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ANTIOXIDANT, ANTIMICROBIAL AND SEWAGE TREATMENT OF SYNTHESISED SILVER NANOPARTICLES FROM LEAF EXTRACT OF *HYGROPHILA AURICULATA (SCHUMSCH) HEINE*.

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Abstract:

In present study silver nanoparticles were synthesized from aqueous leaf extract of ***Hygrophila auriculata*** *plant leaf extracts will be collected were used and compared for their extracellular of metallic silver nanoparticles. Stable silver nanoparticles formed to be treated aqueous by solution of $AgNO_3$ with the plant leaf extracts as reducing agent Ag-to Ag^+. UV spectroscopy is used to monitor the quantitative formation of silver nanoparticles. The synthesized silver nanoparticles is characterized with FT-IR, XRD, SEM and EDX Characterization by the ab ove said instrument analysis confirmed the presence, size and stability of the silver nanoparticles. The silver nanoparticles were spherical in shape with particle size range from 5 to 40 nm. After characterization, the silver nanoparticles were tested at various concentrations to check their bactericidal activity against clinical isolates of five bacterial pathogens. The silver nanoparticles exhibited good bactericidal activity at all concentration against all the tested organisms. Maximum zone of inhibition was of observed against Vibrio cholera (18mm at 400μg) and minimum level of antibacterial activity was observed against Proteus mirabilis (8mm at 100μg). This result suggested the potential use of silver nanoparticles against other clinical pathogens.*

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INTRODUCTION:

Nanotechnology is an important modern research field and a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. Silver is a healthy in traditional Chinese and Indian Ayurveda system of medicine.. Biological diversity can be thus used as a major resource for biotechnological products and processes, which may be suitable for large scale synthesis. Nowadays, there is a growing need to develop eco-friendly processes, which do not use toxic chemicals in the synthesis protocols. Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. The major advantage of using plant extracts for Silver nanoparticles synthesis is that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. The first use of the concept in nanotechnology was in "there is plenty of room at the Bottom" a talk given by physicist Richard Fynman at an American physical society meeting at Caltech on December 29, 1959.

The biological and medical research communities have exploited the unique properties of nanomaterials of various applications. Term such as biomedical nanotechnology, biotechnology and nanomedicine are used to describe the hybrid field. The integration of nanomaterials with biology has led to the development of diagnostic device, contrast agent, analytical tools, physical therapy application and drug delivery vehicle**.**

Nanotechnology on chip id more dimension of lab of a chip technology. Magnetic nanoparticles, bound to a suitable antibody are used to label specific molecules, structures or microorganism. Gold nanoparticles tagged with short segments of DNA can be used for the detection of genetic sequence in a sample. Nanopore technology for the analysis of nucleic acids converts strings of nucleotides directly into electronic signatures.

