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Synthesis Of Silver Nano Particles Using Murraya Koenigii (Green Curry Leaves), Zea Mays (Baby Corn) And Its Antimicrobial Activity Against Pathogens

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Abstract: Plant mediated synthesis of silver nanoparticles and the study of their size and propertiesis of fundamental importance in the advancement of recent research.In this paper we describe the synthesis of silver nanoparticles using plant extract of ***Murraya koenigii*** (Green Curry Leaves), ***Zea mays*** (Baby corn).The synthesized AgNPs have been characterized by UV-Vis spectroscopy and scanning electron microscopy (SEM). Synthesized silver nanoparticles was confirmed by sampling the reaction mixture at regular intervals and the absorption maxima was scanned by UV–vis spectra, at the wavelength of 200–500 nm. Electron microscopy analysis of these particles shows that they are 10-20 nm in range and assembled in very irregular shape of variable morphology. The antibacterial activity of the nanoparticles is evaluated against water borne pathogens Escherichia coli and Staphylococcus aureus.Low concentration of Ag nanoparticles, inhibited the growth of E. coli whereas the growth-inhibitory effects on S. aureus were mild. MIC value of silver nanoparticles against pathogenic strains of *Escherichia coli* and *Staphylococcus aureus* were also investigated.
Keywords: Silver nanoparticles; Murraya koenigii; Zea mays; UV–vis spectrophotometer; pH; SEM; Antimicrobial activity.

Introduction And Experimental

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity bio molecular detection and diagnostics, antimicrobials and therapeutics; catalysis and micro-electronics.Currently there are several methods for the production of nanoparticles like chemical and physical methods. But there are evidences regarding the harmfulness of these methods to environment[1].Development of plant mediated reliable green,nontoxic and environmentally friendly process for the synthesis of silver nanoparticles are best suited to the environment. D. Jain and *et.al*. described a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM AgNO3 solution through the extract of papaya fruit as reducing as well as capping agent. Nanoparticles were characterized using UV–Vis absorption spectroscopy, FTIR, XRD and SEM. This was for the first time, that any plant fruit extract was used for synthesis of nanoparticles[2]**.** Amal Kumar Mondal and *et.al*. successfully synthesized Silver nanoparticles from AgNo3 through a simple green and natural route using the latex of 6 different plant taxa belonging to 6 diffent

families Alstonia scholaris,Hevea brasiliensis,Ficus religiosa ,Calotropis gigantean,Musa paradisiaca,Achras sapota. From this latex, nano particle was synthesized using it reducing as well as capping agent [3].

In this experiment, the rapid synthesis of stable silver nanoparticles has been demonstrated using the extracts Murraya koenigii (Green Curry Leaves) and Zea mays (Baby corn). The **Curry tree** (***Murraya koenigii***) is a tropical to sub-tropical tree in the family Rutaceae, which is native to India and Sri Lanka. It is a small tree, growing 4–6 m (13–20 feet) tall, with a trunk up to 40 cm diameter. The leaves are Fresh and pleasant and enhances the taste of the dish in which they are used. The leaves of *Murraya koenigii* are also used as a herb in Ayurvedic medicine. They are believed to possess anti-diabetic properties[4]. **Babycorn (**Zea mays), **young corn**, or **cornlettes**, is a cereal grain taken from corn (maize) harvested early while the ears are very small and immature.Baby corn typically is eaten whole-cob included in contrast to mature corn, whose cob is considered too tough for human consumption. Baby corn ears are typically 4.5 cm to 10 cm (1.75 inches - 4 inches) in length and 7 mm to 17 mm (1/4 inch - 2/3 inch) in diameter[5]**.** Baby corn is most common in Asian cuisine. In Thai cookbooks, it is referred to as candle corn.

Materials And Methods

To achieve a “green” synthesis of the nanomaterials, reaction medium chosen was distilled water. The reducing agents used were plant extracts. The reagent used for the synthesis (AgNO3) was analytical grade and commercially available (Merck). The plants used were Murraya koenigii (Green Curry Leaves) and Zea mays (Baby corn).

