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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM THE MANGROVE AVICENNIA MARINA AND ITS APPLICATIONS

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ABSTRACT

In recent years, green synthesis of silver nanoparticles (AgNPs) has gained much interest from chemists and researchers and also increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. The present investigation attempted on bio-synthesis of sliver nano particles. These nano particles were biologically synthesis by using mangrove plant *A. marina* leaf extract. Screening of Mangrove for nano particles synthesis tested as evident by the Visual observation and UV-VIS spectroscopic data after 24 hrs of reaction. The peak of colour intensity was observed at 24 hrs of incubation. There was no significance change of colour intensity beyond 24 hrs. The synthesis of sliver nano particles was confirmed by the brown colour with peak of absorption surface at 420 nano meter. The nano particles were spherical with size of 10 to 30 nano meter and with good stability. The Nano particles were successfully deposited on the poly urethane foam filter and tested for water purification. The chemical characteristics of control and nanoparticle treated samples was clear, colourless and odourless. TDS under permissible limit. After treatment pH of the sample becomes neutral. Micro as well as Macro nutrient of the samples under acceptable limit.

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INTRODUCTION

Nanotechnology exhibits the top down phenomena, which means reducing the size of the smallest structures to the nanoscale (Niemeyer and Doz, 2001; Elibol *et al*., 2003; Wadhwa, 2009). Nanotechnology and nano science could be used across all the other science fields, such as chemistry, biology, physics, material sciences, and engineering. (Rajasekharreddy *et al*., 2009). Bio-Nanotechnology is a new and rapidly advancing field of research lies at the interface between biology and nanotechnology (Sahayaraj and Rajesh, 2011). In the recent past, silver nanoparticles have gained interest due to their distinctive properties such as good conductivity, chemical, stability, catalytic, antibacterial, antifungal, antivirl and anti-inflammatory activities (Chen *et al*., 2008; Vijay raj *et al*., 2012). But studies related to the synthesis of nanoparticles using mangrove and mangrove associate plants are very limited.

Marine environmental conditions are extremely different from terrestrial ones; it is surmised that mangrove plants have different characteristics from those of terrestrial Nanoparticles are the basic essential elements in the wall of nanotechnology and it exhibits fabulous advanced characteristic features based on their properties such as size, morphology and other size dependent (Gnanadesigan *et al*. 2011a; Ravikumar *et al*. ,2011a, b). Among the different mangrove plants, *Avicennia marina* is previously proved to have antibacterial, antiplasmodial, antiviral activities (Ravikumar *et al*., 2011) and also it is proved to have high content of secondary metabolites such as polyphenols, flavonoids, alkaloids and tannins (Ravikumar *et al*. 2010). Water is an essential requirement for life. To an average individual of our modern civilization, it is too often taken for granted that it is always available at the tap (Walton, 1970). Fresh water being a finite resource (Wetzel, 1975), comprising only 3 percent (1, 04,900) of the total water on land (Deming, 1978), solicits prime importance to be given for its conservation. Among fresh

water bodies in India, the number of ponds is numerous, found mostly in the villages, places of worship and other human in habitations (Gulati &Schultz, 1980). This makes them quite vulnerable for human impact and changes day by day, measuring which would probably give a clear picture about the pollution stress them. Water quality is no less important than the water quantity from the days of Hippocrates (Wolman ,1976).The National Sanitation foundation (NSF),USA developed Water Quality Index (WQI) in 1970 based on the analysis of nine parameters namely DO, Faecal Coliform, BOD,pH,ΔT,phosphate, Nitrate, Nitrate, Total solids and turbidity (Stapp and Mitchell, 1995). This is found to be more suitable for testing the water quality in temperate regions like USA.Then a modified form of NSF-WQI called HWQI was formulated with nine parameters-DO, pH, BOD, Nitrate, Turbidity, Total solids, fluoride and chromium. It was founded that the inclusion of the parameters fluoride and chromium is suitable for water quality analysis in those localized areas where their concentrations are above the permissive limits (Dhermendra *et al*., 2008).

Using four of the nine parameters namely DO, pH, Nitrate and Total solids to provide a fast and reliable overall analysis of water quality index -3 (HWQI-3) was evolved (Princy *et al*., 2001) Calcium and magnesium which determine the hardness of water are considered as influence the growth and distribution of plants and animals. (Wetzel, 1975). Today a number of techniques are used for treatment of water i.e. chemical and physical agent such as chlorine and its derivatives, Ultraviolet light (Droste 1997). Boiling, Low frequency ultrasonic irradiation (Gupta *et al*., 2006) Distillation, Reverse Osmosis, Water sediment filters (fiber and ceramic) Activated carbon, Solid block, Pitcher and faucet-mount filters, Bottled water, Ion exchange water Softener, Ozonisation, Activated alumina 'Altered' Water. Halogens such as chlorine (Cl) and bromine (Br) are well known and widely used as antibacterial agents, but the direct use of halogens as bactericides has many problems because of their high toxicity and vapour pressure in pure form. The most common cation in water affecting human and animal health is NH4+. In drinking water ammonia removal is very important to prevent oxygen depletion and algae bloom and due to its extreme toxicity to most fish species (Jung *et al* ., 2004).It can be replaced with biologically acceptable cations, like Na+, K+ or Ca2+ in the zeolite. During the past few decades, several investigations have been carried out concerning the use of synthetic and natural zeolites, polymer films and metal ions (Ag+, Cu++, Zn++, Hg++, Ti+++, Ni++, Co++) as bactericides for water disinfection (Feng *et al*., 2000 ; Shearer *et al* ., 2000 ; Mc Lean *et al* ., 1993 ; Chohan *et al* ., 2004 ; Ulkuseven *et al* ., 2002 ; Chen *et al*., 2003 ; Cik *et al*., 2001 ; Islam *et al* ., 2003) . Research is underway to use advance nanotechnology in water purification for safe drinking. Nanotechnology, the deliberate manipulation of matter at size scales of less than 100 nm, holds the promise of creating new materials and devices which take scales, because of their high reactivity due to the large surface to volume ratio (Ichinose *et al*., 1992).Nanoparticles are expected to play a crucial role in water purification (Stoinmenov *et al*., 2002) The environmental fate and toxicity of a material are critical issues in materials selection and design for water purification. No doubt that nanotechnology is better than other technique used in water treatment but today the knowledge about the environmental fate, transport and toxicity of nanomaterials (Colvin 2003) is still in infancy.

