A Study on Antimicrobal Activity and Photocatalytic Degradation of Methyl Orange Dye Using Areca Nut Extract

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Abstract

Nowadays plant mediated synthesis of nanoparticles has great interest and achievement due to its eco-benign and low time consuming properties. In this study silver nanoparticles were successfully synthesized by using areca catechu nut extract under different pH. The aqueous nut extract was added to silver nitrate solution; the color of the reaction medium was changed from pale yellow to brown and that indicates reduction of silver ions to silver nanoparticles. In present investigation, simple and eco friendly method for green synthesis of silver nanoparticles by using areca nut extract. It is a reducing agent at room temperature along with photocatalytic degradation of methyl orange dye. Photocatalytic degradation of methyl orange has to measured spectrophotometrically. Disc diffusion method was used to confirm the antimicrobial action of silver nano particles against tested microbial strains.

keywords- Silver nanoparticles, arecanut extract, green synthesis, antimicrobial activity

I. INTRODUCTION

Pollution is a worldwide problem that has to be controlled. On the positive side, dyes can give beautiful color to various products, and therefore they are widely used in many fields such as textiles, paper, plastic, food, painting, and medicine. However, the waste effluent from industries using dye may still contain up to 15% of the dye. Many synthetic dyes are highly toxic and can bring about serious water pollution, destroy community structure of aquatic organisms, and further become a hazard to all mankind. It is reported that around 25% of diseases facing humans suffering today because of long-term exposure to environmental pollution.

Dyes are used in large quantities in many industries including textile, leather, cosmetics, paper, printing, plastic, pharmaceuticals, food, etc. to colour their products, which generates wastewater, characteristically high in colour and organic content. The textile industry alone accounts for two third of the total dye stuff production. The discharge of coloured wastes into streams not only affects their aesthetic nature but also interferes with the transmission of sunlight into streams and therefore reduces photosynthetic action. Further, colouring pigments may contain various chemicals which exhibit huge toxic effects towards microbial populations and carcinogenic to mammals. In general, dyes are poorly biodegradable. Conventional biological treatment processes are not very effective to dye removal .Basic methyl orange is acidic or anionic dye has been widely used in textiles, paper, food and pharmaceutical industries and research laboratories.

Dyes belong to the class of synthetic organic compounds and are widely used in the textile industry. The removal of these non-biodegradable organic chemicals from the environment is a crucial ecological problem. Many techniques, such as activated carbon sorption, flocculation, electrocoagulation, UV-light degradation and redox treatments, are being routinely used for abating dyes. However, due to the ineffectiveness of these techniques in some way or the other, the present scenario requires better and improved wastewater treatment measures. Azo dyes are well known carcinogenic organic substances. One of this class of dyes, Methyl Orange, which is the main focus of the study, inadvertently enters the body through ingestion and metabolizes into aromatic amines by intestinal microorganisms which can even lead to intestinal cancer. Methyl Orange (MO) is a watersoluble azo dye, widely used in the textile, printing, paper manufacturing, pharmaceutical and food industries, and also in research laboratories.

Recently, metal nanoparticles were reported as effective photocatalysts for degrading chemical complexes, under ambient temperature with visible light illumination. This can be achieved by increasing the optical path of photons leading to a higher absorption rate of nanoparticles in the presence of a local electrical field. These nanoparticles showed new and improved properties based on their morphological structures and characteristics as compared to bulk materials. Ag nanoparticles are good, highly efficient and stable photocatalysts under ambient temperature with visible light illumination for degrading organic compounds and dyes. Moreover, scientists have also shown considerable interest in using nanoparticles for the photocatalytic degradation of dyes. Of all marine plants, seaweed-mediated biosynthesis of Ag nanoparticles have been found to be efficient, cost effective and environmental friendly .The biosynthetic method employing marine seaweed extract has received more attention as being simple, ecofriendly and less time-consuming compared to usual chemical and physical methods. The purpose of present study was extracellular synthesis and photocatalytic degradation of methyl orange using silver nanoparticles synthesized from areca catechu nut.

