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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *MURRAYA KOENIGII* AND ITS APPLICATION IN EFFLUENT TREATMENT

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ABSTRACT
Nanoparticles have a promising action in many 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. Addition of 1mM silver nitrate solution to the above extracts yielded the production of silver nanoparticles. The silver nanoparticles synthesized from Murraya koenigii showed growth inhibitory effects against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia*. The zone of inhibition is in the order 13mm, 13mm, 15mm and 9mm respectively for silver nanoparticles. The zone of inhibition for silver nitrate alone is in the order 11mm, 9mm, 11mm and 8mm respectively. They are characterized using UV-Vis spectrometry, SEM and EDX measurements.. It also plays an important role in effluent treatment by the effective degradation of harmful textile dyes.

KEYWORDS: Nanoparticles, *Murraya koenigii,* antibacterial assay and dye reduction.

1. INTRODUCTION

Murraya koeingii is a valuable medicinal plant which has been used in traditional system of medicine since long time. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders. The branches of *M. koenigii* are very popular for cleaning the teeth used as datun. 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.

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.

Currently silver nanomaterials have a variety of uses in everyday life, nanosilver infused storage containers, nano silver 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.

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 . 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. Due to strong antibacterial property silver nanoparticles are used in clothing, food industry, sunscreens, cosmetics and many household appliances. Few studies have showed that silver nanoparticles kill fungal spores by destructing the membrane integrity.

Dyes are complex aromatic molecular structures which are intended to be stable and consequently are difficult to degrade. The largest consumer of the dyes is the textile industry accounting for two third of the total production of dyes.

Textile industry consumes a large volume of water and chemicals during wet processing stages and delivers considerable quantities of colorants along with other chemicals. Dyes being tinctorially stronger are visible in water at concentrations as low as 1 ppm. One of the major factors responsible for release of water-insoluble as well as water-soluble dyes in the wastewaters is the improper dye uptake as well as the degree of fixation on the substrate which is governed by several factors such as depth of the shade, application method, material to liquor ratio and pH etc.

It is estimated that approximately 2% of the dyes produced are discharged directly in aqueous effluent, and 10% is subsequently lost during the coloration process. It is reasonable to assume that approximately 20% of the colorants enter the environment through effluents from the wastewater treatment plants.

2. OBJECTIVES

- Biosynthesis of silver nanoparticles from aqueous extract of curry leaves, *Murraya koenigii*.
- Characterization of silver nanoparticles by UV-Vis spectroscopy, SEM-EDX.
- To determine the antibacterial efficacy of synthesized silver nanoparticles against different pathogens.
- Effluent treatment by the degradation of dyes by silver nanoparticles.

4. MATERIALS AND METHODS

Fresh curry leaves (Fig. a) were collected from New Civil Nagar, Palakkad, Kerala, India. It was placed in a clean polythene bag. Textile dyes of six different colours viz. red, yellow, green, purple, blue and orange were collected from a major textile industry in Palakkad.

Fig. a: Freshly collected Curry Leaves

Curry leaf extract was prepared with 10g of fresh curry leaves taken in three separate beakers each. It was thoroughly washed with tap water and then with distilled water for atleast two times and cut into small pieces. The chopped leaves were boiled in 75ml of distilled water for 3 minutes in first beaker, for 5 minutes in second beaker and for 10 minutes in third beaker separately.

The leaf broth was then cooled and filtered. It was then stored at 4°C after covering the beaker with aluminium foil for further assay. The leaf broth appeared light green in colour.

Stock solution was prepared by dissolving 1mM silver nitrate and volume made upto 250ml with distilled water. 5ml of curry leaf extract of different concentrations (3 minute boiled, 5 minute boiled and 10 minute boiled separately) was added to 100ml of 1mM silver nitrate solution and allowed to react at room temperature. The formed nanoparticle solutions was shown in Fig. b.

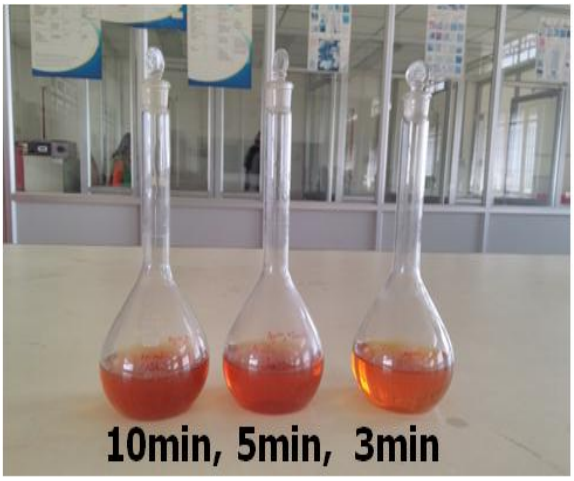

Fig. b: Nanoparticle solutions of three different concentrations

Pure cultures of two Gram negative (*Escherichia coli* and *Klebsiella pneumonia*) organisms and two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) organisms from Bioline Laboratory, Coimbatore were taken for the study.

