JIGNASA STUDENT STUDY PROJECT (2021-2022)

Name of the Topic:

Green synthesis of silvernanoparticles using Achyranthes and its antimicrobial activity.

Abstract

Stable silver nanoparticles have been synthesized using achyranthes acting as both reducing and stabilizing agent without using any synthetic reagent. The reaction is performed using water, which is an environmentally safe solvent. The influence of different parameters such as time, change of concentration of silver nitrate and concentration of achyranthes on the formation of silver nanoparticles has been studied.

The synthesized silver nanoparticles are characterized by UV–Vis spectroscopy. UV–Vis analysis of the sample confirmed the formation of silver nanoparticles exhibiting a sharp peak at a wavelength of 420 nm. The antimicrobial activity of silver nanoparticles stabilized in achyranthes is tested against *Escherichia coli*, *Staphylococcus aureus*,and is found to be possessing inhibiting property. The antifungal activity of these particles against *C. albicans* and *C. tropicalis* was also evaluated.

Under the Guidance of

R. Shyamala Chandra

Asst.Prof of Biochemistry

DEPARTMENT OF BIOTECHNOLOGY

Kakatiya Government College , Hanamkonda.

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Names of the Students

S.No	Name of the Student	H.T.No	Group
1	**M.Bhavani**	006-20-3013	**3rd Yr BTZC**
2	**T.Mahesh**	006-20-3111	**3rd Yr BTZC**
3	**M.Abhinandan Goud**	006-20-3012	**3rd Yr BTZC**
4	**Swarna Priya**	006-21-3109	**2nd Yr BTZC**
5	**P.Parameshwar**	006-22-	**1st Yr BTZC**
6	**Udaya Krishna**	006-22-	**1st Yr BTBC**

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Introduction:

Background

The field of nanotechnology is one of the most active research areas in modern materials science. Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution and morphology. There have been impressive developments in the field of nanotechnology in the recent past years, with numerous methodologies developed to synthesize nanoparticles of particular shape and size depending on specific requirements. New applications of nanoparticles and nanomaterials are increasing rapidly. Nanotechnology can be termed as the synthesis, characterization, exploration and application of nanosized (1-100nm) materials for the

development of science. It deals with the materials whose structures exhibit significantly novel and improved physical, chemical, and biological properties, phenomena, and functionality due to their nano scaled size. Because of their size, nanoparticles have a larger surface area than macro-sized materials. The intrinsic properties of metal nanoparticles are mainly determined by size, shape, composition, crystallinity and morphology. Nanoparticles, because of their small size, have distinct properties compared to the bulk form of the same material, thus offering many new developments in the fields of biosensors, biomedicine, and bio nanotechnology. Nanotechnology is also being utilized in medicine for diagnosis, therapeutic drug delivery and the development of treatments for many diseases and disorders. Nanotechnology is an enormously powerful technology, which holds a huge promise for the design and development of many types of novel products with its potential medical applications on early disease detection, treatment, and prevention.

Silver Nanoparticles

Silver nanoparticles are one of the promising products in the nanotechnology industry. The development of consistent processes for the synthesis of silver nanomaterials is an important aspect of current nanotechnology research. One of such promising process is green synthesis. Silver nanoparticles can be synthesized by several physical, chemical and biological methods. However for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality.

Importance of Silver nanoparticles

1) It is used for purification and quality management of air, biosensing, imaging, drug delivery system.

2) Biologically synthesized silver nanoparticles have many applications like coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling, and as antimicrobials.

3) Though silver nanoparticles are cytotoxic but they have tremendous applications in the field of high sensitivity bimolecular detection and diagnostics, antimicrobials and therapeutics, catalysis and micro-electronics.

4) It has some potential application like diagnostic biomedical optical imaging, biological implants (like heart valves) and medical application like wound dressings, contraceptive devices, surgical instruments and bone prostheses.

5) Many major consumer goods manufacturers already are producing household items that utilize the antibacterial properties of silver nanoparticles. These products include nanosilver lined refrigerators, air conditioners and washing machines.

