiman *et al*., (2014) synthesized silver nanoparticles by green synthesis technique using *Elettaria cardamom* seed extract as reducing agents without use of chemicals. In UV-Vis spectra, the peak was obtained at 445nm which corresponds to the absorbance of silver nanoparticles. Ojha *et al.,* (2012) reported the maximum absorbance of silver nanoparticles reduced by Clove extract at 417 nm by using UV visible spectrophotometer. The active biomolecusles present in plants are responsible for the bioreduction of $AgNO_3$ (Ag^+) leading to the formation and capping of AgNPs (Ag^0). For example one molecule of eugenol (in clove extract) releases two electrons and these two electrons will be taken by 2 Ag^+ ions and these will get reduced to 2 Ag^0. They all confirmed the synthesis of AgNPs by the appearance of change in color (Kaur *et al*., 2013). Reaction mixture of plant extract and $AgNO_3$ solution changes the color by adding various concentrations of metal ions. These color change arose because of the excitation of surface plasmon vibrations in the silver nanoparticles. It showed a change from yellow to dark brown in color. The dark brown color of silver colloid was accepted to surface plasmon resonance (SPR) arising due to the group of free conduction electrons induced by an interacting electromegnatic field (Elumalai *et al.,* 2010).

Nanoparticles were also characterized by using SEM analysis, which provides information about surface morphology and size of developed nanoparticles. It was renowned that particles were spherical in shape but some particles with other shapes were also observed. Minimum size of NPs observed was 82.87 nm (Fig. 3). Our results had good resemblance with Arunachalam *et al*., 2013.

EDX analysis provides information about the presence and crystalline nature of synthesized nanoparticles. Optical absorption peak observed at 3 keV due to surface Plasmon resonance which depicts that nanoparticle contains silver. Beside silver, peaks of carbon, oxygen and nitrogen may also observe due to presence of bound biomolecules (Fig. 4).

Further in present study, antibacterial activity was performed to find out the extent and efficiency of the synthesized nanoparticles against bacterial strains. For this purpose two gram negative bacterial strains were used, namely *Bordetella pertussis* and *Xanthomonas axonopodis*; pathogens causing whooping cough in humans and citrus canker in citrus family respectively. Values of inhibition zones against *Bordetella pertussis* ranged from 12.5 mm to 32mm (Table 1). It was found that the largest inhibition zone against bacterial strain *B. pertussis* was formed by lowest concentration of clove extract (5mL) AgNPs, which was 32mm (Plate 1). For the bacterial strain *Xanthomonas axonopodis*, the values of Inhibition zones varied between 12.5 and 23.4mm (Table 1) and the largest inhibition zone was formed by the highest concentration of black cardamom AgNPs extract (25mL) which was noted to be 23.4mm (Plate 2).

