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Research Article

SYNTHESIS OF SILVER NANO PARTICLES FROM CURRY LEAF (*MURRAYA KOENIGII*) EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT
Nanoparticles have a promising action in a variety of areas and fields. Plant based nanoparticle synthesis become advantageous over chemical synthesis. The curry leaf extract was prepared from fresh curry leaves by boiling it for 3 minutes, 5 minutes and 10 minutes separately. Freshly prepared leaf extract was added to 1mM silver nitrate solution and the reaction takes place at room temperature which leads to the synthesis of silver nanoparticals. The synthesized silver nanoparticles were characterized by UV-Vis spectrometry, SEM and EDX measurements. The UV-Vis spectra of silver nanoparticles formed in the reaction media has absorbance peak at 435 nm. Silver nanoparticles with an average size of 146 nm was synthesized. The silver nanoparticles showed antibacterial activity against *Bacillus species, E.coli, Staphylococcus species*, *Klebsiella species* and *Micrococcus species*. It has been demonstrated that curry leaf extract is capable of producing silver nanoparticles that shows good stability in solution, under the UV-Visible wavelength nanoparticles shown quiet good surface Plasmon resonance behavior. The synthesized silver nanoparticles were characterized by UV-Vis, SEM and EDX measurements. This green synthesis method is alternative to chemical method, since it is cheap, pollutant free and eco-friendly. The results showed that Curry leaf plays an important role in the reduction and stabilization of silver to silver nanoparticles. Further, these synthesized silver nanoparticles from Curry leaf shows antibacterial activity on both Gram positive and Gram negative bacteria.

Keywords: *Nanoparticles; Curry Leaves; Antibacterial Activity; SEM; EDX*

INTRODUCTION
Engineered nanomaterials in the range of 1-100 nm in size possess novel physical and chemical properties that have been used to create unique devices. The distinctive quantum properties of nanomaterials strongly influence their physico-chemical properties, conferring an electrical, optical and magnetic property which is not present in their corresponding bulk counterparts. Biological fictionalization of nanomaterials has come to be of significant interest in the recent years due to the potential for developing sensitive imaging and signaling pathway detection systems (Oberdorster *et al.*, 2005).

The bio-fictionalization of nanomaterials' surfaces can result in aqueous soluble materials which can be further modified with active molecules making them compatible, active, specific capture field agents and useful in biological systems.

While all of this seems promising it is still unknown how engineered nanomaterials of different size, structure, and geometries interact with cells and the molecular events involved in nanoparticle-membrane receptor binding, endocytosis and subsequent signaling activation.

Silver's (Ag) extremely small size and large surface area allow for different properties than the bulk material.

Nano-silver possesses a high extinction coefficient, high surface plasmon resonance and anti-microbial properties which are less toxic then the bulk form. In the future, nano-silver's high surface plasmon resonance has a possibility for many color based biosensor.

The high surface plasmon resonance is beneficial for sensors, because silver has a typical excitation wavelength and when something disrupts the surface the excitation wavelength changes and we are able to detect what has been bound. Currently silver nanomaterials have a variety of uses in everyday (Lesniak *et al.*, 2005) consumer's lives such as: nanosilver infused storage containers, nanosilver coated surfaces of medical devices to reduce hospital related infections, bandages, footwear and countless household items which claim to be anti-microbial. Nanosilver is a popular additive in many health products due to its unique ability to fight infectious diseases, slow the growth of bacterium, mold and germs.

While all of these properties appear to make nanosilver the new "wonder-drug" of the nanotechnology world. New applications of nanoparticles and nanomaterials are emerging rapidly.

Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics, antimicrobials and therapeutics, catalysis and micro-electronics. However, there is still need for economic, commercially viable as well environmentally clean synthesis route to synthesize silver nanoparticles.

A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted process, electrochemical, sonochemical, microwave assisted process and recently via green chemistry route.

With the development of new chemical or physical methods, the concern for environmental contamination are also heightened as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts.

Thus, there is a need for 'green chemistry' that includes a clean, nontoxic and environment-friendly method of nanoparticle synthesis. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for inspiration.

Biological methods of synthesis have paved way for the "greener synthesis" of nanoparticles. Due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nanotechnological applications. The use of environmentally benign materials like plant extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol (Bhainsa *et al.*, 2006).

Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications.

Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, and safe for human therapeutic use (Kumar *et al.*, 2009). Easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.

Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds. Silver nano particles are reported to have many therapeutic uses. T

here are reported to possess anti-viral (Jayaseelan *et al.*, 2011), antibacterial (Sukirtha *et al.*, 2012), antifungal (Gengan *et al.*, 2013), anti-parasitic (Wijnhoven *et al.*, 2009; Perelshtein *et al.*, 2008), larvicidal activity (Li *et al.*, 2011) and anticancer (Durán *et al.*, 2007; Ravindra *et al.*, 2010) properties. Due to strong antibacterial property silver nanoparticles are used in clothing, food industry, sunscreens, cosmetics and many household appliances (Vivekanandhan *et al.*, 2010). Few studies have showed that silver nanoparticles kill fungal spores by destructing the membrane integrity.

Nanotechnology has a wide range of applications in the fields of biology, medicine, optical, electrical, mechanical and optoelectronics.

Silver nanoparticles have also been used for a number of applications such as nonlinear optics, spectrally selective coating for solar energy absorption, biolabelling and antibacterial activities.Silver nanoparticles have shown promise against gram positive S. aureus.

Metal nanoparticle embedded paints have good antibacterial activity (Kumar *et al.,* 2008). Current research is going on regarding the use of magnetic nanoparticles in the detoxification of military personnel in case of biochemical warfare (Salata, 2005).

Photocatalytic activity of nanoparticles has been utilized to develop self- cleaning tiles, windows and anti-fogging car mirrors. The high reactivity of Titania nanoparticles when illuminated by UV light have been used for bactericidal purposes in filters.

An important opportunity for nanoparticles in the area of computers and electronics is their use in a special polishing process, chemical-mechanical polishing or chemical-mechanical planarization (CMP), which is critical to semiconductor chip fabrication. The interaction of silver nanoparticles with HIV I, it was found that the silver nanoparticles inhibited the binding of the virus to the host cells in vitro (Elechiguerra *et al.*, 2005).

Magnetic nanoparticles are also used in targeted therapy where a cytotoxic drug is attached to a biocompatible magnetic nanoparticle for tumor cell treatment (Pankhurst *et al.,* 2003). Porous nanoparticles have also been used in cancer therapy. Alivisatos (2001) reported the presence of inorganic crystals in magnetotactic bacteria.

This principle can be extended to develop a process for the removal of heavy metals from waste water. Bioremediation of radioactive wastes from nuclear power plants and nuclear weapon production, such as uranium has been achieved using nanoparticles (Duran *et al.*, 2007). Biominerals have been formulated by using several bacteria such as *Pseudomonas aeruginosa*, *E.coli* and *Citrobacter sp*. Metal sulfide microcrystallites were formulated using S. pombe which could function as quantum semiconductor microcrystallite.

These crystals have properties like optical absorption, photosynthetic and electron transfer. Magnetosome particles isolated from magnetotactic bacteria have been used as a carrier for the immobilization of bioactive substances such as enzymes, DNA, RNA and antibodies (Mohanpuria *et al.,* 2008). Gold nanoparticles are widely used in various fields such as photonics, catalysis, electronics and biomedicine due to their unique properties.

Curry leaves has recently been found to be a potent antioxidant due to high concentrations of carbazoles, a water soluble heterocyclic compound (Rai *et al.,* 2008) which is responsible for the reduction and stabilization of metal ions.

The effective reduction of silver ions into AgNPs by curry leaf extract has motivated to extend the research work for the fabrication of AgNP impregnated microcrystalline cellulose (MCC) as a functional fillers. A promising result was obtained for the *Murraya koenigii* extract that actively reduces the silver ions in to silver nanoparticles.

Recently, Philip *et al.,* (2011) also reported the rapid synthesis of silver and gold nanoparticles using *Murraya koenigii* leaf extract (Christensen *et al.,* 2011). Curry leaves are well known as a spice but has also been used in traditional medicine as a treatment for a variety of ailments. Detailed investigation is necessary in order to extend the reduction mechanism of *Murraya koenigii* leaf extract for further applications.