1. Objectives

- To introduce green synthesis of silver nano particles from areca nut extract.
- To degrade methyl orange dye using areca nut extract
- To define the anti microbal property of arecanut

2. Scope of the study

In the modern world, the environmental pollution has been recognized as one of the major problems. The increasing demand for water and declining supply has made the treatment and recycle of industrial effluents an attractive option. Color is one of the most important environmental pollution problems in water courses, although some of this color is usually present. Some dyed effluents are related with the manufacture and use of dyestuff. Azo dyes, the major chemical class of dyes with the maximum variety of colors, have been used widely for textile, dyeing and paper painting. These dyes cannot be easily degraded. Several combinations of treatment methods have been developed so far for efficiently process textile wastewater; decolorization being among the main targets to achieve. They are environmental friendly techniques since they produce no solid wastes. Adsorption techniques have newly gained a considerable significance due to their efficiency in the removal of pollutants too stable for conventional methods. Biological treatment is a better natural wastewater treatment process than other wastewater treatment methods.

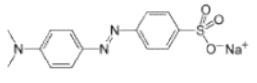
II.MATERIALS AND METHODS

A .MATERIALS

The materials used are as follows

1) Methyl orange dye
   Methyl Orange (MO), is a water soluble toxic azo dye having IUPAC name as Sodium pdimethylamino azobenzenesulphonate, and molecular formula and molecular weight $C_{14}H_{14}N_3NaO_3S$ and 327.34 g mol-1 respectively. Methyl orange was collected from nearby laboratory.

Table 1 properties of Methyl Orange dye

Property	Details
Nature	Anionic Dye
λ_{max}	470 Nano meter
Spectral Range	200-800Nano meter
M. Wt.	327.34 g/mole
Molecular Structure	

2) Areca nut

Areca nut was collected from chalissery, palakkad district of kerala state. The husk of areca nut was removed and the nut was then dried under sunlight. After drying , the areca nut was powdered using a blender and stored in an air tight container for further analysis.

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Fig 1 areca nut sample

Fig 2 Dried areca nut sample

3) Chitosan
Chitosan was collected from nanotechnology research lab, bengaluru of Karnataka state. It was stored in an air tight glass container for further analysis.

Fig 3 chitosan sample

4) silver nitrate solution
Silver nitrate ($AgNO_3$) is procured from nearby Laboratory.

Code used for experimental analysis

- IS 10500-2012

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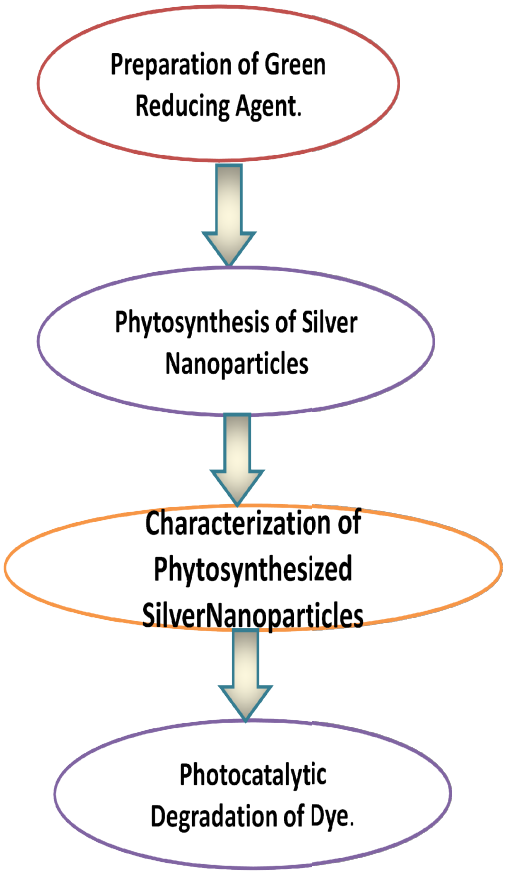
B Methodology

In this study, silver nanoparticles have to be synthesized from areca nut extract under different pH. The aqueous nut extract was added to silver nitrate solution; the color of the reaction medium will change from pale yellow to brown and that indicates reduction of silver ions to silver nanoparticles.