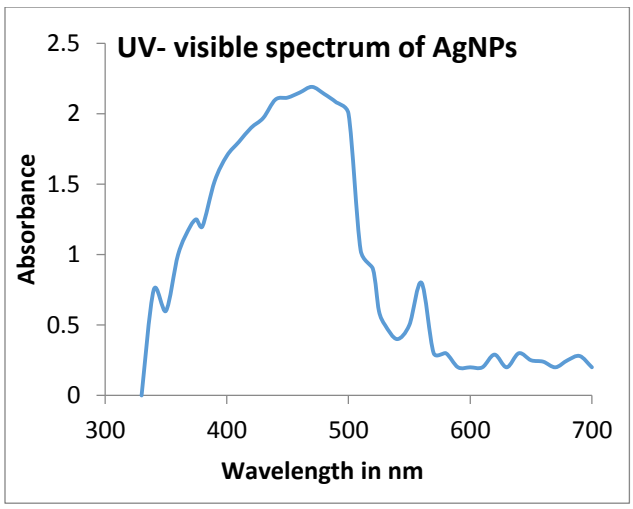

Fig. 2. UV- Vis spectrum of treated solutions

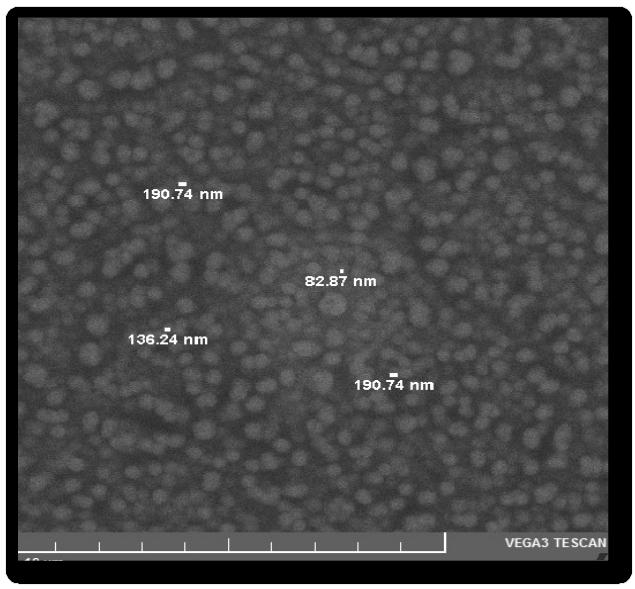

Fig. 3. SEM micrograph of silver nanoparticles by clove extract.

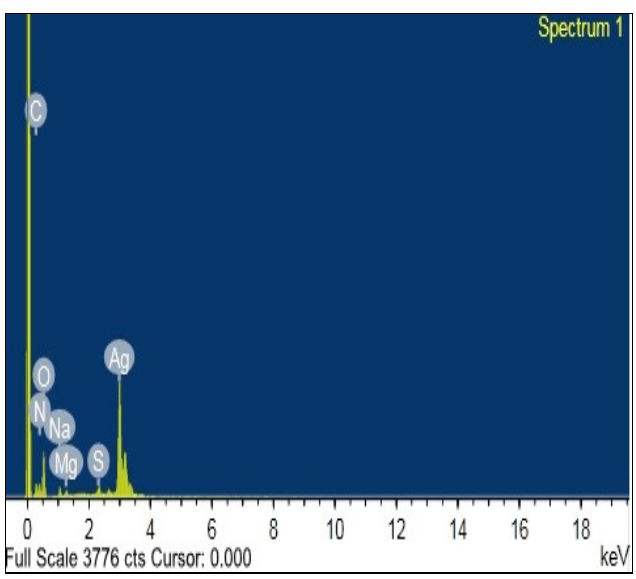

Fig. 4. EDX pattern of material containing nanoparticles by Cinamon extract

Table 1. Inhibition zones formed by different plant extracts and their respective AgNPs against *Bordetella pertussis* and *Xanthomonas axonopodis.*

Sr. #	Plant material	Inhibition zones (in mm) against	
		Bordetella pertussis	***Xanthomonas axonopodis***
1	Clove extract	$24.53^{d}\pm0.75$	$16.06^{d}\pm0.6$
	AgNPs of 5ml clove extract	$32.00^{a}\pm0.55$	$18.60^{bc}\pm0.6$
	AgNPs of 30ml clove extract	$29.00^{b}\pm0.70$	$15.50^{e}\pm0.45$
2	Cinnamon extract	$20.03^{ef}\pm0.65$	$13.50^{g}\pm0.43$
	AgNPs of 5ml Cinnamon extract	$15.40^{i}\pm0.45$	$17.50^{c}\pm0.55$
	AgNPs of 30ml Cinnamon extract	$14.10^{j}\pm0.37$	$17.10^{c}\pm0.32$
3	Tea extract	$17.50^{g}\pm0.95$	$16.60^{d}\pm0.66$
	AgNPs of 5ml tea extract	$16.50^{h}\pm0.49$	$14.03^{fg}\pm0.52$
	AgNPs of 30ml tea extract	$19.40^{f}\pm0.43$	$12.50^{h}\pm0.10$
4	Black pepper extract	$18.50^{fg}\pm0.85$	$18.03^{bc}\pm1.04$
	AgNPs of 5ml Black pepper extract	$13.40^{jk}\pm0.35$	$16.30^{d}\pm0.93$
	AgNPs of 30ml Black pepper extract	$12.50^{k}\pm0.45$	$14.03^{fg}\pm1.11$
5	Red chilli extract	$27.50^{c}\pm1.01$	$14.50^{f}\pm0.34$
	AgNPs of 5ml Red chilli extract	$16.00^{h}\pm1.00$	$17.00^{c}\pm0.73$
	AgNPs of 30ml Red chilli extract	$13.50^{jk}\pm0.90$	$12.50^{h}\pm0.09$
6	Black cardamon extract	$25.00^{d}\pm0.54$	$15.50^{e}\pm0.93$
	AgNPs of 5ml Black cardamon extract	$15.20^{i}\pm0.99$	$19.10^{b}\pm1.03$
	AgNPs of 30ml Black cardamon extract	$16.40^{h}\pm0.58$	$23.40^{a}\pm1.00$