Given lacking qualitative and quantitative data on various nanoparticles using biological agents, objective of this study were to (1) synthesize the silver nanoparticles using aqueous extract of curry leaves, *Murraya koenigii* (2) characterization of silver nanoparticles by using UV-Vis spectroscopy, SEM-EDX (3) analyze antimicrobial properties against Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

Sample Collection

Curry leaves were collected from Karimkunnam, Kottayam district of Kerala state, India. The fresh leaves were collected in poly ethylene zipper bags, later washed two times with distilled water. The plant materials were thoroughly washed with distilled water and fresh weight were determined.

The samples are then oven dried (KOA4, KEMI lab equipments, Ernakulam, India) at 60°C for 24 h. The dried samples were powdered using a waring blender (Magic V2, Preethi Kitchen Appliances Pvt Ltd, Chennai, India) and stored in air-tight polyethylene bottles until further analysis.

Extraction Method

Curry leaf extract was prepared with 10 g of fresh curry leaves taken in 3 separate beakers each. It was thoroughly washed with tap water and then with distilled water for at least 2 times and cut into small pieces. The chopped leaves were boiled in 75ml of distilled water for 3 minutes in 1st beaker, for 5 minutes in 2nd beaker and for 10 minutes in 3rd beaker separately.

The leaf broth was then cooled and filtered. It was then stored at 4°C after covering the beaker with aluminum foil for further use. The obtained curry leaf extract which appeared light green in color was stored 4°C for further use.

Synthesis of Sliver Nanoparticiles

Stock solution was prepared by dissolving 1mM sliver nitrate (AgNO3; Merck, Mumbai, India) and volume made up to 250 ml with distilled water. 5 ml of curry leaf extract of different concentration (3 min boiled, 5 min boiled and 10 min boiled separately) was added to 100 ml of 1mM AgNO3 solution and allowed to react at room temperature.

Test Microorganisms

The organisms used comprise of two gram-negative organisms (*Klebsiella* and *E.coli*) and three gram-positive organisms (*Staphylococcus*, *Bacillus* and *Micrococcus*). The test organisms were obtained from the Department of Biotechnology, Mar Augusthinose College, Ramapuram.

Escherichia Coli

These are gram negative, facultative or anaerobic rods commonly found in the lower intestine of warm blooded organisms. The organisms are relatively heat sensitive and are readily destroyed at high temperature. The optimal temperature for growth is 37°C. E. coli is responsible for intestinal tract infection and diarrhea.

Staphylococcus Species

These are spherical in shape, non-motile, gram positive and facultative anaerobes which are positive in the catalase test. The coagulase test is used to broadly demarcate Staphylococcus species into coagulase positive and coagulase negative species. *Staphylococus* species grow readily on ordinary media with a temperature range of 10 to 40°C, the optimum being 37°C and a pH of 7.4-7.6. *Staphylococus aureus* strains have emerged resistant to the penicillinase-stable penicillins (cloxacillin, dicloxacillin, methicillin, nafcillin, and oxacillin).

Klebsiella Species

The genus Klebsiella consists of non-motile, capsulated rods that grow well on ordinary media forming large, dome shaped, mucoid colonies of varying degrees of stickiness. *Klebsiella* species are widely distributed in nature, occurring both as commensals in the intestines and as saprophytes in soil and water. *Klebsiella* species can cause diseases like pneumoniea, ozena, rhinoscleroma etc.

Micrococcus Species

These are positive cocci which occur mostly in pairs, tetrads or irregular clusters. They are catalase and oxidase positive. They are aerobic with a strictly respiratory metabolism. They are parasitic on mammalian skin and are ordinarily non-pathogenic.

Bacillus Species

The genus Bacillus consists of anaerobic bacilli forming heat resistant spores. They are gram positive but tend to be decolourised easily so as to appear gram variable, or even frankly gram negative. They are generally motile with peritrichous flagella. *Bacillus anthracis*, the causative agent of anthrax, is the major pathogenic species. *Bacillus cereus* can cause food borne gastroenteritis. Some species may be responsible for opportunistic infections.