An environment friendly and biosynthesis silver nanoparticles using *Avicenia marina* leaves, the formation and characterization of $AgNO_3$ were confirmed by UV-Vis spectroscopy, FTIR analysis (Packialakshmi *et al*., 2014). Research is underway to use advance nanotechnology in water purification for safe drinking. Nanotechnology, the deliberate manipulation of matter at size scales of less than 100 nm, holds the promise of creating new materials and devices which take advantage of unique phenomena realized at those length scales, because of their high reactivity due to the large surface to volume ratio (Ichinose *et al*., 1992). Nanoparticles are expected to play a crucial role in water purification (Stoimenvo *et al*., 2002). The environmental fate and toxicity of a material are critical issues in materials selection and design for water purification. No doubt that nanotechnology is better than other technique used in water treatment but today the knowledge about the environmental fate, transport and toxicity of nonmaterial's (Colvin *et al* ., 2003) is still in infancy.Advances in nanoscale science and engineering suggest that many of the current problems involving water quality could be resolved or greatly diminished by using nonabsorbent, nanocatalysts, bioactive nanoparticles, nanostructured catalytic membranes, submicron, nanopowder, nanotubes, magnetic nanoparticles, granules, flake, high surface area metal particle supramolecular assemblies with characteristic length scales of 9-10 nm including clusters, micromolecules, nanoparticles and colloids have a significant impact on water quality in natural environment (Mamadou and Savage 2005).

Nanotechnology used for detection of pesticides (Nair and Pradeep 2004) chemical and biological substances including metals (e.g. Cadmium, copper, lead, mercury nickel, and zinc), Nutrients (e.g. Phosphate, ammonia, nitrate, nitrite), Cyanide Organics, Algae (e.g. Cyanobacterial toxins) Viruses, Bacteria, Parasites, antibiotics and Biological agents are used for terrorism. Innovations in the development of novel technologies to desalinate water are among the most exciting and seem to have promis (Diallo *et al*., 2005). Opportunities and challenges of using nanomaterials in the purification of surface water, groundwater and industrial wastewater streams is a matter of continuing concern. Misconceptions and one of the many impressions that people have about the future of nanotechnology is the expectation that nanoparticles can be used to kill harmful organisms, repair body tissue, in water quality improvement and to cure disease. Recent applications of nanoparticulate silver have included open wound and burn treatment and preliminary studies have shown that a 20 ppm silver suspension (~30 nm diameter) in purified water has a 100% cure rate for malaria (Balitimore ASAP) Titanium dioxide, especially as nanoparticulate anatase, is also an interesting antibacterial, with notable photocatalytic behavior. But ultrafine anatase has also been identified as cytotoxic and *in-vivo* studies have shown that it can be severely toxic in the respiratory system (Oberdorste 2001; Ishibashi 2000). Nanocapsules and nanodevices may present new possibilities for drug delivery, gene therapy, medical diagnostics, antimicrobial activity etc. The effect of particle size on the adsorption of dissolved heavy metals to iron oxide and titanium dioxide nanoparticles is a matter laboratory-scale experiments. Iron oxide and titanium dioxide are good sorbents for metal contaminants. Spherical aggregates of nanoparticles that have a similar size and shape to the resin beads already used in water purification. Ligands, fulvic acids, humic acids and their aggregates have a significant impact on

contaminant mobility, reactivity and bioavailability. Nanoparticles can also be designed and synthesized to act as either separation or reaction media for pollutants. The high surface area to mass ratios of nanoparticles can greatly enhance the adsorption capacities of sorbent materials. Nanotechnology is a deliberate manipulation of matter at size scales of less than 100 nm holds the promise of creating new materials and devices which take advantage of unique phenomena realized at those length scales. In addition to having high specific surface areas, nanoparticles also have unique adsorption properties due to different distributions of reactive surface sites and disordered surface regions. Their extremely small feature size is of the same scale as the critical size for physical phenomena for example, the radius of the tip of a crack in a material may be in the range 1-100 nm.The way a crack grows in a larger-scale, bulk material is likely to be different from crack propagation in a nanomaterial where crack and particle size are comparable. Fundamental electronic, magnetic, optical, chemical and biological processes are also different at this level. (Dhermendra *et al*., 2008).

Objectives

- Nano technology is an exsisting fast growing are of research. Most of the studies on Bio- synthesis of nanoparticles are restricted to terrestrial organisms in particular plants, but not that much with the mangrove habitats of extreme marine environment. In order to tolerate the extrimities, the mangroves produce novel chemicals of unique biological activities (Kathiresan *et al*., 2013; kathiresan and Ranjendran 2005).Therefore, the present investigation was undertaken to study the potential of mangroves in the synthesis of nanoparticles.
- Whether, the Mangrove plants ***Avicennia marina*** capable of producing silver nanoparticles?
- If yes what is the concentration is most efficient one?
- To characters synthesized by ***Avicennia marina*** **leaf** extracts using UV- spectrum, SEM,
- FTIR and XRD
- To prepare water filter system for water disinfection.