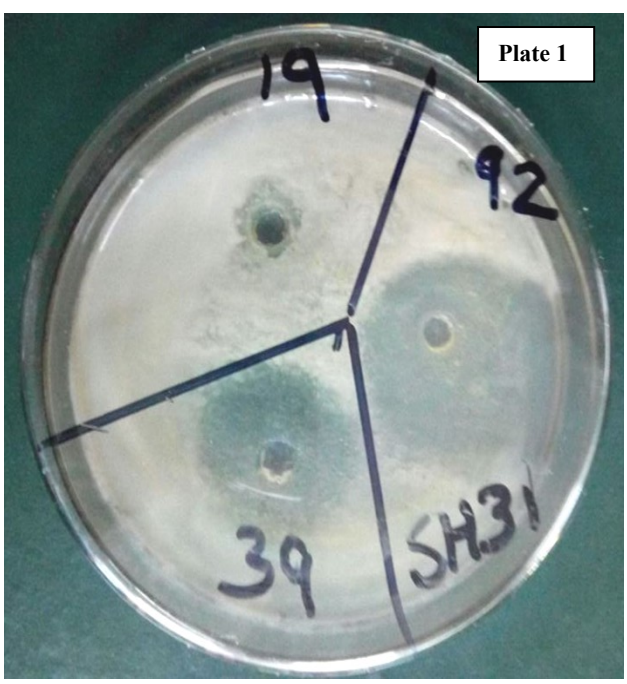

Plate 1. Antibacterial activity of Clove AgNPs against *Bordetella pertussis,* 19($AgNO_3$), 39 (plant extract), 92 (AgNPs).

Plate 2. Antibacterial activity of black cardamon AgNPs against *Xanthomonas axonopodis,* 31($AgNO_3$), 32(plant extract), 33(AgNPs)

Ojha *et al*., (2012) studied the synthesis of nanoparticles by clove and their antibacterial activity which was tested against bacterial strains *E.coli, S.aureus, S.typhi*. It resulted in appearance of inhibition zones of 15mm, 12mm and 18mm respectively. Jobitha *et al*., (2012) examined the antibacterial activity of the silver nanoparticles against *Bacillus subtilis* and *Klebsiella planticolla* by using standard zone of inhibition (ZOI) microbiology assay. The Nanoparticles showed inhibition zone against all the studied bacteria. Maximum zone of inhibition was found to be 23-25 mm for *Bacillus subtilis* and *Klebsiella planticolla.*

Antibacterial activity of silver nanoparticles was found to be dependent on the size of silver particles. AgNPs bind with cytoplasmic membrane and killed the bacterial cell. Electrostatic attraction of silver nanoparticles causes damage to bacterial cell membrane to the formation of pits on the surface, and these structural changes take place due to cell expiration. The prokaryotic bacteria have a mesosome cell organelle, and they are present in the inside of plasma membrane produced enzymes as well as major function of cellular respiration, DNA replication, cell division and increased the surface area of the bacterial cell membrane. AgNPs interfere with the bacterial cell membrane and bind with mesosome cell organelle and after that reduce the meosomal function. AgNPs interact with thiol groups in protein which induced the inactivation of the bacterial protein synthesis as well as DNA replication. Similarly, oxygen associates with silver and reacts with the sulfhydral (-S-H) groups on cell wall to remove the hydrogen atoms causing the sulfur atoms to form an R-S-S-R bond, blocked the respiration, and causing the lethal effect of bacterial cells. AgNPs naturally interact with the membrane of bacteria and disrupt the membrane integrity; silver ions bind to sulfur, oxygen, and nitrogen of essential biological molecules and inhibit bacterial growth (Gopinath *et al*., 2013).

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