Silver nanoparticles as an antimicrobial agent

Ag NPs are highly antimicrobial to several species of bacteria, including the common kitchen microbe, E. coli. According to the mechanism reported, silver nanoparticles interact with the outer membrane of bacteria, and arrest the respiration and some other metabolic pathway that leads to the death of the bacteria.

Application of Nanoparticles

Once materials are prepared in the form of very small particles, they change significantly their physical and chemical properties. In fact in nano-dimension, percentage of surface molecule compare to bulk molecule is high and this enhances the activity of the particle in nano dimension and therefore, the normal properties of the particle like heat treatment, mass transfer, catalytic activity, etc are all increases. But compare to non-metal nanoparticles, metal nanoparticles have more industrial application. Nanoparticles offer many new developments in the field of biosensors, biomedicine and bio nanotechnology-specifically in the areas

- Drug delivery
- As medical diagnostic tools,
- As a cancer treatment agent (Gold nanoparticles)

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particle structures ranging from approximately 1-100 nm. Nanoparticles (NPs) have wide range of applications in areas such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single electron transistors, light emitters, nonlinear optical devices, and photo-electrochemical applications .

Nowadays, there is a growing need to develop eco-friendly processes, which do not use toxic chemicals in the synthesis protocols.

The use of naturally occurring biocompatible plant materials has been the focus of recent research activity in the synthesis of silver nanoparticles and evaluate for its antimicrobial and anti cancer activity.

Synthesis of NPs using ecofriendly green synthesis methods has proven to be more beneficial over the hazardous chemical based methods.

Materials and Methods

Plant Material

Achyranthes aspera (common names: chaff-flower, prickly chaff flower, devil's horsewhip, Sanskrit:□□□□□□□□apāmārga) in telugu Utthareni: □□□□□□□□• This is a species of plant in the family Amaranthaceae. It is distributed throughout the tropical world .

Importance of achyranthes

Achyranthes aspera is a traditional and essen tial medicinal weed at all over india. All parts of this plant which includes leaves,seeds,roots have been used in both traditional and medicinally.Anchyranthes asprea plant and seeds are rich in carbohydrates and protines,fattyacids and different aminoacids are the chemical components.

- ❖ Anti fungal
- ❖ Anti bactiral
- ❖ Anti – allergic
- ❖ Anti asthmatic properties to treat various types of health essius

• Achyranthes used in the treatment of

asthama,cold,cough,headache,dogbite,snakebite,scropienbite & skin diseases

• The excessive intake of this plant supplements can causes vomiting

Synthesis of silver nanoparticles from achyranthes leaves extract

Collection of Plant Materials

Fresh leaves of achyranthes plant, that is, free from diseases were collected from Kakatiya Government college campus and then washed thoroughly 2-3 times with tap water and once with sterile water. Dried leaves of achyranthes were collected and made it to powder by using morter & pestle.