1. **photocatalytic degradation of methyl orange**



a. **Preparation of Green Reducing Agent**.
The collected fresh Areca nut of 100g wet weight was cut into fine pieces and washed with distilled water and boiled with 1000mL of double distilled water for 10 min at 60∘C. Boiled mixture was filtered

through Whatman No. 1 filter paper and collects the supernatant of nut extract and stored at 4◦C for further nanoparticles synthesis process.

b. Phytosynthesis of Silver Nanoparticles.

Aqueous solution of silver nitrate was prepared using double distilled water at a concentration of 1mg. 10mL of freshly prepared extract was added to 90mL of aqueous solution of silver nitrate and kept at room temperature for the reduction of silver ions to silver nanoparticles. Nanoparticles formation was visually identified by color change and followed the UV spectrum analysis. The pH of extract was altered to study its effects on synthesis of silvernanoparticle. The various pH(4.6,5.6, 6.6,7.6, and 8.6)of the 10mL of extract were added into 90mL of 1mM silver nitrate solution. The pH was adjusted by using 0.1N NaOH and 0.1N HCl. Formation of silver nanoparticles was measured by UV spectrophotometer at different wavelengths.

c. Characterization of Phytosynthesized SilverNanoparticles

The reduction of silver ions was monitored by measuring double beam UV-Vis spectra of the reaction medium at different wavelenthgs from 360 to 700nm at different functional time. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 7000rpm for 15min and dried at 100◦C. The morphology and size of the silver nanoparticles were found by Scanning Electron Microscope. Charaterization was performed by

a) UV-visible spectroscopy
b) FTIR analysis
c) SEM analysis

d. Photocatalytic Degradation of Dye.

In photocatalytic activities of synthesized Ag NPs, the reactions are implemented in a 15 mL potential quartz cuvette and absorbance values are monitored using UV-noticeable (UV-vis) spectrophotometer. Catalytic activity of biosynthesized Ag NPs was studied by degradation of MO dye under the sunlight irradiation. Initially, the dye solution was prepared by dissolving 1 mg of Methyl orange(MO) powder in 50 mL de-ionized water (keeping 10 mg/l concentration) and then Ag NPs (10 mgs) were added to dye solutions. The mixture was stirred magnetically for 45 min in darkness before exposing to sunlight. It was prepared and kept under the similar condition for comparing any change in color of the dye solution
Percentage of dye degradation was estimated by the following formula:
%Decolourization = $[100 \times (C_0 - C)]/C_0$
where C_0 is the initial concentration of dye solution and C is the concentration of dye solution after photocatalytic degradation

e. Determination Antimicrobal activity of areca nut extract

Disk diffusion (Kirby Bauer) method is used to determine the antimicrobial activity. Sabouraud dextrose broth cultures were prepared in test tubes. Sabouraud Dextrose Agar is used for the cultivation of yeasts, moulds and aciduric bacteria from clinical and non clinical
samples. The ingredients used to make sabouraud agar are:

- Dextrose (glucose) – 40 gm/L
- Distilled water – 1 Liter
- Agar – 15 gm
- Peptone – 10 gm
  Final pH 5.6 +/- 0.2 at 25ºC

Preparation of culture :

i. Gather all the necessary ingredients and combine them in about 900 ml of deionized water.
ii. Add hydrochloric acid to adjust the pH to the desired level. The final volume should be 1 liter.
iii. Bring to heat to dissolve the medium.
iv. Autoclave for 15 minutes at 121 degree Celsius.
v. Allow to cool down before placing into Petri dish to test tube for slants.