Characterization of Silver Nanoparticles

UV –Vis Spectroscopy

The periodic scans of the optical absorbance between 385 and 500nm with a UV- Vis spectrophotometer (Model 118, Systronics, Mumbai, India) at a resolution of 1 nm were performed to investigate the reduction rate of silver ions by curry leaf extract. The reaction mixture was diluted 20 times and used for UV-Vis spectrophotometry. Deionised water was used to adjust the baseline.

SEM-EDX Analysis

SEM- EDX Analysis was carried out in instrument JSM 6390 with acceleration voltage 20 kV. SEM reveals information about the sample including external morphology, chemical composition and crystalline structure and orientation of materials making up the sample. SEM provides detailed high resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or back scattered electron signal. The EDX spectrum of the silver nanoparticles was performed to confirm the presence of elemental silver signal and provides quantitative compositional information.

Antibacterial Assay

Nutrient broth, nutrient agar and Muller Hinton agar plates were made according to standard microbiological protocol. Filter paper discs of approximately 6 mm diameter were soaked with 50 µl of the plant extract, AgNO3 and silver nanoparticle separately and allowed to dry at room temperature for 15 minutes. Muller Hinton Agar plates were prepared and the test microorganisms were inoculated by the spread plate method. Prepared discs were placed in the previously prepared agar plates. Each plate of every test organisms contained discs impregnated with Ag nanoparticle, leaf extract, silver nitrate solution and an antibiotic disc. The discs were pressed down to ensure complete contact with the agar surface and distributed evenly so that they were not closer than 24 mm from each other, center to center.

The agar plates were then incubated at 37ºC. After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of inhibition were measured, including the diameter of the disc where the antibiotic was used as control (NCCLS, 1997).

Statistical Analysis

The survey results were analyzed and descriptive statistics were done using SPSS 12.0 (SPSS Inc., an IBM Company, Chicago, USA) and graphs were generated using Sigma Plot 7 (Systat Software Inc., Chicago, USA).

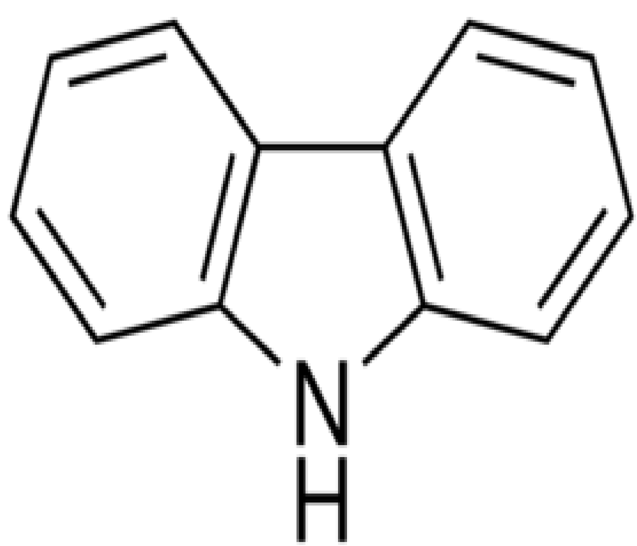

Figure 1: Structure of carbazole in curry leaves
(http://www.sigmaaldrich.com/catalog/product/sigma/c5132?lang=en&region=IN)

Figure 2: *Murraya koenigii* plant with fruits in the field (top left); young leaves (top right); young leaves collected for making extract (bottom left); extracts of fresh leaves (bottom right). Photo credit: speedyremedies.com; suburbantomato.com.

RESULTS AND DISCUSSION

Results

Synthesis of Sliver Nanoparticles

After the addition of curry leaf extract to $AgNO_3$ solution a visible color change from transparent to dark brown was observed which indicates the formation of silver nanoparticle. After 90 minutes there was no change in the intensity of color developed, which indicates the completion of reduction reaction.