Medicinal plants are nature of gifts to cure more than number of diseases in human beings [1]. The very large number of plants on the earth's surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new therapeutic agents[2]. *Hygrophila auriculata (K. Schum) Heine (*synonym*: Asteracantha longifolia Nees,Barleria auriculata Schum, Barleria longifolia Linn*), is described in the Ayurveda Literature as Ikshura, Ikshagandha and Kokilasha having eyes like the kokila or the Indian Cuckoo. The plant is a sub shrub, usually growing in marshy places along water courses. The stem is reddish brown and the shoot has 8 leaves and six thorns at each node. In India being used as vegetable in some states like Odisha, Chhattisgarh and West Bengal. The seeds are used as ingredients in various aphrodisiacs and tonic confections, and in the treatment of blood disorders, biliousness, gonorrhoea, spermatorrhea and fever. The seeds are ground into a paste and given in buttermilk to cure diarrhea. AKSIR-ULIMRAZ, a preparation having Talamkhana (seeds) as one of the ingredients, is used to prevent leukorrhea. The ashes of the plant are also used against dropsy and gravel. A tincture of the whole plant is beneficial in urinary affections, dysuria, and painful micturition. A root decoction drunk to combat rheumatism, gonorrhea, and hepatic obstruction. The leaves are diuretic, sweet, tonic, aphrodisiac, hypnotic and useful in the treatment of cough, diarrhoea, thirst, urinary calculi, urinary discharges, inflammations, joint pain, eye diseases, pains, ascites, anemia, and abdominal disorders. An aqueous extract of the herb is taken orally as diuretic, spasmolytic and hypotensive. The herb exhibits anti-hepatotoxic activity in dogs. The oil extracted from the whole plant is antibacterial. [3]. The pre-flowering or flowering succulent aerial parts are boiled and consumed by the rural people of these states to increase the haemoglobin level Hygrophila stimulates the male genital system and is beneficial in the treatment of sexual debility, premature ejaculation and erectile failure. It is also a potent remedy for kidney stones. The plant is a wild herb commonly found in moist places on the banks of rivers, ditches and paddy fields throughout India, Sri Lanka, Burma, Malaysia and Nepal. Following various folk claims as a cure for numerous diseases, efforts have been made by researchers to verify the efficacy of the plant by scientific biological screening. The plant Contains various groups of phyto-constituents viz. phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, alkaloids, enzymes, amino acids, carbohydrates, hydrocarbons, flavonoids, terpenoids, vitamins, glycosides, etc. and is useful in the treatment of anasaraca, diseases of urinogenital tract, dropsy of chronic Bright's disease, hyperpiesia, vesicle calculi ,flatulence, diarrhoea, dysentery, leucorrhoea, gonorrhoea, asthma, blood diseases, gastric diseases, painful micturition, menorrhagea, etc [3-7]. To study the synthesis of silver nanoparticles on plant extract ,characterization of silver nanoparticles by UV-Vis spectroscopy analysis,FTIR analysis,XRD analysis,SEM analysis and EDAX analysis,study of the antimicrobial activity of silver nanoparticles by

agar well diffusion assay method ,the ability to kill the microorganism on silver nanoparticles on serial dilution technique by spread plate method and to study the anti-oxidant activity of silver nanoparticles by DPPH assay method.

METERIALS AND METHODS:

Plant Collection

Fresh leave *Hygrophila auriculata (K. Schum) Heine* plant collected in Thenampillai at Trichy, Tamil Nadu, and India in the Month of December 2015.

Preparation of Plant Extract

The fresh leaves were washed with running tap water in 15 minutes and dry in show at room temperature for one week. The leaves are cut into small pieces and made into fine powder, 20g of the powder are weighted and dissolved in 100ml distilled water in a 500 mL Erlenmeyer flask and boiled for 30 min. The extract was filtered with glass cloth before using Whatman No.1 filter paper, and were stored in an airtight container and kept away from sunlight for further use.

Preparation of Silver Nanoparticles

The silver nitrate was purchase from the fish scientific company. 1mM of Silver nitrate ($AgNo_3$) was prepared in 1000ml bottle. The 100ml leave extract were mixed with 900ml silver nitrate solution in (1:9) ratio. The pH was found to be 5.62. Then the mixture was kept in dark condition for 12 hours. The colour of the solution changed from yellow to brown indicating that the silver nanoparticles were synthesized. Then it was centrifuged at 7000 rpm for 15 min at 28ºc. The pellet was collect and kept in hot air oven to dry.

Characterization of Silver Nanoparticles

The characterization of silver nanoparticles was carried out by different instrument and technique. It includes visual observation, UV- Vis Spectrophotometer, FTIR, XRD, SEM, EDAX.

UV- VIS Spectrophotometer

To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken 300 to 600 nm using a UV–Vis spectrophotometer. The deionized water was used as the blank.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis were carried out to identify the possible biomolecules responsible for reduction of the Ag^+ ions and capping of the bio reduced silver nanoparticles synthesized by crude plant extract. The residue was dried and mixed with potassium bromide (KBr)

X-Ray diffraction analysis (XRD)

The X-ray diffraction pattern indicated the crystalline structure of silver nanoparticles. The dried fine crystalline powder was need for XRD analysis .The XRD spectrum confirmed the presence of silver nanoparticles. The diffracted intensities were recorded from 2Ɵ angle range.

SEM (Scanning Electron Microscope) analysis

The silver nanoparticles were also characterized by scanning electron microscopy (SEM). The direct electron microscopic visualization allows measuring the sized and shaped of silver nanoparticles formed.

EDAX (Energy- Dispersive X-ray Spectroscopy) analysis

EDAX is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of X-ray excitation and a sample. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing unique set of peaks on its X –ray spectrum.