The curry leaf extract of three different concentrations (3 minute boiled, 5 minute boiled and 10 minute boiled) were analysed using UV-Vis spectroscopy. The periodic scans of optical absorbance between 200nm and 900nm was performed to investigate the reduction rate of silver ions by curry leaf extract. Deionised water was used to adjust the baseline.

For SEM-EDX analysis, the curry leaf extract with highest UV-Vis spectroscopy peak (400nm) was selected. This analysis reveals about the sample including the external morphology, chemical composition and crystalline structure & orientation of materials making up the sample. It 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 measurement was made to confirm the presence of elemental silver signal and provides quantitative compositional information.

Mueller Hinton Agar plates were prepared and sterilized. Filter paper discs were sterilized and were soaked in 50µl of plant extract, $AgNo_3$ and silver nanoparticle separately and allowed to dry at room temperature for 15 minutes.

The test organisms were inoculated using a sterile swab, and the organism was uniformly spread throughout the plate. Antibiotic discs were placed in the previously prepared plates. The discs were pressed down to ensure complete contact with the agar surface. The plates were then incubated at 37°C. The resulting zone of inhibition was uniformly circular with a confluent lawn growth. The diameters of zones of inhibition were uniformly measured.

1g of each dye was taken in 10ml of distilled water and prepared the stock solution. From the stock solution, 0.7ml of each dye solution was pipetted into screw cap tubes. 1ml of silver nanoparticle solution was added to each of the tubes with dye solution. Then using a colorimeter, the OD values were measured at 540nm with an interval of 24 hours for 5 days. The value of absorbance shows the intensity of dye present in the solution.

3. RESULT

4.1. Synthesis Of Silver Nanoparticles

After the addition of curry leaf extract to silver nitrate solution, a visible colour change from transparent to dark colour was observed. This colour change indicates the formation of siver nanoparticles. After 90 minutes, no change in the intensity of colour was developed. This indicates the completion of the reaction.

4.2. Characterization of Silver Nanoparticles

The UV absorption spectrum of silver nanoparticles from curry leaf extract of different concentrations was obtained. The SEM image and EDX spectrum of silver nanoparticles were also obtained.

4.3. Antibacterial Assay

The silver nanoparticles synthesized from *Murraya koenigii* showed growth inhibitory effects against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The zone of inhibition is in the order 13mm, 10mm, 13mm and 11mm respectively for silver nanoparticles. The zone of inhibition of silver nitrate alone is in the order 11mm, 9mm, 11mm and 8mm respectively (as shown in Fig.. c).

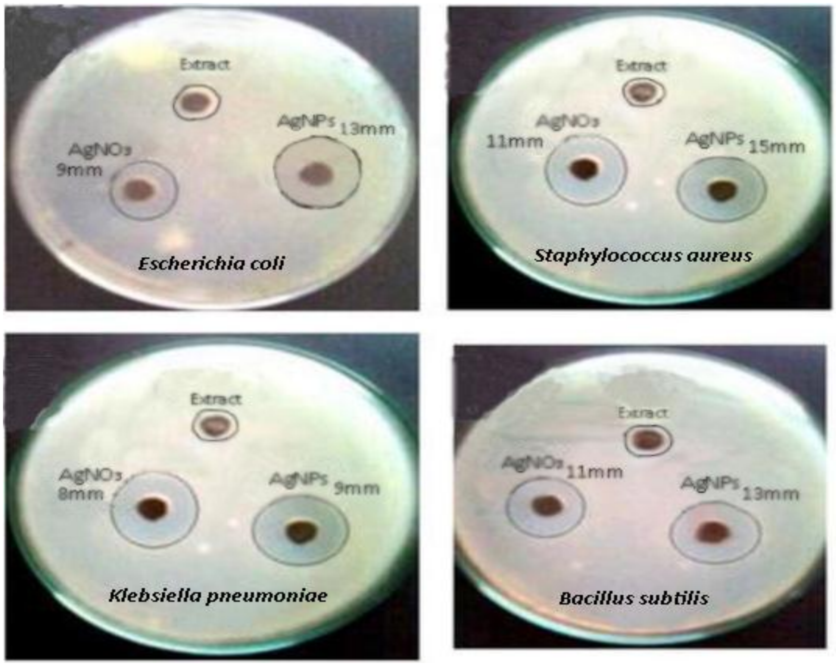

Fig. c: Mueller Hinton Agar plates showing Zones of Inhibition

4.4. Reduction Of Textile Dyes

The OD values obtained for each dye at an interval of 24 hours for 5 days is tabulated as follows:-