Charaterization of Sliver Nanoparticles

UV Spectrometry

The UV absorption spectrum of silver nanoparticles from Curry leaf extract of different concentrations were obtained as follows

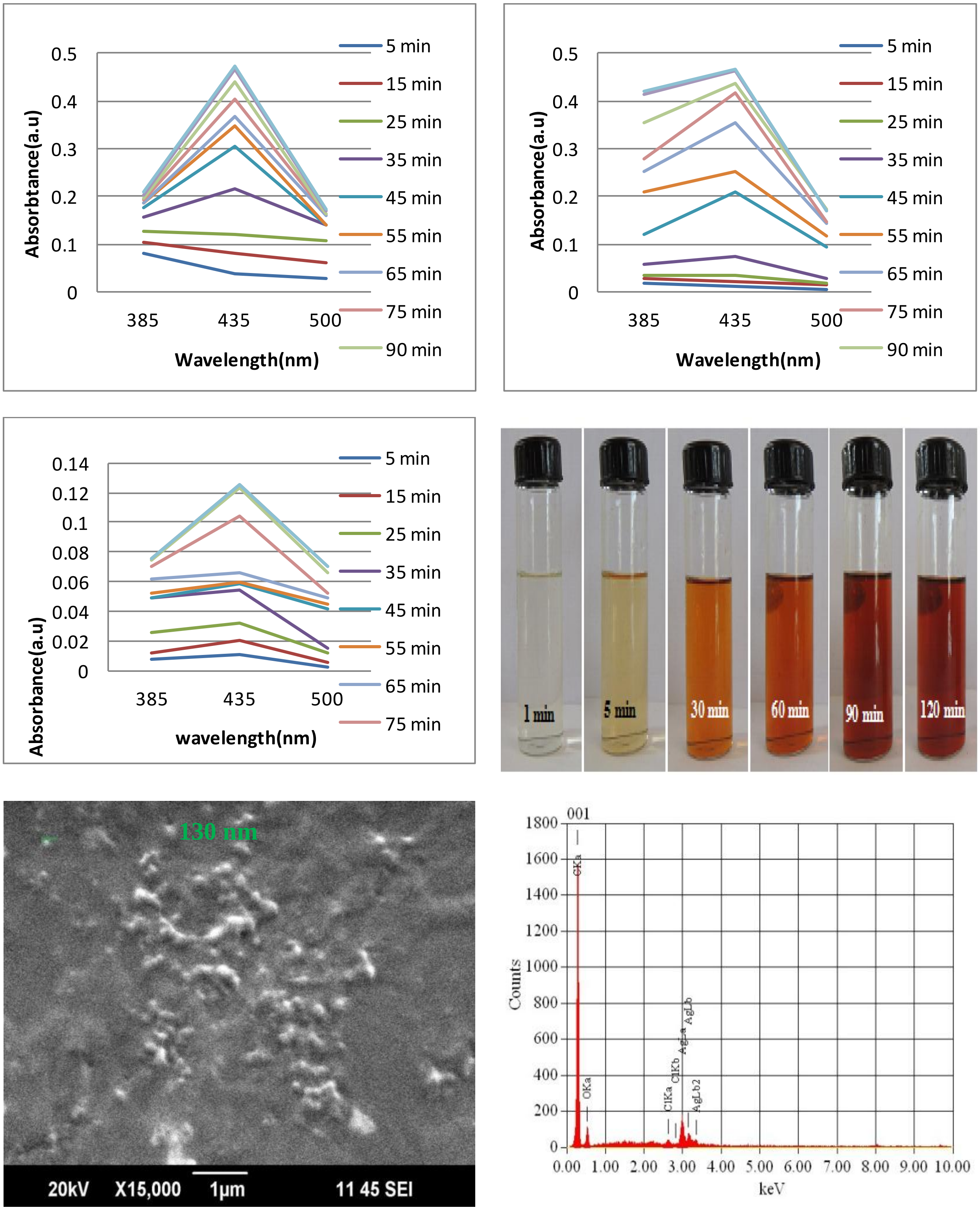

Figure 3: UV absorption spectrum of sliver nanoparticles formed from curry leaves extracted at 10 minutes (top left); 5 minutes (top right); 3 minutes (middle left); mixture of curry leaves extract and sliver nitrate over 2 hrs incubation (middle right); SEM micrograph of sliver nanoparticles (bottom right); EDX spectra (bottom left)