vi. The sabouraud dextrose agar plate is inoculated by streaking or exposure the medium to ambient air. Ideally, a mold is incubated at a room temperature, which is typically 22 to 25 degree Celsius. It is incubated at 28 to 30 degree Celsius. The incubation time needed for the organism to grow determine its fungal species.
Those cultures maintained for 72 hours were taken. The surface of the Sabourauds Dextrose agar plates was scrubbed with sterile cotton swabs to prepare lawn cultures. This was done 5 minutes after the agar surface had dried. Wells were dug using cork borer aseptically. These wells are saturated with areca nut extract with concentrations ranging from 100 g to 1000 g. The plates were incubated at 37^{0}C for 24-48 hours. The diameter of zones of the inhibition was measured using a scale to the nearest millimeter.

III. RESULTS AND DISCUSSION

1 .Preparation of Areca nut extract

5 g of dried areca nut sample was boiled for 5 min in 100 ml ultra pure water and filtered through Whatmann No. 1 filter paper. The Areca nut extract was in yellow colour. The filtered areca nut extract was used for the synthesis of silver nanoparticles.

Fig 4 areca nut extract

4.2 Silver nanoparticles synthesis

Aqueous solution (1mM) of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 3 ml of extract was added to 40 ml of 1 mM AgNO3 solution for the reduction of Ag+ ions. The synthesis of silver nanoparticles was carried out at room temperature (25° C + 2° C) for 24 h in dark.

2. UV-visible studies

The different quantities of leaf extract were used as reducing agent for the synthesis of Ag NPs. The nut extract of various amounts such as 5, 10 and 15 mL was mixed with 100 mL aqueous solution of $AgNO_3$ (1 nm), a constant increase in absorbance peak intensity was measured in UV-vis spectra. With respect to the addition of arecanut extract, the intensity of solution color yellow is increased within 1h at the room temperature. The formation of AgNPs was obviously identified as a color of arecanut extract and $AgNO_3$ solution turned from yellow to dark brown. The color changes occurred in the solution after the addition of arecanut extract is due to the excitation of NPs Surface Plasmon Resonance.

al., 2011), which strongly indicates the formation of Ag NPs.

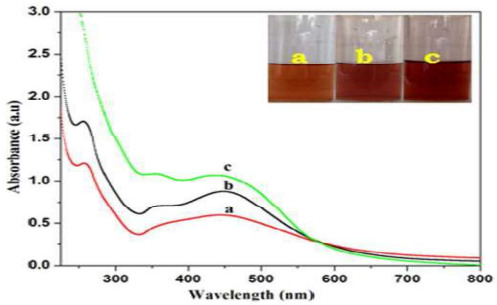


Fig 5 UV-vis absorption spectra of Ag NPs synthesized by arecanut extract, (a) 5 mL (b) 10 mL and (c) 15mL

The characteristic absorbance peaks at 448, 444 and 437 nm were absorbed the prepared Ag NPs with nut extracts of 5, 10 and15 mL respectively.

3. FT-IR Studies

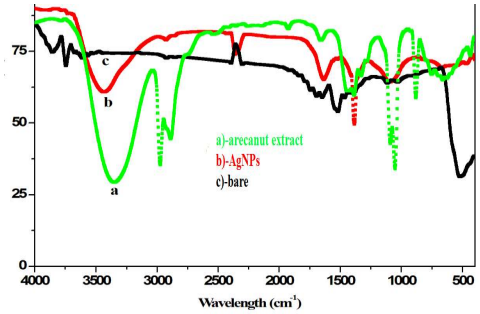


Fig 6 FT-IR spectra of (a)Arecanut extract, (b) synthesized Ag NPs and (c)

Table 2 FT-IR Stretching vibration and functional groups of Arecanut extract, synthesized Ag NPs and Ag