To stimulate the emission of characteristic X-rays from a specimen, a high- energy beam of charged particles such as electrons or protons (see PIXE), or a beam of x ray, is focused into the sample being studied.

The number and energy of the X rays emitted from a specimen a specimen can be measured by an energy – dispersive spectrometer.

Antimicrobial Activity of Silver Nanoparticles

Silver with its potent antimicrobial activity has been used in the synthesis of silver nanoparticles which finds extensive use in the preparation of food processing, topical ointments and medical implants (Weiss et al. 2006; Wong 2012). The antimicrobial activity of silver nanoparticles has been known for long time. The silver nanoparticles synthesized from plant powders, such as, were tested for their antimicrobial activity against pathogenic organisms, like Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli, by the well diffusion method. The pure cultures of the organism were sub cultured on nutrient broth at 35 °C on a rotary shaker at 200 rpm. Each strain was swabbed uniformly on the individual plates using sterile cotton swabs. Wells of size 6 mm were made on nutrient agar plates using gel puncture. Using a micropipette, 100µg, 200µg, 300µg, 400µg of the nanoparticle solution samples were poured into wells on all plates.

After incubation at 37 °C for 24 h, the different levels of zone of inhibition were measured.

Application of Silver Nanoparticles in Sewage Treatment

Antibacterial effect of silver nanoparticles was assessed against the bacteria present in sewage water samples by spread plate method. Then, 1 ml from the sample was taking out and added into 9ml of distilled water. This step was continuously repeated until fifth dilution. 0.1 ml from each dilution bottle the bacteria were isolated from samples before treating with silver nanoparticles and CFU (colony forming units) were recorded. Then sample was treated with different concentrations (0.5, 1 and 2 ml) of silver nanoparticles for varying time intervals (3, 4 and 5 hrs) and both the sets were grown on petriplates containing 20 ml of nutrient agar medium. The plates were incubated at 370C for 24 hrs and CFU were recorded after 24 hrs.

Anti Oxidant Activity of Silver Nanoparticles

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potential of the Silver nanoparticles was determined using the modified method by Brand-Williams et al. (1995)**.** Different concentrations (20, 40, 60, 80 and 100 ug/mL) of silver nanoparticles and standard (Tris HCL 0.1M were taken in different test tubes. In the above samples, 1 mL of freshly prepared DPPH (1 mM) dissolved in methanol was added and vortexes thoroughly. Finally, the solution was incubated in dark place for 30 min. The absorbance of stable DPPH was recorded at 517 nm. The DPPH (containing no sample) was used as a control prepared using the same procedure. Free radical scavenging Appl Nanosci 123 activity was expressed as the percentage of inhibition that was calculated using the equation of DPPH radical scavenging activity ð%Þ ¼ ðAc _ AsÞ=Ac _ 100; ð1Þ where Ac is the control absorbance of DPPH radical ? Methanol; as is the sample absorbance of DPPH radical? Sample Silver nanoparticles /standard Tris HCL.

RESULT AND DISCUSSION:

Characterization of Silver Nanoparticles

The formation of silver nanoparticles from of the *Hygrophila auriculata* plant aqueous extract in the solution of 1mMsilver nitrate was observed with change in the colour brown colour indicate that synthesis of silver nanoparticles**.**

A B C D

Fig 1: synthesis of silver nanoparticles

A- *Hygrophila auriculata* plant extract.
B- Only silver nitrate solution.
C- Mixing plant extract and silver nitrate.
D- Synthesis of silver nanoparticles.