Time (hours)	Different Colours of Textile Dyes					
	RED	YELLOW	GREEN	ORANGE	PURPLE	BLUE
24	1.28	0.14	0.30	0.53	0.43	1.14
48	1.21	0.11	0.23	0.39	0.31	1.10
72	0.65	0.08	0.20	0.10	0.23	0.45
96	0.61	0.05	0.15	0.09	0.17	0.33
120	0.30	0.03	0.09	0.07	0.15	0.14

Graph is plotted as shown in Fig.. d

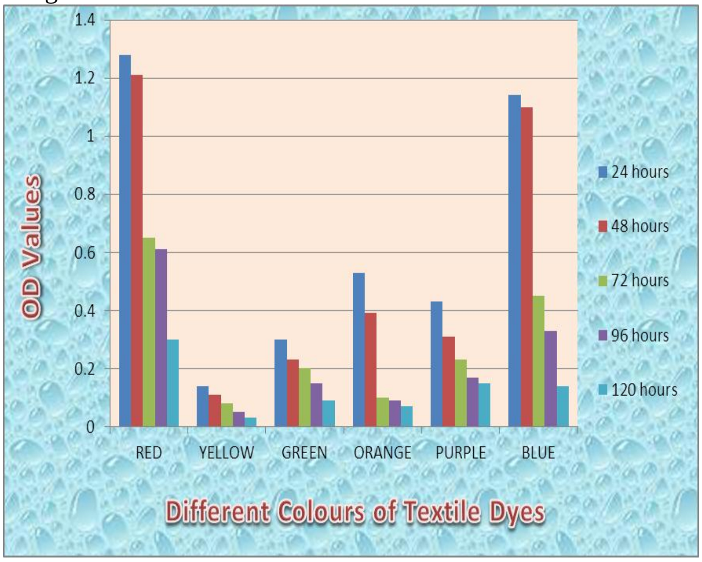

Fig. d: Graph showing OD values of different dyes.

DISCUSSION AND SUMMARY

Curry leaves has recently been found to be a potent antioxidant due to high concentrations of carbazoles, a water soluble heterocyclic compound. 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 stirred. To synthesize silver nanoparticles, the extract was added to 1mM silver nitrate solution and allowed to react at room temperature. A colour change was observed from colourless to yellowish and then finally to dark brown. This occurred due to the reduction of silver ions present in the solution, The reduction was due to the terpenoids present in the leaf extract. The reduced silver particles are in the range of nano size. Synthesized silver nanoparticles are characterized using UV-Vis spectrometry to examine the shape and size controlled nanoparticles in its aqueous solutions. The peak occurs at 400 nm (λ max) which corresponds to absorbance of silver nanoparticles. SEM analysis showed that nanoparticle formed is spherical in shape. The EDX analysis confirmed the presence of elemental silver in the sample.

The silver nanoparticles from *Murraya koenigii* showed growth inhibitory effects against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia.* The zone of inhibition is in the order 13mm, 10mm, 13mm and 11mm respectively for silver nanoparticles. The zone of inhibition of silver nitrate alone is in the order 11mm, 9mm, 11mm and 8mm respectively. This was found to be less effective to the above pathogens when compared with silver nanoparticles.

The decreasing OD values of different dyes showed that the absorbance has decreased throughout the time. This indicates the reduction of dyes by silver nanoparticle solution. On each consecutive day, the concentration of dyes get reduced and so the OD value also shows a decreasing trend in the absorbance value.

The textile industry accounts for two-thirds of the total dye stuff market. During the dyeing process, approximately 10-15% the dyes used are released into the wastewater. The presence of these dyes in the aqueous ecosysytem is the cause of serious environmental and health concerns. The presence of very small amounts of dyes in water is highly visible and affects the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies and degradation products of these dyes are often carcinogenic. The accumulation of textile dyes in water bodies affects the aquatic ecosystem by reducing the photosynthetic activities of the aquatic plants and the livelihood of the aquatic species like fishes surviving in it leading to the damage of the food chain of the aquatic ecosystem.

All these harmful effects can be reduced to a considerable extent by means of degradation of the textile dyes by silver nanoparticles.

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