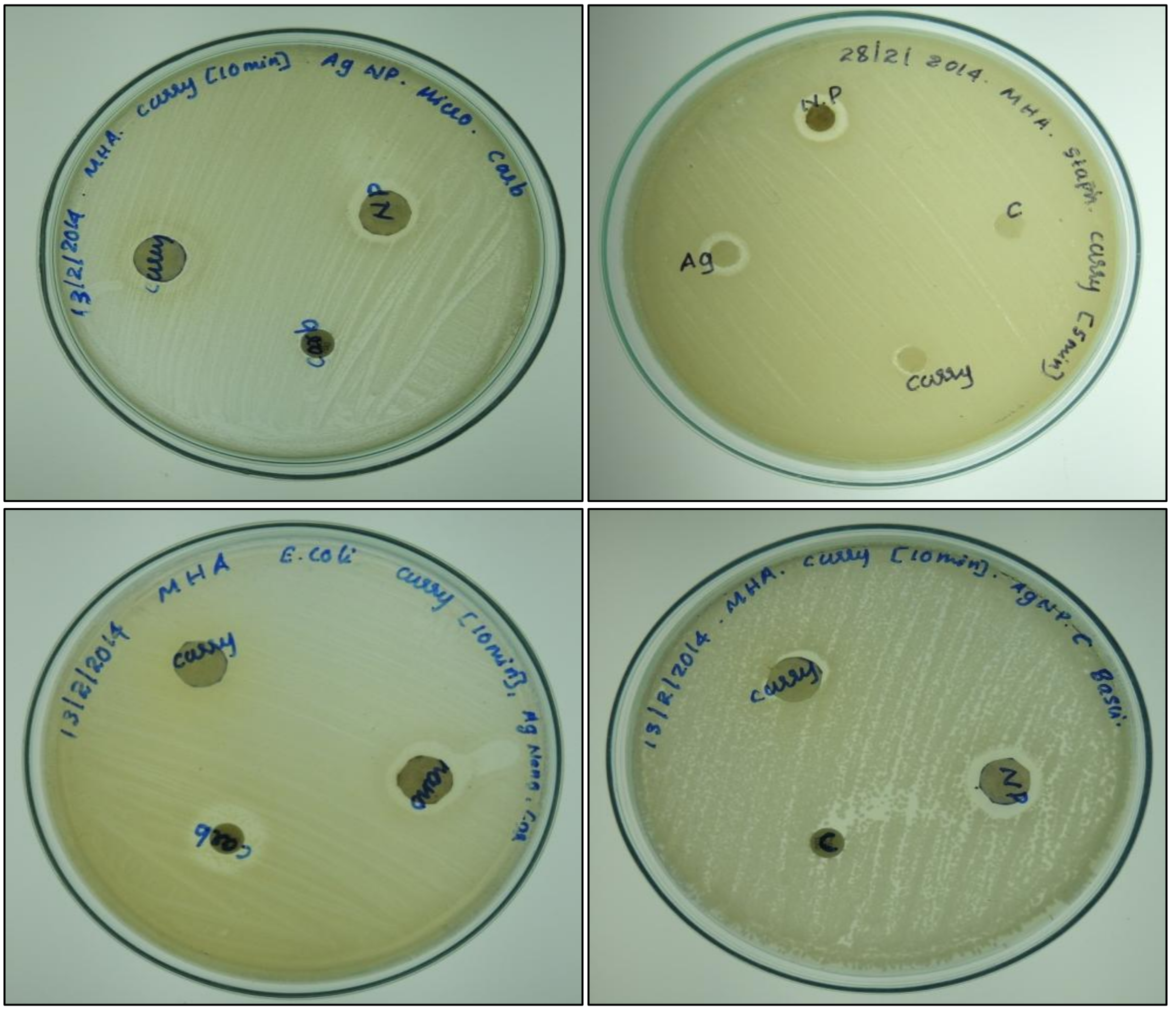

Figure 4: Antimicrobial activity of sliver nanoparticles against *Micrococus species* (top left); *Staphylococcus species* (top right); against *E.coli* (bottom left); against *Bacillus species* (bottom right)