MATERIALS AND METHODS

Material

Avicenna marina is a mangrove plant was collected from Hare Island, Thoothukudi, and East- Coast of India. It is a small tree with long creeping roots which give out at intervals narrow conical leaflets suckers (pneumatophores). Leaves opposite, coriaceous, entire, Ovate or lanceolate, flowers small, yellow sessile in capitates peduncle heads of close cymes, sometimes forming terminal trichotomous panicles; bracts small. *Calyx* short, 5 partite; lobes ovate, concave, imbricate. *Corolla*- tube short cylindrical; lobes 4, sub equal or the posterior slightly the larger. *Stamens* 4, adnate to the corolla- throat; filaments- short; anther- cells ellipsoid, parallel. *Ovary* imperfectly 4- celled the central axis 4- winged; ovules 4, pendulous between the axial wings; style tapering; stigma bifid. Fruit a compressed capsule, dehiscing by 2 leathery valves. *Seed* solitary, erect; albumen 0; cotyledons large, longitudinally plicate; radical villous; seed often germinating on the plant. (Plate – 1)

Preparation plant extract

The collected samples were washed thrice with tap water and twice with distilled water to remove the adhering salts and other associated contaminants. Take 20 g of finely cut leaves in a 500 ml Erlenmeyer flask with 100 ml of sterile distilled water and then boiled the mixture for 2 min before finally decanting it.

Screening of plant extract for silver nano particles synthesis

According to the literature studies, it is well known that the Silver nanoparticle solution has dark brown or dark reddish in colour. In Avicennia *marina* before addition of Silver nitrate solution its colour was dark grey but after its treatment with AgNo3, it colour changes to dark brown which showed the formation of Silver nano particles. For reduction of silver ions 5 ml of leaf broth was added to 45 ml of five different concentrations of (0.1mM,0.25mm, 0.5mM, 0.75mM) $AgNo_3$ in the ratio of(1:9, 2.5:7.5, 5:5,7.5:2.5). The reduction of pure silver ions was monitored by measuring the absorbance of the solution at regular intervals after diluting a small aliquot (0.2ml) of the sample 20 times. The absorption was measured at the range of (200 to 500 nm) Spectrophotometer (Elico, Chennai).Reduction process was started immediately and formation of nanoparticles was indentified from the colour change of the solution. The suspended nanoparticle was separated by centrifugation at 10,000 rpm for 2 hours. After decanting the mother liquor, the nanoparticles were thoroughly washed with distilled water and the pure particles were separated and stored at 4^0c.

Characterization of silver Nanoparticles synthesized by a mangrove *A. marina*

XRD

The X ray diffraction (XRD) measurement of silver synthesized by *A marina* was carried out using cu-ka radiation source in power diffracto meter (ANALYTICAL X' Per PRO model X – ray diffracto meter) on films of the solutions drop coated onto glass substrate in this instrument operating at a voltage of 50KV and current of 3mA.

FTIR

For FTIR analysis, 100ml of nanoparticle was centrifuged at 5ooo rpm for 10min. The suspension was again centrifuged at 10,000 rpm for 60 min and the pellet was obtained. This was followed by redispersion of the pellet of silver and silver nanoparticle into 1ml of deionized water. Thereafter, the purified suspension was freeze, dried to obtain dried powder finally; the dried nanoparticles were analyzed by FTIR.

UV-Visible Spectroscopy

According to this technique many molecules absorb ultraviolet or visible light. The percentage of transmittance light radiation determines when light of certain frequency is passed through the samples. This spectrophotometer analysis records the intensity of absorption (A) or optical density (OD 420nm) as a function of wavelength. Absorbance is directly proportional to the path length, L, and the concentration, c, of the absorbing

species. Beer's Law states that A=€ C L Where € is a constant of proportionality, called the absorbtivity coefficient.

SEM

SEM is the scanning electron microscope that creates various images by focusing a high energy beam of electrons onto the surface of a sample and detecting signals from the interaction of the incident electron with the sample's surface. SEM images have greater depth of field yielding a characteristic 3D appearance useful for understanding the morphology material. Magnification is of order 10,000 X and resolution 10 nm.

Application of nanoparticles

Water purification

Preparation of nano-coated foam filters: The measurement of 10 X10 cm and thickness of 6mm polyurethane foams were soaked in Nanoparticles solution for overnight. For the saturated coating of foams 250 ml of the nanoparticles solution was required. The sheet was washed repeatedly with water to remove any absorbed ions and was air dried.

Filteration with polyurethane foams: Normal tap water was used in the filteration process. Filteration experiments were performed in 500 ml Erlenmeyer conical flasks. The polyurethane foams, treated with nanoparticles were taken in a funnel. The funnel was packed with polyurethane foams coated with nanoparticles place in the upper ends of conical flask. The whole set up was tightly fixed to hold the water for proper filteration. The funnel was washed and sterilized under UV light for 30 min. The conical flasks were autoclaved before use. The normal tap water was pumped vertically upwards through the funnel. And the filtered water was continuously collected in conical flasks. The water was analysed for quality parameter as described below.

Assessment of water quality: The water quality of filtered and unfiltered water through nanofilter was assessed for colour visually, odour based on acceptability by involving (five voluntaries).

Determination of pH (APHA, 1976): The pH of water is measured with the help of pH meter which gives grounds to judge the properties of the water especially with reference to the presence of carbonate and bicarbonates of sodium, calcium and magnesium.

Determination of Electrical conductivity (APHA, 1976): This is measured as electrical conductivity (EC) OF the water and expressed as Micro mho/

Determination of chloride (APHA, 1965): Potassium Chromate Indicator (5%) and 0.05 N standard silver nitrate solution.

Procedure: To the solution from carbonate and bicarbonate add 1 ml of potassium chromate indicator and titrate with 0.05 N Agno3 standard solutions under bright light till the first brick red tinge appears.

Estimation of potassium: Potassium is determined in the flame photometer with 'potassium' filter. The sample is directly atomized.