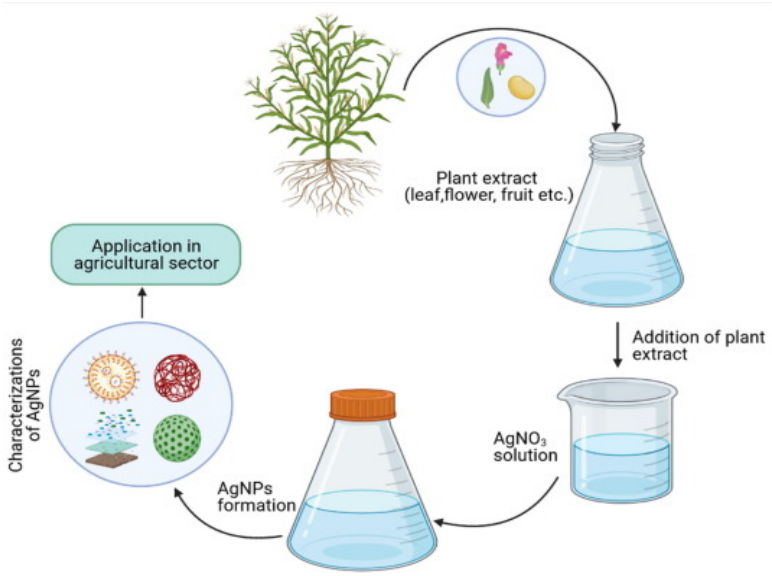
Preparation of the Extract

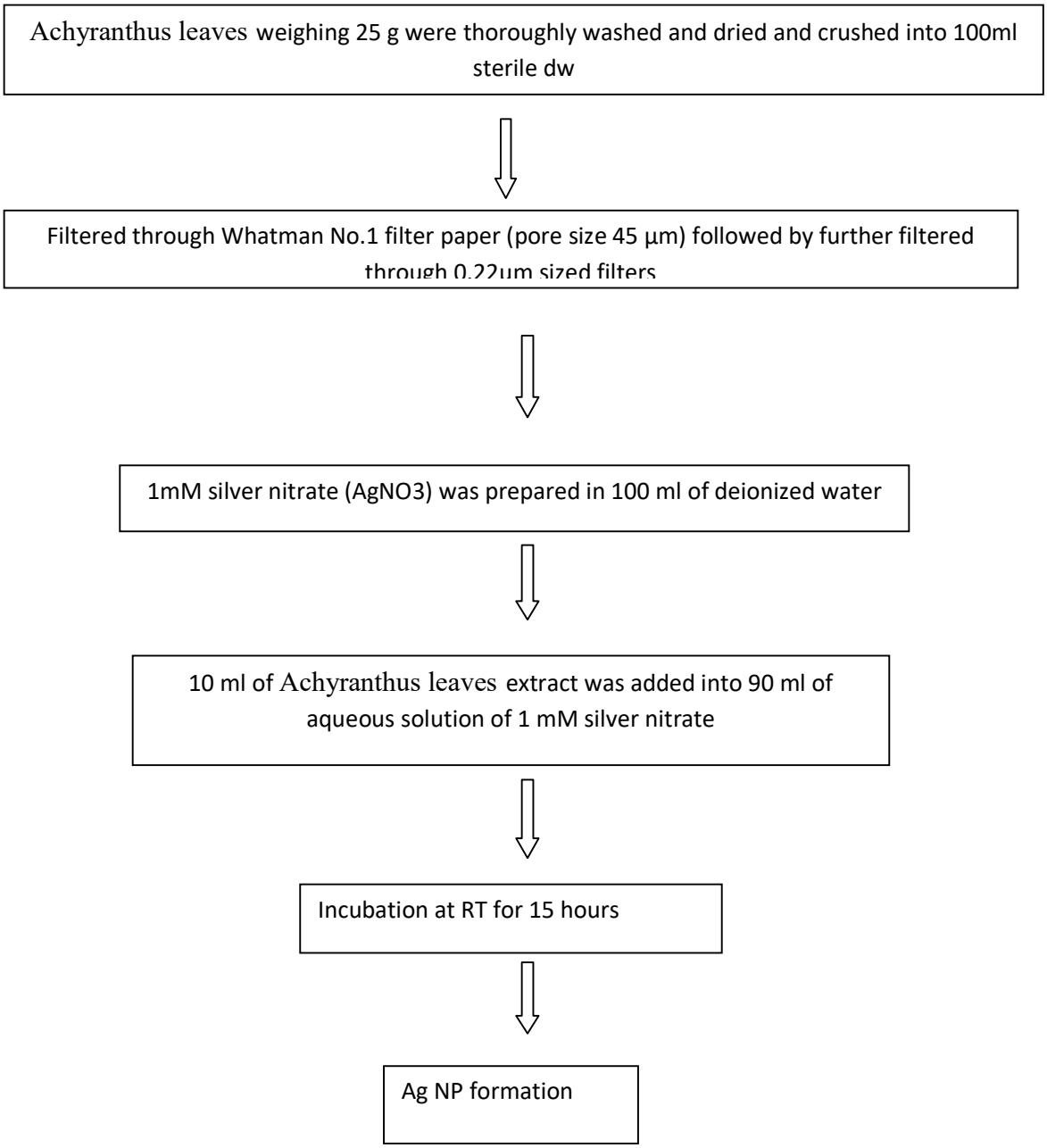
Weighing 25 g of achyranthes leaves was thoroughly washed in distilled water, dried, cut into fine pieces and was smashed into 100 ml sterile distilled water and filtered through Whatman No.1 filter paper (0.45 μm) and was further filtered through 0.22 μm sized filters. The extract was stored at 4^0 C for further experiments.