Wavenumber (cm-1)			Stretching	Functional
Arecan	Ag	Bar		
3348	3343	-	O-H	Alcohol and Phenol
2974	2926	-	C-H	Alkenes
2353	2358	-	C-NH⁺	Amines
-	-	1516	N=H	Amide-II, Proteins
1656	1633	-	C=O	carboxylic
1384	1382	-	C-C	Aliphatic
1049	1093	-	C-O	Alcoholic, carboxylic acids, esters, ethers
881	825	-	C-N	Aromatics
596	582	520	M-O	Ag-O
435	470	-	M-O	Ag-O

In green synthesis process, no chemical reducing agent (nitrocompound) has been used. This results in the absence N=H stretching mode

4. SEM studies

Figure shows the different magnifications of SEM images and EDX spectrum of synthesized Ag NPs, surface morphology and particle size of the Ag NPs. It is observed that Ag NPs are predominantly spherical in shape and quite well distributed with agglomeration of smallest particles. The agglomeration of particles may be involved due to the capping agent of plant biomolecules binding with Ag NPs. However, the result reveals that Ag NPs retain the shape of spherical as shown in Figure 4.6a and 4.6b. Moreover, the blue shift in absorption peak has been observed due to the decrease in particle size caused by high quantity of arecanut extract was confirmed by UV-vis. In order to ascertain the above, higher volume extracts are alone tried in FE-SEM. EDX analysis exhibits the strong signal at 3 keV is matching to the binding energies of Ag and confirming the formation of the synthesized Ag NPs.

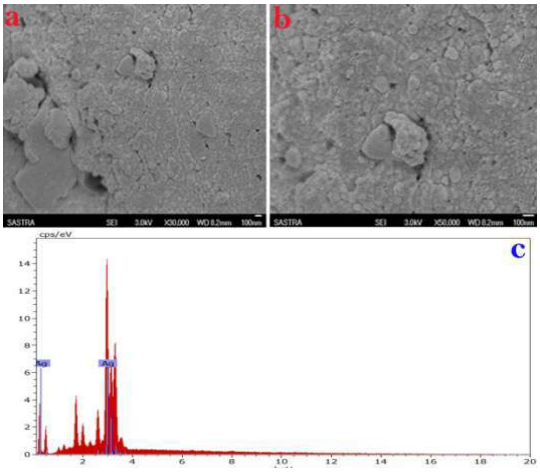


Fig 7 SEM images of different magnifications and EDX spectrum of synthesized Ag NPs

5. Photocatalytic activity

The methyl orange (MO) dye was selected for the estimation of photocatalytic activity of the synthesized Ag NPs in aqueous medium under the solar irradiation method and dye degradation
was monitored by recording the UV-vis spectra. The characteristic absorption peak of MO is at 664 nm. The degradation of the MO in presence of biosynthesized AgNPs is visualized by gradual change from deep orange to colorless. The dye degradation was confirmed by the decrease in absorption peak intensity (664 nm) at 210 min of exposure to solar light while no change in control was observed during the solar light exposure. The dye degradation (%) of biosynthesized NPs was calculated by using the formula

$$Dye\ degradation\ (\%) = [(C_0\text{-}C_t)\ /C_0] \times 100$$

Where, 'C_0' is the initial concentration of MO solution and 'Ct' is the concentration of the dye solution after't' hours of exposure in solar irradiation. There are absorbance changes in MO after irradiation of sunlight using bare Ag and AgNPs. Dye degradation of bare Ag at different time of intervals from 30-210 min is measured around 52 %, while green synthesized AgNPs showed a great degradation of MO around 86 %within 210 min of sunlight irradiation.

Generally, when the (MO dye + Ag NPs) mixture was exposed to solar light, the photons hit on the surface of Ag NPs and released the electrons. The excited electrons from the surface of NPs were accepted by the solution containing oxygen and converted to oxygen anionic radicals. These radicals break the dye molecule and transform as simpler organic molecules resulting to degradations of dye. Within the 210 min solar light exposure, more than 86 % of dye was degraded, due to the generation of more number of oxygen anionic radicals caused by the tiny NPs.