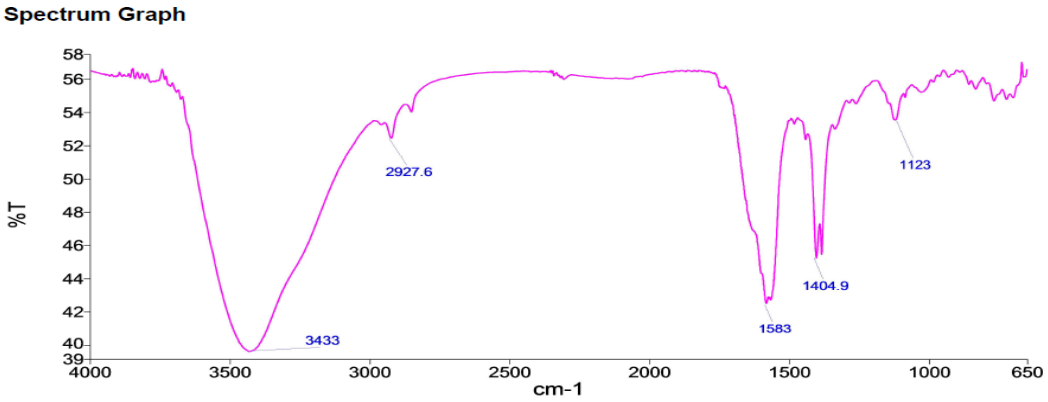
Fig 2: UV- Visible spectrophotometer

UV- Visible spectrophotometer

The addition of *Hygrohila auriculata* leaf extract to silver nitrate (AgNO3) solution resulted in colour change of the solution from transparent to brown due to the production of silver nanoparticles. The colour changes arise from the excitation of surface plasmon vibrations with the silver nanoparticles. Primary conformation of the silver nanoparticles was carried out by UV- Visible spectrophotometric analysis. The nanoparticles show maximum absorbance beak at 450 nm on UV - Vis spectra which is shown in Fig 2. The UV-vis spectra recorded from the *Hygrohiala auriculata* plant. The strong surface Plasmon resonance centred at 440 nm clearly showed

Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectrum obtained for *Hygrophila auriculata* leaf extract (Figure 2) displays a number of absorption peaks, reflecting its complex nature. Strong absorption peaks at 3433 cm^{1} result from stretching of the O-H stretch, H bonded band of alcohols and phenol functional groups are presented. The absorption peaks at about 2927.6 cm−1 could be assigned to stretching vibrations from the O-H band of carboxylic acids function are present. The peaks at 1,583 cm−1 indicate the N-H bend and 1ºamines function are presented. The intense band 1404.9cm−1 could be assigned to the C-C stretching vibrations of aromatics. The FT-IR spectrum also shows bands at 1123 cm−1 identified as amide I and amide II which arise due to carbonyl (C-O) and) stretching vibrations in the amide linkages of the proteins, respectively.. FT-IR study indicates that the carboxyl (−C=O), hydroxyl. (−OH), and amine (N-H) groups in Hygrophila auriculata leaf extract are mainly involved in reduction of Ag+ ions to Ag nanoparticles. The FT-IR spectroscopic study also confirmed that the protein present in *Hygrophiala ariculata* leaf extract acts as a reducing agent and stabilizer for the silver nanoparticles and prevents agglomeration. The carbonyl group of amino acid residues has a strong binding ability with metal, suggesting the formation of a layer covering silver nanoparticles and acting as a stabilizing agent to prevent agglomeration in the aqueous medium.



FTIR VALUE	Bond	Functional group
3433	O–H stretch, H bonded	alcohols, phenols
2927.6	O–H stretch	carboxylic acids
1583	N–H bend	1° amines
1404.9	C–C stretch (in–ring)	Aromatics
1123	C–O stretch	alcohols, carboxylic acids, esters, ethers

Fig 3: FTIR spectrum

SEM Analysis

SEM analysis shows uniformly distributed silver nanoparticles on the surfaces of the cells. The silver nanoparticles were spherical in shape with particle size range from 5 to 40 nm. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements. Fig.4.