Procedure: Filter the sample in polythene beaker, atomize in flame photometer and note the deflection. Express the results in mg/l

Determination of Sodium: Sodium is determined in the flame photometer with sodium filter. The sample is directly atomized.

Procedure: Atomized distilled water and the stock 100 ppm solution and set the galvanometer reading at 100 respectively. By working different strength of the solution, note the deflect on and then plot the curve. From the standard curve the value could be found out for any reading. Filter the sample if necessary in polythene beaker, atomise in flame photometer and note the deflection, express the results in mg/l

To determine the total alkalinity in water sample

Reagents:

- Sulphuric acid (0.02N)
- Sulphuric acid (0.01N)

2.8 ml of conc. H_2 SO_4 diluted to 1L using distilled water. Take 200 ml from this solution and dilute to 1L using distilled water.

Phenolphthalein indicator

1 g of phenolphthalein is dissolved in 100 ml of ethyl alcohol. After complete dissolution, add 100 ml distilled water. Add NaOH reagent (0.2272) in drops till a faint pink colour appears.

Procedure

- Estimation of alkalinity should be done immediately after the collection of the sample.
- In 5o ml of sample, add 2-3 drops of phenolphthalein indicator.
- If the solution shows pink colour, titrate it against sulphuric acid.
- Appearance of slight pink colour indicates the presence of hydroxides or carbonates whereas colourless sample confirms the presence of free CO_2.
- Note down the colourless end point as ***p***
- Now add 2-3 drops of methyl orange indicator to the same flask and proceed with the titration till the solution turns from yellow to orange.
- Record the value as t and calculate the alkalinity express the results in mg/l

To determine the total acidity in water sample

Reagents

- Sodium hydroxide (0.1N)
- Dissolve 4 g of NaOH in little distilled water and make up to one liter.
- Sodium hydroxide (0.05N)
- Take 5 ml of 0.1 N sodium hydroxide and make up to one liter.
- Phenolphthalein indicator :

- Dissolved 0.1 g of phenolphthalein in 100 ml of ethyl alcohol. To this, add 100 ml distilled water. Mix well and store for future use.

Procedure

- Transfer 100 ml of the sample in a 250-ml volumetric flask.
- To the flask content, add 3 drops of methyl orange indicator.
- Observe for the colour change from orange to yellow.
- If the solution changes to pink colour, titrate it with 0.05N Sodium hydroxide.
- The end point is sharp with a colour change from pink to yellow.
- Note the reading. Let it be m.
- Now contitue to the titrate the sample after adding. Phenolphthalein indicator
- Observe for the colour change from yellow to pink. Note the reading. Let it be *p*.

To Determination the iron content of the sample

Reagents

- concentrated hydrochloric acid (12N)
- $KMnO_4$ (0.1N)
- 3.16 g of $KMnO_4$ dissolved in distilled water and make Up to 1 l.
- Hydroxylamine hydrochloride solution
- 10 g hydroxylamine hydrochloride dissolved in distilled water. Make up to 100 ml.
- Ammonium acetate buffer solution
- 100g of ammonium acetate dissolved in 60ml distilled water. To this, add 280 ml of glacial acetic acid.
- E.Phenanthroline solution.

50g of 1, 10- phenanthroline monohydrate dissolved in 50ml distilled water. Ensure complete mixing and heat up to 80^0C in a water bath.

Standard iron solution

Dissolve 1.404 g of FAS in 20 ml of sulphuric acid dilute with 50 ml of distilled water. Add $KMnO_4$ solution in drops till faint pink colour appears (see to it that the colour retains). Make this solution to 1 l. keep this stock solution (200 to mg Fe/ I) to prepare standard iron solution from 1 to 5 mg.

Procedure

- In 50 ml of sample, add 2 ml of HCl and 1 ml of hydroxylamine hydrochloride solution.
- Mix well and heat the contents till boiling point.
- Put off the flame when the content is half of its original volume.
- After cooling, add 2 ml each of ammonium acetate buffer solution and phenanthroline solution.
- Dilute the content with distilled water and make up to 100 ml.
- Keep this flask for ten minutes and measure the absorbance on spectrometer (510 nm) using distilled water as blank.
- Repeat the same procedure for standard iron solution series and note down their respective absorbance at 510 nm.
- Using these values draw a standard curve.
- Evaluate the total iron content by comparing sample value with different dilutions of known standard solution.

To Determination the Manganese content of the sample:
Reagents

a. Manganese stock solution

Prepare 1% sulphuric acid solution. To this, add 1 g of pure manganese metal. Stir well and ensure complete dissolution. Make up this solution to one liter. One ml of this solution contains 1 mg manganese.

b. Manganese working solution: Transfer 5 ml of manganese stock solution to a 500 –ml volumetric flask. Make up to the mark using distilled water. One ml of this solution contains 1 mg manganese.

c. Special reagent: Mix 200 ml of concentrated nitric acid to 100 ml of distilled water. To this, add 37.5 g of mercuric sulphate, 100 ml of 85 % phosphoric acid and 17.5mg of silver nitrate. Stir well and make up to 500 ml using distilled water.
d. Ammonium persulphate crystals.

Procedure

- Prepare several dilutions of manganese working solution in a series of beakers with their concentrations ranging from 5 ml to 50 ml at 5-ml interval. Make up the content of each beaker to 100 ml using distilled water.
- Take 100 ml of distilled water as blank.
- Similarly pipette out 100 ml sample in a beaker.
- To all the beakers, add 5 ml of special reagent.
- Continue to heat until the content of each beaker is reduced to 40 ml.
- Now add 1 g of ammonium persulphate to each beaker and boil for another minute.
- Carefully remove the beakers from the hot plate and cool them under running tap water.
- Measure the absorbance value of all the solutions, sample, standards and blank in spectrophotometer at 545 nm.
- Draw a standard curve by plotting the absorbent value of standards against their concentrations.
- From the standard curve, evaluate the concentrations of manganese in the sample and express the results as mg/l.