Preparation Of The Extracts And Synthesis Of Silver Nanoparticles

Aqueous extract of *Green Curry Leaves* and *Baby corn* were prepared using 50 gm each. They were surface cleaned with running tap water, followed by distilled water, dried, cut into fine pieces and boiled with 150 ml of distilled water for 5 min, at an interval of 30seconds. The extract was filtered through Whatman No.1 filter paper (pore size 25μm) and used for further experiments.1mM aqueous solution of Silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. Now 10 ml of extract was added to 30ml of AgNO3 and was mixed well. It is then exposed to sunlight, until the colour of the solution becomes dark, after which the pH reading was taken. A control setup was also maintained. It was then incubated in dark (to minimize the photoactivation of silver nitrate), at 37 °C under static condition for 72hours.

Characterization Of Silver Nanoparticles

Synthesized silver nanoparticles was confirmed by sampling the reaction mixture at regular intervals and the absorption maxima was scanned by UV–vis spectra, at the wavelength of 200–500 nm in Beckman-DU 20 spectrophotometer. Further, the reaction mixture was subjected to centrifugation at 75,000 × *g* for 30 min, resulting pellet was dissolved in deionized water and filtered through Millipore filter (0.45 μm). An aliquot of this filtrate containing silver nanoparticles was used for SEM analysis.

Anti Bacterial Activity Of Silver Nanoparticles

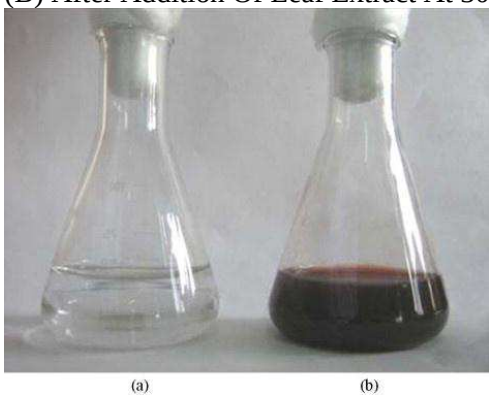
The potential of silver nanoparticles as effective antimicrobial agents is well known. The antibacterial activities of silver nanoparticles were carried out against pathogenic strains of Escherichia coli and Staphylococcus aureus , by disc diffusion method[6] **.** Nutrient agar medium plates were prepared, sterilized and solidified. After solidification the bacterial cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (20 μl and 40 μl) and placed in the nutrient agar plate and kept for incubation at 37 degree C for 24 hours. Zones of inhibition for control and silver nanoparticle were measured.MIC was also determined using the initial bacterial inoculums with different concentrations of the AgNPs (10, 20, 30, 40, 50 and 100 μl) in 5 different test tubes respectively. Minimal inhibitory concentration (MIC) is the lowest concentration of an anti microbial that will inhibit the visible growth of microorganism after overnite incubation.

Results And Discussions

Silver nanoparticles, an effective germ fighter are widely recognized as being especially effective because of their enormously high surface area.Silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of Surface Plasmon vibrations in silver nanoparticles. As the plant extracts of Curry leaves and Baby corn were mixed in the aqueous solution of the silver ion complex, it started to change the colour from pale watery to dark yellowish-brown due to reduction of silver ion which indicated formation of silver nanoparticle. This has been further supported by Elumalai and *et.al* in the synthesis of silver nanoparticles using leaves of Euphorbia hirta and their antibacterial activities[7].

Figure 1: Aqueous Solution Of 10−3M Agno3 With Baby Corn Leaf Extracts

(A) Before Adding The Leaf Extract
(B) After Addition Of Leaf Extract At 30 Min.



Change In pH

The pH reading of the plant extracts at different time interval has been obtained. This has been illustrated in the table below. It has been noticed that the bioreduction of silver was carried out in acidic pH as there was not much variation during the synthesis process. This result is supported by an earlier study by Ranjan et al. who reported at that lower pH, polydispersity of the nanoparticles were reflected in the UV- vis peaks[8].