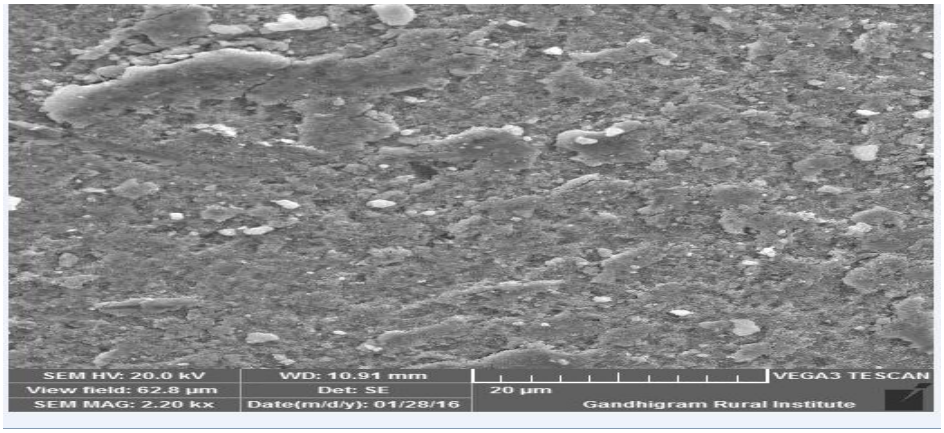


Fig: 4 SEM Analysis

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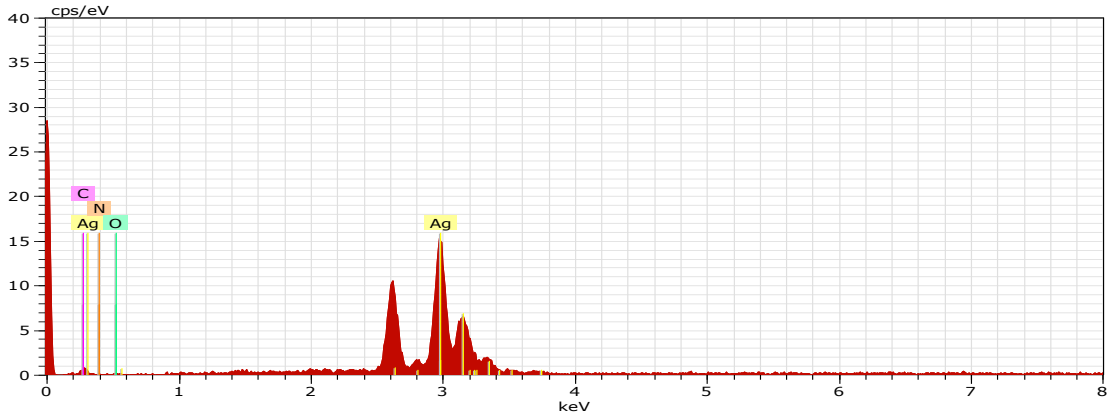


Fig 5: EADX Spectrum

EDAX Spectrum Analysis

In the present study, for the conformation of Silver nanoparticles, EDAX spectroscopy analysis was performed, which confirmed the presence of elemental Agr,C,H,O by the sharp signals (Fig:5) carbon , nitrogen, silver, oxygen, present at **3Kev**

XRD Analysis

Fig: 6 show the Analysis through X-ray diffraction was carried out to confirm the crystalline nature of the silver nanoparticles. The XRD pattern showed numbers of Bragg reflections that may be indexed on the basis of the face-centered cubic structure of silver. A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 38. 28°, 44.04°, 64.34°, and 77.28° corresponding to (111), (200), (220), and (311) Bragg reflections, respectively, which may be indexed based on the face-centered cubic structure of silver. X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag+ ions by the carob leaf extract are crystalline in nature. The unassigned peaks at 2θ = 27.96°, 32.28°, and 46.18° denoted by are thought to be related to crystalline and amorphous organic phases. It was found that the average size from XRD data and using the Debye-Scherrer equation was approximately 18 nm. The presence of structural peaks in XRD patterns and the average crystalline size around 18 nm clearly illustrate that the Silver nanoparticles synthesized by our green method were nanocrystalline in nature. The average particle size of silver nanoparticles synthesized by the present green method can be calculated using the Debye-Scherrer equation [19,22] **D ¼ Kλ=β cosθ**; where D is the crystallite size of Silver nanoparticles , λ is the wavelength of the X-ray source (0.1541 nm) used in XRD, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with a value from 0.9 to 1, and θ is the Bragg angle.

Antimicrobial Activity of Silver Nanoparticles

Antimicrobial agents are so wide spread that they are likely to play an important protective role. These agents have a variety of activities ranging from gram negative selective to gram positive selective to broad spectrum in nature. The antimicrobial activity of silver has been recognized by clinicians for over 100 years. It is only in last few decades mode of action of silver as an antimicrobial agent has been studied. ***Raut Rajesh et al***. In the present investigated the antibacterial activity of phytosynthesised silver nanoparticles against *Staphylococcus aureas*, *Escherichia coli*, *Pseudomoneas aeruginosa*, *vibro cholera* and *Proteus mirabilis*. We reported antimicrobial activity of silver nanoparticles against *Escherichia coli* and *Staphylococcus aureas*. The effect was dose dependent. The silver nanoparticles also exhibited the antibacterial activity against both gram-positive and gram-negative and formed the zone of inhibition of diameters, respectively.