Synthesis of Silver nanoparticles from achyranthus leaves extract

The aqueous solution of 1mM silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 10 ml of achyranthes leaves extract was added into 90 ml of aqueous

solution of 1 mM silver nitrate for reduction into Ag+ ions and kept for incubation period of 15 h at room temperature. Here the filtrate acts as reducing and stabilizing agent for 1 mM of AgNO3.





Schematic diagram of synthesis of Ag NP.

Preparation of 1mM Silver nitrate solution:

Composition:

Silver nitrate 99.5%

Chloride 0.0005%

Sulfate 0.005%

Iron 0.002%

Preparation:

Molarity is moles per liter. Since the molar mass of AgNO3 is 169.87g/mol, a 1 M solution of AgNO3 would be 169.87 g (1 mole of AgNO3) in 1 liter. For preparation of 200ml of solution 0.034g of silver nitrate is taken.

Characterization of silver nanoparticles:

UV-Visible Absorbance Spectroscopy

Synthesis of silver nanoparticles by reducing the silver ions solutions with achyranthus leaves extract may be easily absorbed by UV-visible spectroscopy. The absorption spectra of leaves extract quantities and metal concentration were measured using 200-1000 range.

UV-Visible spectroscopy analysis was carried out on a Systronic UV-Visible absorption spectrophotometer 117 with a resolution of ±1 nm between 200 and 1000 nm processing a scanning speed of 200 nm/min. Equal amounts of the suspension (0.5 mL) were taken and analysed at room temperature. The progress of the reaction between metal ions and the leaf extract was monitored by UV-Visible spectra of silver nanoparticles in aqueous solution with different wavelength in nanometers from 200 to 800 nm. The reduction of silver ions and formation of silver nanoparticles occurred within an hour of reaction. Control was maintained by using $AgNO_3$.

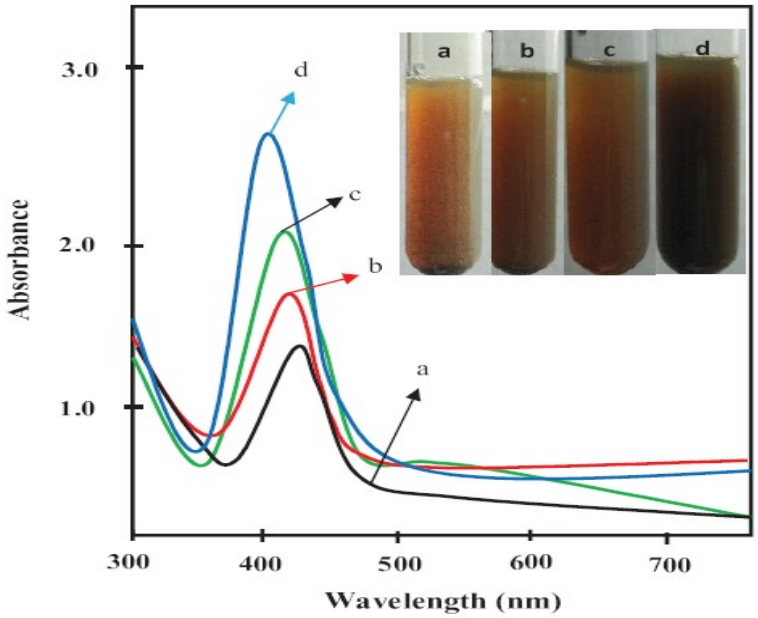


Fig:1 UV-Vis Spectra of Silver nanoparticles using achyranthus leaf extract of different concentrations (a) 2.0 ml (b) 2.5 ml (c) 3.0 ml (d) 3.5 ml