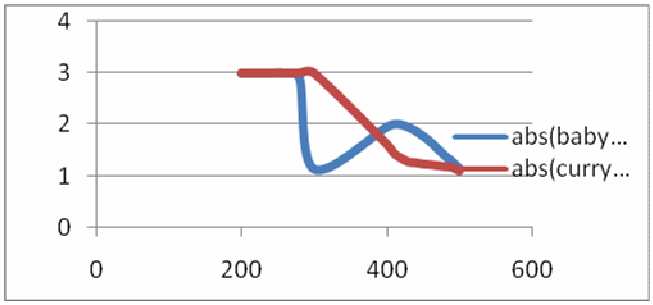
Table1: pH Reading Of The Plant Extracts At An Interval Of 60 Secs

Sample	Curry Leaves	Baby Corn
pH reading taken at an interval of every 60 seconds	5.75	5.62
	5.79	5.94
	5.80	5.97
	5.71	5.96
	5.78	5.96
	5.82	5.96
	5.39	5.98
	5.54	6.17
	5.57	6.21
	5.59	5.70
	5.60	5.65
	5.61	5.63
	5.59	5.63
	5.62	5.62
	5.64	5.62

Absorption Spectra Of Plant Extracts

It is generally recognized that UV–Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions. The figure below shows the UV-Vis spectra recorded from the reaction medium. The characteristic absorption peak at 420-440nm in UV–vis spectrum confirmed the formation of silver nanoparticles. Broadening of peak indicated that the particles are polydispersed. This has been further supported by Amal Kumar Mondal and *et.al* [3]**.**

Figure 2: UV–vis spectra of aqueous silver nitrate of plant extracts at different wavelength



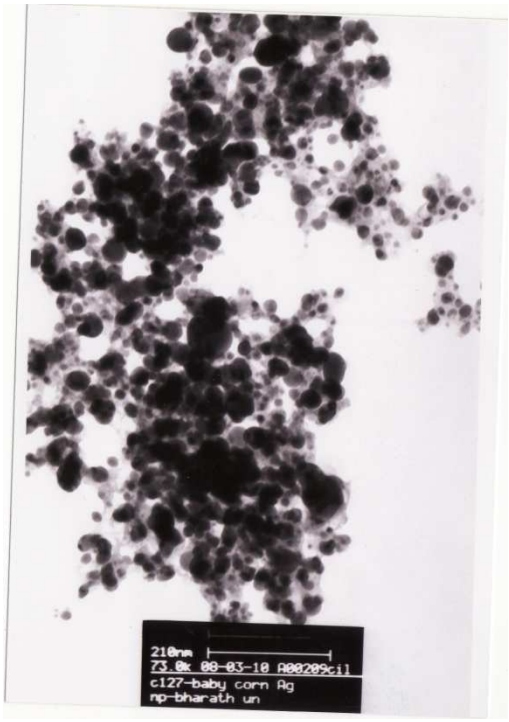
X-scale **-Wavelength** in lambda Y-scale represents **Absorbance** of the extracts

Sem Analysis:

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. from Madras Veterinary College, Veppery, Chennai. The SEM image showed relatively spherical shape nanoparticle formed with diameter range 20-30 nm. Similar results were also obtained by D.Jain and et.al which showed the particle size between 25-50nm as well the cubic structure of the nanoparticles**.**

Figure 3: Image of Scanning Electron Microscopy

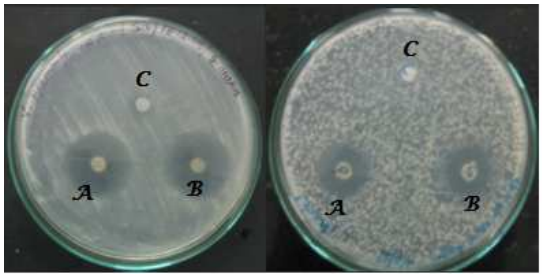
(a) Green Curry



(b) Baby corn

Antibacterial Activity

The antibacterial activity of silver nanoparticles was investigated against pathogenic bacteria of Gram positive (*S. aureus,*) and Gram negative(*E. coli)* strains using disc diffusion method. The AgNPs synthesized displayed significant antibacterial activity that was observed by the zones of inhibitions produced**.** These figures below illustrate the Anti-Microbial activity of Silver Nanoparticles on the pathogenic bacterial strains.