To compare the degradation efficiency of bare and synthesized Ag NPs, the plots are drawn between $(C_0\text{-}Ct)/C_0$ and time are shown in Figure 4.7 The degradation efficiency of AgNPs is three times higher than bare Ag, due to larger surface to volume ratio of AgNPs.

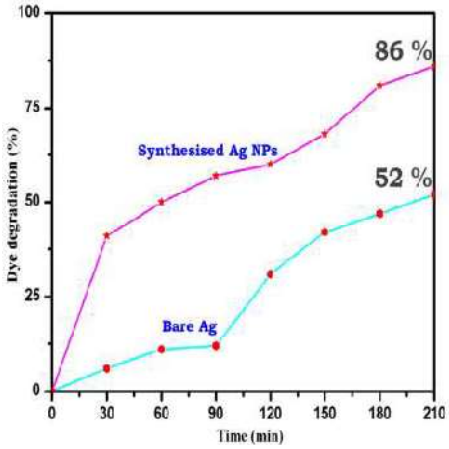


Fig 8 Plots drawn between $(C_0\text{-}Ct)/C_0$ and times for bare Ag and synthesized Ag NPs.

Fig 9 photocatalytically degraded MO

6. Antimicrobal activity

With the help of disc diffusion method, the antimicrobal activity of Ag NPs was studied against various pathogenic microorganisms such as *B. cereus, B. subtilis* (Gram positive), *P.aeruginosa* and E. aerogenes (Gram negative). Figure shows the zone of inhibition around the disc for the synthesized AgNPs and obtained values are presented in the Table 4.2. It was observed that the synthesized Ag NPs showed better antibacterial activity against all microbes and its value. The reasons which enable the Ag NPs antibacterial activity are

(i) Release of Ag+ ions and

(ii) Smaller particle size

However, *B. cereus* has higher inhibition of zone. This is because of accumulation of the large amount of Ag+ ions on negatively charged cell membrane leads to lose the permeability control and causes cell death. Moreover, Ag NPs are reacting with sulphur contain proteins in the core of the cell as well as phosphorous contain compounds such as DNA spirit affect the respiratory chain and cell separation in bacteria at last causing to the death of the cell.

Fig 10 Disc diffusion method

Table 3 Zone of incubation by Extract, AgNO3, AgNPs and antibiotic against human pathogenic bacteria

Sino:	Pathogenic bacteria	Inhibition zone (mm)			
		Extract	NPs	Ag	Antibiotic
1	*Bacillus cereus*	11	16	7	17
2	*Bacillus subtilis*	16	18	8	12
3	*Pseudomonas Aeruginosa*	12	15	11	14
4	*Enterobacter aerogenes*	10	14	8	18

IV CONCLUSION

The Silver nanoparticle was successfully synthesized from Areca nut extract. Presence of the Silver nanoparticles was confirmed by a change in colour from yellow to brown. Silver nanoparticles are widely used in waste water treatment process. The rapid green synthesis of silver nanoparticles using Areca nut extracts provides a simple, efficient, economic, and environmental friendly method. The prepared silver nanoparticles were characterized by SEM, FTIR, and UV-Visible spectrophotometer techniques. Synthesis of silver nanoparticles using green method, despite obvious limitations, has a significant potential and a number of substantial advantages relative to traditional methods of nanoparticles synthesis. The green synthesized Ag NPs possess potential in photocatalytic degradation of methyl orange dye and antibacterial activity. Almost 86% of dye was removed photocatalytically at 210 min. From the technical point of observation the obtained AgNPs have potential utilization in industrial and wastewater treatment applications. In totality, the study suggests achievement of an ecofriendly and highly efficient method which may be considered useful for the removal of dyes from textile industries.

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