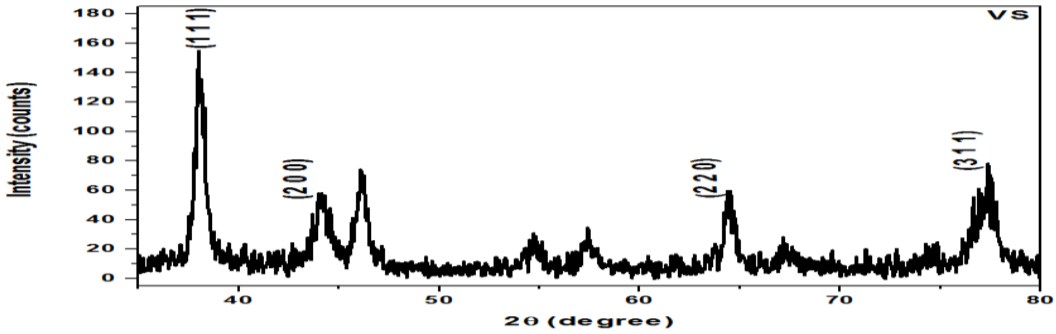


Fig. 6:XRD Pattern of synthesised silver Nano particles.

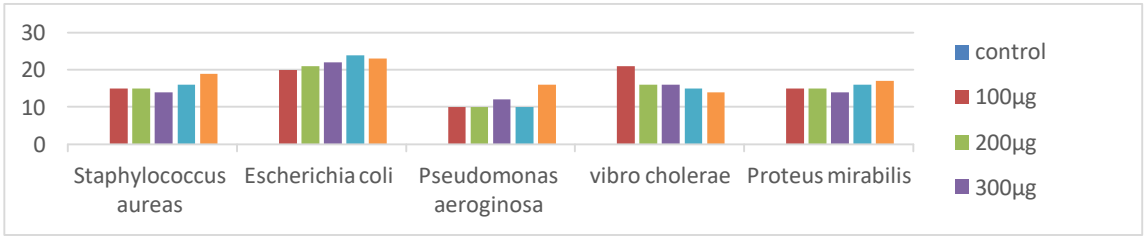
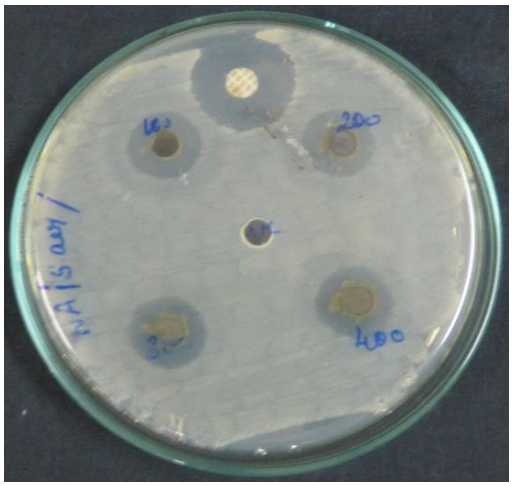
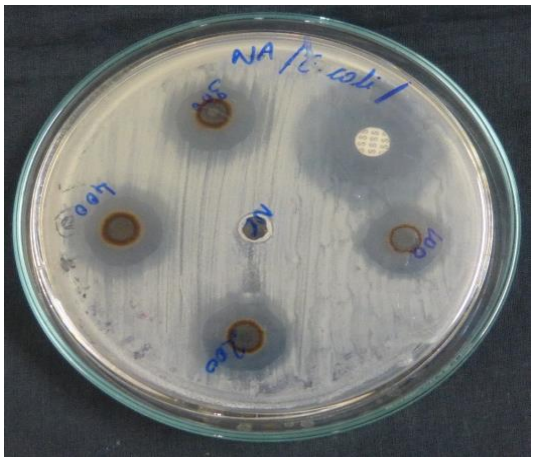