(a) ***S. aureus*** (b) ***E. coli***

Fig. 4: Figure 4: Anti-Microbial activity of Silver Nanoparticles against bacterial strains (a) S. aureus and (b) E. coli, by using (A) 20 *µl* and (B) 40 *µl* of silver nanoparticles and (C) taken as control sample

Table2: Zone of inhibition (mm) of silver nanoparticles against pathogenic bacterial strains

	20 µl	***40 µl***
E. coli	15	17
S. aureus	22	24

The results suggest that S.aureus was inhibited at the low concentration of Ag nanoparticles, whereas the growth-inhibitory effects on E.coli were mild. Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems.

MIC (Minimum inhibitory concentration)

The **minimum inhibitory concentration** (MIC) was read after 24 h of incubation at 37°C. The MIC values of Ag-NPs against the bacterial strains were observed in the range of 20-30 µl, indicating very well bacteriostatic activity of the antibacterial agents. The absorbance values for both blank and the test tubes were measured at 600nm. Bacterial growth was measured as increase in absorbance at 600 nm determined using a spectrophotometer. Against bacterial strains of *S. Aureus* , the MIC value was found to be 20 µl/5ml (v/v),or 0.72 µg/1ml (w/v) while the strains of *E.coli* were in the range of 30µl/5ml or 1.08 µg/1ml(w/v).

Discussion

Nano-science is the study of phenomena and manipulation of materials at atomic molecular and macromolecular scales. The bio-reduction of aqueous Ag+ ions by the extracts of ***Murraya koenigii*** (Green Curry Leaves), ***Zea mays*** (Baby corn)has been demonstrated. The reduction of the metal ions through these extracts leading to the formation of silver nanoparticles of fairly well-defined dimensions. This green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Further, the above Ag nanoparticle revealed to possess an effective antibacterial property. The present study emphasizes the use of the synthesis of Ag nanoparticles with potent antibacterial effect. For detail study and deep characterization of nano particles, UV-vis spectroscopy & scanning electron microscopy measurements (SEM) have been analysed.

Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the plant leaf extract observed by the UV-Vis spectroscopy revealed the presence of silver

nanoparticles. As the plant extracts were mixed in the aqueous solution of the silver ion complex, it started to change colour from water colour to yellowish brown; colour was changed due to excitation of surface Plasmon vibrations, which indicated formation of silver nanoparticles. UV-Vis spectroscopy is well known to investigate shape and size controlled of nanoparticles. Klabunde and co-workers demonstrated that reactive metal oxide nanoparticles show excellent bactericidal effects[9]**.** Studies conducted by researchers in the recent past revealed that metal oxide nanoparticle formulations possessed significant antibacterial activity[9]. Recently it was shown that highly concentrated and nonhazardous nanosized silver particles can easily be prepared in a cost-effective manner[10] and tested as a new type of bactericidal nanomaterial.

Utilizing silver in treatment of various infections has been in existence for a long time. In this study, the antibacterial activity of the silver nanoparticles was evaluated. The results of this study clearly demonstrated that the colloidal silver nanoparticles inhibited the growth and multiplication of the tested bacterial strains such as *S. aureus & E. coli.*

The size and shape of the nanoparticles forms a basis for the wide variety of applications. The size of the plant mediated AgNPs were observed to be in the range of 25 – 55 nm with a spherical shape that were found to be dispersed. This was further confirmed by SEM analysis of the synthesized particles. Nanoparticles have a large surface area compared with the total volume. The surface area to volume ratio is interesting because chemical reactions typically occurs on surfaces, so nanoparticles that have a high surface to energy ratio can be used in many interesting ways such as in catalysis[11]**.** Hence, the biological approach appears to be cost efficient alternative to conventional physical and chemical methods of silver nanoparticles synthesis and would be suitable for developing a biological process for large-scale production. These silver nanoparticles may be used in effluent treatment process for reducing the microbial load[12]. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic nanomaterials.

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