Total evaluate the nitrite content of the sample using sulphanilamide

Reagents

- Sulphanilamide solution
- 1 g of Sulphanilamide dissolved in 100 ml of 10 % hydrochloric acid.
- N-1 napthyl ethylene diamine hydrochloric solution.

- g of N-1-napthyl ethylene diamine hydrochloride solution dissolved in 100 ml distilled water.
- Standard nitrite solution
- 500 mg of disodium salt of EDTA dissolved in 100 ml distilled water.

Procedure

- Add 1 ml of Sulphanilamide solution to 45 ml sample in a 50 –ml volumetric flask. Allow it to stand for 5 minutes.
- Then add 1 ml of N-1-napthyl ethylene diamine hydrochloride solution the absorbance value on. Mix thoroughly and make up to 50 ml.
- Swril the flask for 2 minutes and record the absorbance value on Spectrophotometer at nm 543.
- Run the distilled water as blank.
- Perfom the same procedure to standard nitrite solutions and note down their absorbance value.
- Draw a standard curve by plotting the concentration of nitrite solution against the absorbance values.
- Evaluate the nitrite content of the sample by comparing its absorbance value with standard curve.

Total evaluate the nitrate content of the sample using sulphanilamide

Reagents

Sulphanilamide solution

- 1 g of Sulphanilamide dissolved in 100 ml of 10 % hydrochloric acid.

N-1 napthyl ethylene diamine hydrochloric solution.

- g of N-1-napthyl ethylene diamine hydrochloride solution dissolved in 100 ml distilled water.

Standard nitrite solution

- 500 mg of disodium salt of EDTA dissolved in 100 ml distilled water.

Procedure

- Add 2 ml of Sodium chloride solution to 10 ml of sample taken in a 25 –ml volumetric flask.
- To these add carefully, 10 ml of concentrated sulpuric acid and 0.5 ml of brucine sulphanilic acid solution.
- While doing this, place the flask in a hot- water bath for 20 minutes.
- Allow it to cool and then measure the absorbance (Spectrophotometer) using distilled water.
- To the various concentration of standard nitate solutions, add reagents as described above.
- Draw a standard cutve by plotting the concentration of nitate solutions against the absorbance values.
- Evaluate the nitrite value of the sample by comparing the absorbance value with that of the standard curve.

To determine the magnesium hardness of the sample: The total hardness and calcium hardness are determined.

Calculation

Magnesium (mg /l) = (Total hardness - calcium hardness) × 0.243.

Total determine the Calcium hardness of the sample

Reagents

- NaOH solution (8%)
- 8 g of NaOH dissolved in 100 ml of distilled water.
- Murexide indicator
- Add 0.2 g of ammonium purpurate to 100 g of sodium chloride. Grind it well in a mortar and pestle.
- EDTA solution (0.01M)
- 3.723 g of disodium salt of EDTA dissolved in distilled water. Make up to 1000 ml.

Procedure

- In 50 ml sample, add 1 ml of sodium hydroxide solution and a pinch of Murexide indicator.
- Swril the flask to ensure complete mixing.
- The colour of the solution turns pink.
- Now titrate it against EDTA solution till the colour changes from pink to purple.
- Note down the end point. Repeat the Procedure till you attain the constant weight.
- Determine the calcium using the following formula.
- Express calcium hardness as $CaCO_3$ mg/l and Ca mg /l.

To determine the total hardness of the water sample:
Reagents

a. Ammonia buffer solution
Dissolve 13.5 g of ammonium chloride in 114 ml of conc. Ammonium hydroxide. Make up to 200 ml using distilled water.
b. Erichrome black ‘T ‘dye in 100 ml of ethyl alcohol (80%).
c. EDTA solution (0.001M)
3.723 g of sodium salt of EDTA in little distilled water and make up to 1000 ml. Use polythene bottle for storage.

Procedure

- In 50 ml sample, add 1 ml of ammonia buffer solution and 4 drops of Erichrome black ‘T’ indicator.
- Now the solution turns wine red in colour.
- Titrate it against EDTA solution till the colour changes from wine red to blue. (The colour changes are very sharp).
- Record the end point, repeat the procedure to attain constant value.
- Calculate the total hardness using formula.

To determine the amount of sulphates in the water sample:
Reagents

a. Methyl red indicator

Dissolve 10 g of barium chloride in 100 ml of distilled water. Filter it through whatman No. 1 filter paper and store for use.

b. Silver nitrate solution

Dissolve 8.5 of silver nitrate powder in little nitric acid solution. Make up to 500 ml using distilled water.

c. Hydrochloric acid (50%)

Add distilled water and conc. hydrochloric acid in the ratio 1:1.

Procedure

- Transfer 100 ml of sample into a 250- ml volumetric flask.
- To this, add 2-3 drops of methyl red indicator.
- Slowly add hydrochloric acid to the flask content and observe for the colour change from red to orange. (This indicates the acidic pH of the solution).
- Add still more hydrochloric acid to the flask and boil it.
- Now add warm barium chloride solution so as to favour the precipitation process.
- Continue to add it till the precipitation is completed.
- Again heat it in a hot- water bath for about 2 hours. Do not cool it. Immediately filter it through ash less filter paper.
- Flush the filter paper with distilled water many times.
- This should be repeated till the filtrate does not contain traces of chloride.
- Every time after washing the filter paper, check the presence of chloride in the filtrate by filterating it against silver nitrate solution. Absence of turbidity indicates nil chloride content and at this stage transfers the filter paper to a silica crucible.
- Ignite it in a muffle furnace at 80^0C for 1 hour. After one hour take the weight of the precipitate.