Discussion

The curry leaves were boiled for 3 minutes, 5 minutes and 10 minutes to obtain the extract in varying concentration. The extract was filtered and stored. To synthesis silver nanoparticles, the extract was added to 1 mM silver nitrate solution and kept to reaction take place. A color change was observed from colorless to yellowish then finally to dark brown. This occurred due to the reduction of silver ions present in the solution. The reduction was due to terpeniods present in curry leaf extract. When no more color change takes place it shows the complete reduction of silver ions to silver metal. The reduced silver particles are in the range of nano size. It is generally recognized that UV spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions (Wiley *et al.*, 2006). Synthesized silver nanoparticles were characterized by UV spectrometry. The peak occurs at 435 nm (λmax) which corresponds to the absorbance of silver nanoparticles. The intensity of the peak at 435 nm was increased with time until the reduction completes. The formation of silver nanoparticles slows after 120 minutes. The maximum peak was found to be 435 nm for *Murraya koenigii.* According to Christensen *et al.,* 2011 for the curry plant, the maximum peak found at 420 nm.

SEM analysis shows that the nanoparticle formed is spherical in shape with diameter 130 nm. The EDX analysis confirmes the presence of elemental silver in the sample. The silver nanoparticle synthesized by curry leaves extract showed growth inhibitory effects against *Bacillus* species, *E.coli*, *Staphylococcus* species, *Klebsiella* species and *Micrococcus* species.

For *Murraya koenigii* the zone of inhibition showed for *Bacillus* species, *E.coli*, *Staphylocoocus* species, *Klebsiella* species and *Micrococcus* species by nanoparticles formed from, 3 minutes concentration leaf extract was 14 mm, 11 mm, 14 mm, 10 mm and 10 mm respectively; from 5 minutes concentration leaf extract was 13 mm, 10 mm, 13 mm, 11 mm and 12 mm respectively; and from 10 minutes leaf extract concentration was 13 mm, 13 mm, 15 mm, 9 mm and 14 mm respectively. The zone of inhibition showed for *Bacillus* species, *E.coli*, *Staphylocoocus* species, *Klebsiella* species and *Micrococcus* species by AgNO3 solution was 11 mm, 9 mm, 11 mm, 8 mm and 11 mm respectively. The zone of inhibition showed for *Bacillus* species, *E.coli*, *Klebsiella* species and *Micrococcus* species by antibiotic Carbenicillin was 14 mm, 12 mm, nil and 15 mm respectively. The zone of inhibition showed for *Staphylococcus* species by antibiotic Streptomycin was 15 mm.

When antibacterial activity of silver nanoparticles from 3 different concentration were observed, nanoparticles formed from 3 minutes extract concentration showed maximum activity against *Bacillus* species; for 5 minutes extract concentration nanoparticles showed maximum activity against *Klebsiella* species and for 10 minutes extract nanoparticles showed maximum activity against *E.coli*, *Staphylococcus species* and *Micrococcus* species. From the above data obtained, against all test organisms used antibacterial activity was enhanced when naoparticles were used when compared to the use of AgNO3 alone.

Antibacterial property for *Staphylococcus* species and *Klebsiella* species were increased by the use of nanoparticles when compared to antibiotics Streptomycin and Carbenicillin. For *E.coli*, antibacterial activity was greater when antibiotic Carbenicillin was used when compared to the use of silver nanoparticles. For *Staphylococcus* species and *Bacillus* species antibacterial activity are in same range when both nanoparticles and antibiotics were used separately.

Silver ions and silver salts are used as antimicrobial agents (Russel *et al.*, 1994). However, the high concentrations of silver salts restrict the use of them in present day medicine. Use of metal nanoparticles decreases the concentration of silver and other metal salts. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio which allows them to interact closely with microbial membranes and is not merely due to release of metal ions in solution or in culture plates (Morones *et al.*, 2005). The mode of action of both silver nanoparticles and silver ions were reported to be similar, although the nanoparticles were reported to be effective at significantly lower concentration than that of the ions (Morones *et al.*, 2005). According to Lok (2007), the attachment of both silver ions and nanoparticles to the cell membrane caused acclimization of envelope protein precursors causing dissipation of the protein motive force.

Conclusions

The results showed that Curry leaves play an important role in the reduction and stabilization of silver to silver nanoparticles. Further, these synthesized silver nanoparticles from Curry leaf shows antibacterial activity on both Gram positive and Gram negative bacteria. This green synthesis method is alternative to chemical method, since it is cheap, pollutant free and eco-friendly.

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