Antibacterial Assay

Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts . In this method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the silver nanaparticles extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial(silver nanoparticles) agent diffuses in the agar medium and inhibits the growth of the microbial strain tested

Composition of MHA media:

Ingredients	In Gram/Litre
Beef Extract	2.00 gm
Acid Hydrolysate of Casein	17.50 gm
Starch	1.50 gm
Agar	17.00 gm
Distilled Water	1000 ml

Final pH 7.3 ± 0.1 at 25ºC

Preparation of MHA

1. Suspend 38 gm of the medium in one liter of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Pour cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth.
5. Allow to cool to room temperature.
6. Check for the final pH 7.3 ± 0.1 at 25ºC.
7. Store the plates at 2-8 ºC.

Antibacterial activity

All equipment and growing media were sterilized by autoclaving at 115°C and 15 psi for 30 minutes.The antibacterial assays were done pathogens like nosocomial pathogens such as *Staphylococcus aureus*, *and Escherichia coli*, by standard agar well diffusion method. Briefly Luria Bertani (LB) agar medium was used to cultivate bacteria. Fresh overnight culture of inoculum (100 μL) of each culture was spread on to Mueller Hinton Agar (MHA) plates. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the silver nanoparticles extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial(silver nanoparticles) agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.The plates were incubated at 37^0 C overnight. Next day the inhibition zones around the discs were measured.

Table .1

Diameter Zone of Inhibition by Ag NPs ,Extract and AgNO3 against human pathogenic bacteria.

S.No	Pathogenic bacteria	Zone of Inhibition		
		Ag NPs	Extract	$AgNO_3$
1	Escherichia coli	14.2	10.9	7.2
2	Staphylococcus aures	15.8	11.7	6.4

Results

The reduction of silver nitrate using the plant leaf extract was viewed by the colour change in the reaction solutions .In UV-V is spectra recorded for the reaction solution of reduced silver nitrate by leaf extract of achyranthes The maximum absorbance peak was seen at 420 for achyranthus.

Discussion

The formation of silver nanoparticles using plant leaf extract of *acyranthes* was viewed by the colour change from colourless to yellowish brown. By using UV-Visible spectrum the maximum absorbance peak for *achyranthus* was seen at 420nm. The bioreduction of Ag^{+} ions to silver nanoparticles is due to the reduction by capping material of plant extract. Similarly, Gole et al. reported that proteins present in the extract can bind to silver nanoparticles through either free amino or carboxyl groups in the proteins. Prasad et al. reported that the carboxyl (–C=O), hydroxyl (–OH), and amine (–NH) groups of leaf extracts are mainly involved in fabrication of silver nanoparticles. Silver nanoparticles obtained from the *Achyranthus* have very strong inhibitory action against *Staphylococcus aureus*, *and Escherichia coli*, The silver nanoparticles of *achyranthus was* against the pathogens growth which was inhibited.

Conclusion

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. Silver nanoparticles play a profound role in the field of biology and medicine due to their attractive physiochemical properties. In the present study, we have demonstrated that use of a natural, low cost biological reducing agent and *achyranthus* leaf

extract can produce metal nanostructures, through efficient green nanochemistry methodology, avoiding the presence of toxic solvents and waste. The biosynthesized silver nanoparticles using *achyranthus* leaf extract proved to be excellent against nosocomial pathogens The antimicrobial activity is well demonstrated by well diffusion method. The present study showed a simple, rapid, and economical route to synthesize silver nanoparticles. The use of *achyranthus*

has the added advantage that this plant can be used by nanotechnology processing industries. Prepared nanoparticles can be used as bactericidal and, in wound healing, water purification, and also in the field of medicine due to these applications, this method is potentially exciting for the large-scale synthesis of nanoparticles.

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