To determine the concentrations of fluorides of the water sample

Reagents

a. Fluoride stock solution

To a 100- ml volumetric flask, add 0.221 g of anhydrous sodium fluoride. Make up to mark using distilled water.

b. Fluoride standard solution

Transfer 100 ml of the stock solution in a 1000- ml volumetric flask using distilled water. Make up to mark. One ml of this solution contains 0.01 mg fluorine.

c. Acid zirconium __ Alizarin solution

i. dissolve 0.7 g sodium alizarin sulphate in 100 ml distilled water.
ii. dissolve 0.45 g zirconyl chloride in 100 ml distilled water.
iii. Sulpuric acid solution: carefully add 70 ml of conc. sulphuric acid to 700 ml distilled water. Mix well and cool.

Add solution (ii) to (i), stir well and finally add solution (iii).Make up the mixed solution to a final volume of 1000 ml.

d. Sodium arsenate solution

To 100 ml distilled water, add 1g of sodium arsenate, stir well and store it in amber bottle.

Procedure

- Prepare several dilutions of fluoride standard solution into a series of 50-ml Nessler's tubes with their concentrations ranging from 1 ml to 12 ml at 2-ml interval.
- Shift the pH of each solution to neutral pH (pH 7) and Make up the volume of each tube to 50 ml using distilled water.
- Take 50 ml of distilled water as blank and the prepared sample in separate tubes.
- Add 1 ml of acid zirconium __ alizarin solution to the standards, sample and blank. Stir well and measure their absorbance.
- Draw a standard graph by plotting their absorbance value against concentrations.
- From the calibration curve, evaluate the concentration of fluoride in the sample.

Note

Residual chlorine interferes with the analysis and can be eliminated using sodium arsenate.

Total evaluate the phosphorus content of the sample

Reagents

- Perchloric acid
- Phenolphthalein indicator
- Sodium hydroxide

Procedure

- To 25 ml of the sample add 1 ml of ammonium molybdate and 3 drops of stannous chloride solution.
- Observe for the appearance of blue colour.
- After 10 minutes, to check the persistence of colour produced, measure the absorbance at 690 nm Spectrophotometerlically.
- Conduct a blank using distilled water.
- To the different dilutions of standard phosphate solution (0.1 to mg/), add 1 ml of ammonium molybdate and 3 drops of stannous chloride solution.
- Measure the absorbance for each dilution.
- Draw a standard graph by plotting the concentration of phosphorous solution against the absorbance value.
- Evalute the total inorganic phosphorous content of the sample by comparing its absorbance value with the standard curve.

To determine the Phenolphathalein alkalinity in water sample

Reagents

a. Sulphuric acid (0.02N)
b. Sulphuric acid (0.01N)

2.8 ml of conc. H_2SO_4 diluted to 1 l using distilled water. Take 200 ml from this solution and dilute to 1 L using distilled water.

c. Phenolphthalein indicator:

1 g of phenolphthalein is dissolved in 100 ml of ethyl alcohol. After complete dissolution, add 100 ml distilled water.

Add NaOH reagent (0.2272) in drops till a faint pink colour appears.

Procedure

- Estimation of alkalinity should be done immediately after the collection of the sample.
- In 5o ml of sample, add 2-3 drops of phenolphthalein indicator.
- If the solution shows pink colour, titrate it against sulphuric acid.
- Appearance of slight pink colour indicates the presence of hydroxides or carbonates whereas colourless sample confirms the presence of free CO_2.
- Note down the colourless end point as ***p***
- Now add 2-3 drops of methyl orange indicator to the same flask and proceed with the titration till the solution turns from yellow to orange.
- Record the value as t and calculate the alkalinity using the formula.

RESULT AND DISCUSSION

Effect of time duration on nanoparticles synthesis

The incubated reaction mixture which turned from initial colour to required colour was considered as the time taken for nanoparticles synthesis.

Visual Observation

The synthesis of silver ions in aqueous solution was confirmed both measuring the change of colour through absorption measurement by UV - Vis spectrophotometer at regular intervals (0-72 hrs).Wavelength between (200- 500nm).

Visual observation and UV and visible Spectrophotometer analysis

The intensity of the colour gradually changed to light brown then to dark brown. In the 1mm concentration (Table-1) (Fig-1) (Plate-2).

Table 1. Optical density at 420 nm, as measures of silver nanoparticle synthesis by *A marina* at different concentration of $AgNO_3$

Sl.NO	different concentration of $AgNO_3$in nm	OD (420nm)
1	0.1	0.313
2	0.25	0.325
3	0.5	0.371
4	0.75	0.392
5	1.0	0.401

Screening of silver nano particles synthesis in different time intervals

According to the literature studies, it is well known that the Silver nanoparticle solution has dark brown or dark reddish in colour. In *Avicennia marina* before addition of Silver nitrate solution its colour was dark grey but after its treatment with AgNo3, it colour changes to dark brown which showed the formation of Silver nano particles. The colour intensity of the synthesized Silver nanoparticles increased time duration, maximum level of OD value (1.101) after 1440 minutes (24 hrs). (Table -2) (Fig -2)

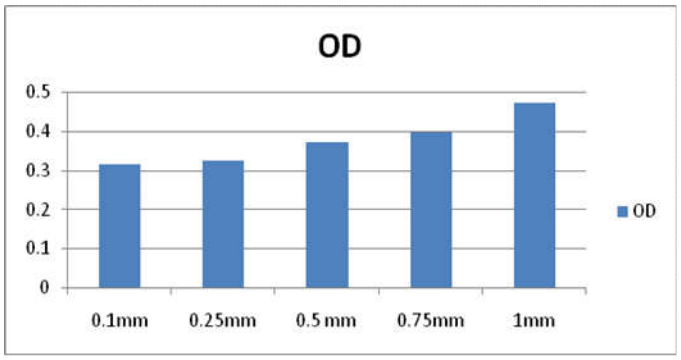

Fig. 1. Visual observation and UV and visible Spectrophotometer analysis

Table 2 Optical density at 420 nm, as measures of silver nanoparticle synthesis by *A marina* at different time intervals

Sl.NO	Time intervals in minutes	OD (420nm)
1	10	0.721
2	20	0.729
3	30	0.741
4	40	0.753
5	50	0.862
6	60	0.879
7	120	0.972
8	240	1.001
9	1440	1.101
10	2880	1.100
11	4320	1.098

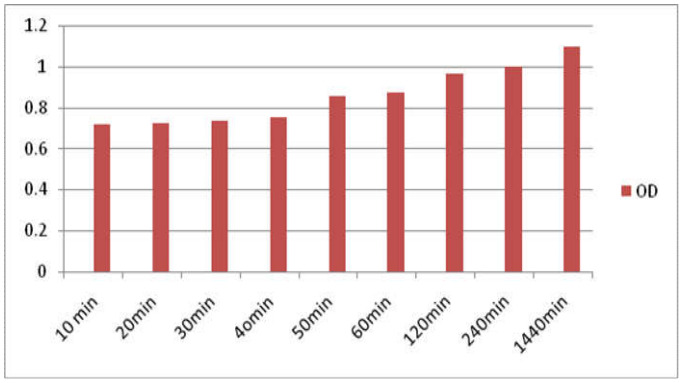

Fig. 2. Screening of nano particles synthesis were studied by visual observation

Characterization of silver Nanoparticles synthesized by a mangrove *A. marina*

UV- Vis Spectrophotometer Analysis

The silver nanoparticles were characterized by UV-Vis spectrophotometer, one of the most widely used techniques for structural characterization of silver nanoparticles (Sun *et al*., 2001). The reduction of Silver irons in to silver nanoparticles by using *Avicennia marina* the observation of the yellowish brown silver nanoparticle solution prepared with the proposed method showed a surface Plasmon absorption band with maximum of 420 nm. Indicating the presence of spherical silver nanoparticles (Plate–3). This structure was further confirmed by SEM images.

Table 3. Wave number (cm^{-1}) of dominant peak obtained from absorption spectrum from *A marina* leaf extract

Peak	Bond	Functional group
615	C-Br stretch	Alkyl halides
1078	C-N stretch	Aliphatic amines
1385	-	-
1556	-	-
1653	N-H bend	Primarminesy a
2922	C-H stretch	Alkanes
3442	0-H stretch	Alcohols, Phenols
3743	-	-
3850	-	-

X-RAY Diffraction analysis

XRD analysis is used to determine the phase distribution, crystalline and purity of the synthesized nanoparticles. Fig-4 shows the XRD patterns of *Avicennia marina*, With reference to the JCPDS data file No. 04-0783 it was concluded that the nanoparticles were crystalline in nature having spherical shape with no such impurities. (Figure 3).

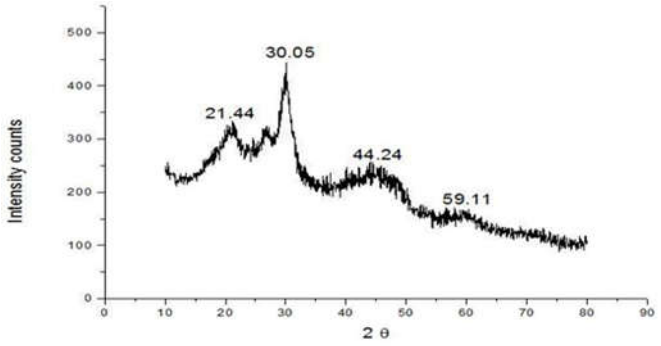

Fig. 3. X- Ray diffraction pattern of prepared silver nanoparticles using *Avicennia marina*

FTIR- Fourtier Infra-Red spectroscopy Analysis

FTIR gives the information about functional group present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic AgNO3 to elemental silver by the action of the different phytochemical which would act simultaneously as reducing, stabilizing and chapping agent. FTIR spectrum clearly indicates the biofabricates the silver nano particles mediated by the plant extracts.(Figure 4 & Table 3).

Scanning Electron Microscope (SEM) Analysis

To determine the morphology of the synthesized silver nanoparticles the sample was analyzed with Scanning electron microscope (SEM). The redispersed silver nanoparticles were dried in an oven for 2 hours to obtain a powdered form. Then, 5 mg of the sample was redispersed in ethanol and the sample was prepared in thin films on carbon coated copper grid. EDX analysis was used to identify nano articles in solution. EDS measurements were preformed on a Compact Detector Unit (CDU) (EDAX, Mahwah, NJ, USA) incorporated into a Hitachi S3000N SEM (scanning electron microscope). The particle solution was diluted 100-fold in water and a drop of 10 µl diluted solution was placed on a carbon stub and air dried. The EDS spectrum was obtained at an acceleration voltage of 20 keV and collected for 19 s. Mapping was completed using pseudo-colors to represent the two-dimensional spatial distribution of energy emissions from the chemical elements present in the sample SEM analysis revealed that synthesized nanoparticles was in the range of 15-25nm (Plate -5).

Application of silver nanoparticles

Water Purification: As shown in Table – 4 and 5. The physico chemical characteristics of control and nanoparticle

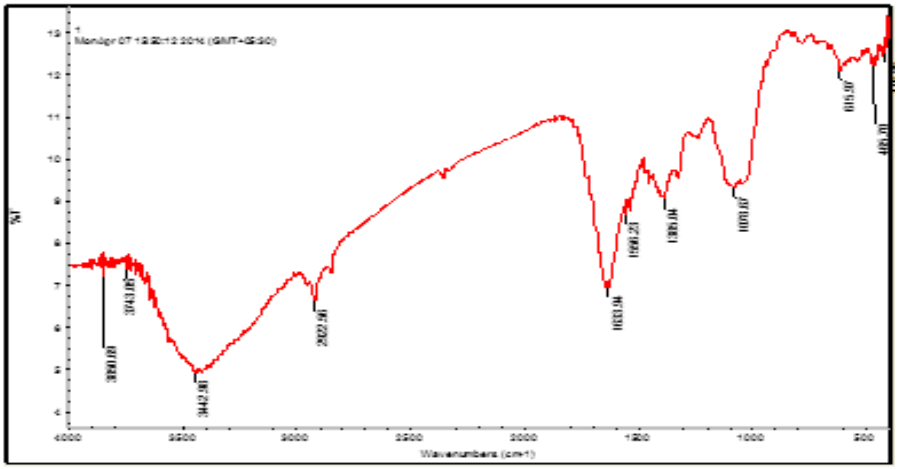

Figure 4. FTIR analysis of silver nanoparticles using *Avicennia marina* extract

Table-4 Water purification potential of biosynthesized silver nanoparticles using *A.marina* leaf extract - Physical Examination of Water Samples

Sl. No	Physical examination	Acceptable limit	Permissible limit in the absence of alternate source	RESULT	
				Control	Sample treated with AgNO3particles
1	Appearance	-	-	Clear	Clear
2	Colour	-	-	Colourless	Colourless
3	Odour	Agreeable	Agreeable	None	None
4	Turbidity NT Unit	1	5	20	0.2
5	Temperature C^0	-	-	33	35.37
6	Total Dissolved Solids mg/L	500	2000	1156	777
7	Electrical conductivity Micro mho	-	-	1699	1142

Table 5. Water purification potential of biosynthesized silver nanoparticles using *A.marina* leaf extract – Chemical Examination of Water Samples

Sl. No	Physical examination	Acceptable limit	Permissible limit in the absence of alternate source	RESULT	
				Control	Sample treated with AgNO3particles
1	pH	6.5 – 8.5	6.5 -8.5	6.8	7.2
2	Total alkalinity mg/L	200	600	324	212
3	Total Hardness mg/L	200	600	424	248
4	Calcium mg/L	75	200	85	76
5	Magnesium mg/L	30	100	51	15
6	Sodium mg/L	-	-	164	116
7	Potassium mg/L	-	-	16	12
8	Iron mg/L	0.3	0.3	0.05	0.12
9	Nitrate mg/L	-	-	9	3
10	Chloride mg/L	250	1000	232	160
11	Fluoride mg/L	1.0	1.5	1	1
12	Sulphate mg/L	200	400	104	71
13	Phosphate mg/L	-	-	0.48	0.19
14	Tidys Test 4 hrs. as $O_{2\ mg/L}$	-	-	0.52	0.68

treated samples was clear, colourless and odourless.The water samples of both Control and biosynthesis silver nanoparticles treated samples were clear, colourless and odourless. TDS were under permissible limit. After treatment pH of the sample becomes neutral. Micro as well as Macro nutrient of the samples under acceptable limit.

Plate 1. Avicennia marina

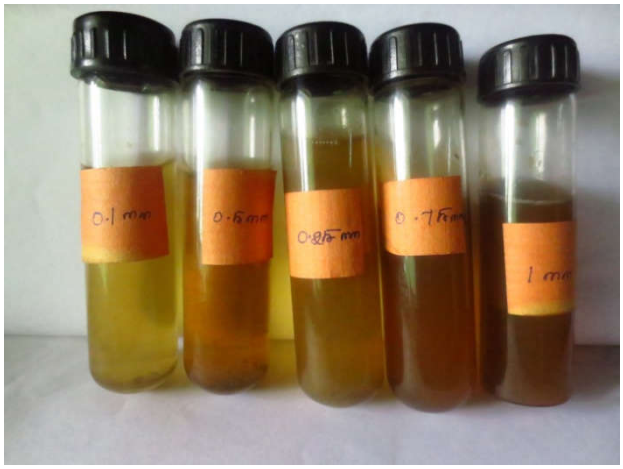

Plate 2. Biosynthesized silver nanoparticles of *A. marina* leaf extract

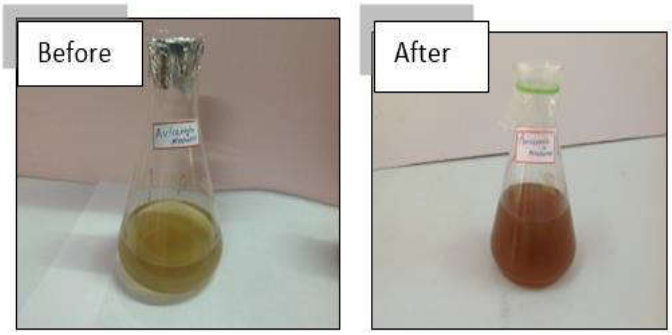

Plate 3. Visual observation

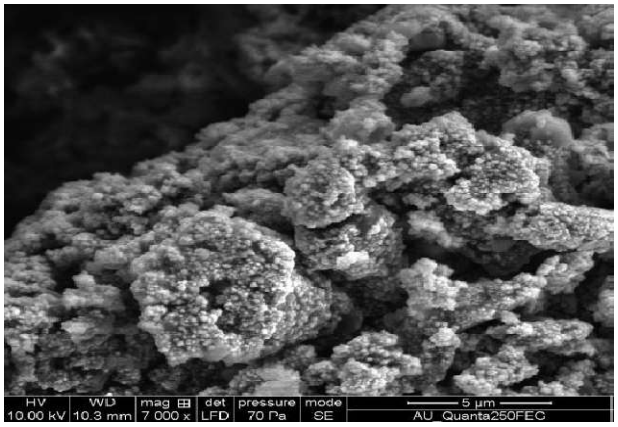

Plate 4. Scanning Electron Microscope (SEM) Analysis

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