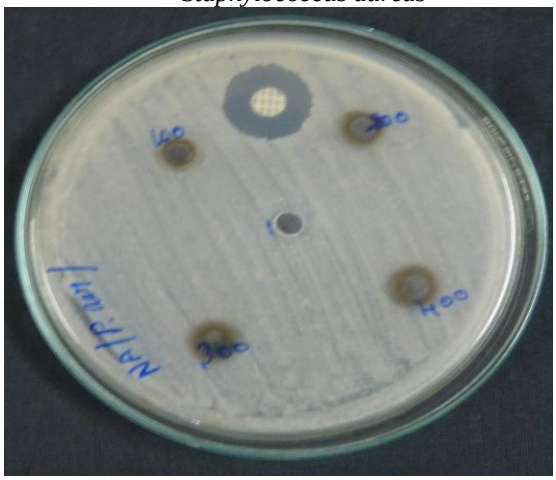
Fig.7: Zone of inhibition of synthesised silver Nano particles aqueous leaf extract of *Hygrophila auriculata*.



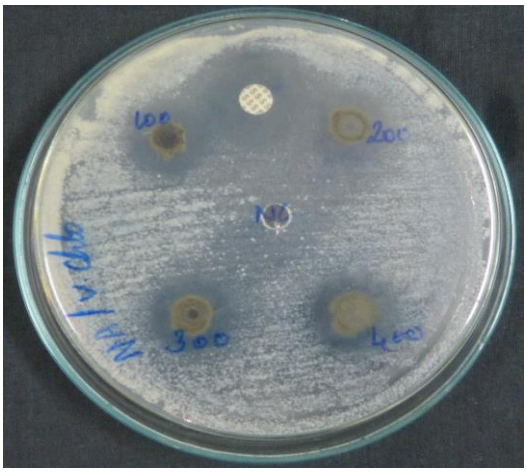
Staphylococcus aureas



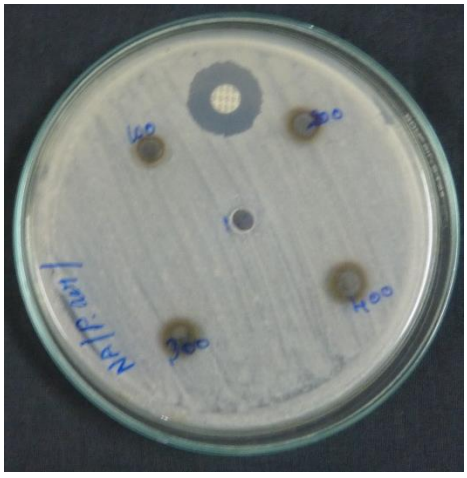
Escherichia coli



Pseudomonas aeruginosa



Vibro cholera



Proteus mirabilis

Fig. 8: Antibacterial activity of synthesised silver Nano particles aqueous leaf extract of *Hygrophila auriculata.*

Table 1: Serial Dilution Sewage on Nutrient Agar

S.No	SEWAGE SAMPLE (ml)	CFU measurement value
1	0.1	470
2	0.1	455
3	0.1	333
4	0.1	378

Table 2: Silver nanoparticles in sewage treatment

Synthesis of Silver nanoparticles ml	Sewage volume	CFU of bacteria value in ml
0.5	0.1	400
1	0.1	343
2	0.1	200
3	0.1	145

Fig 9: Before addition of the silver Nano particles in Sewage on Nutrient agar

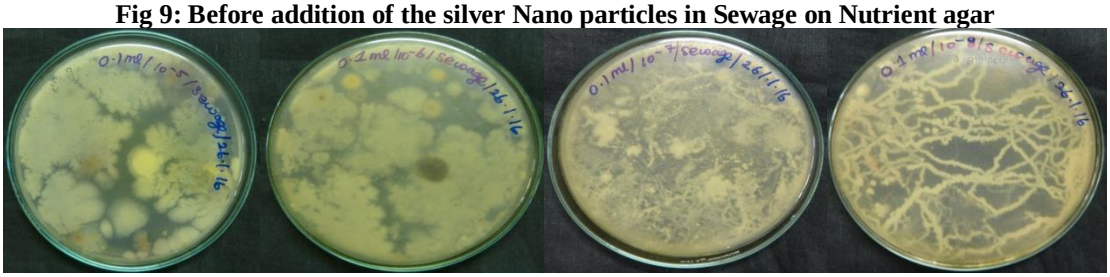
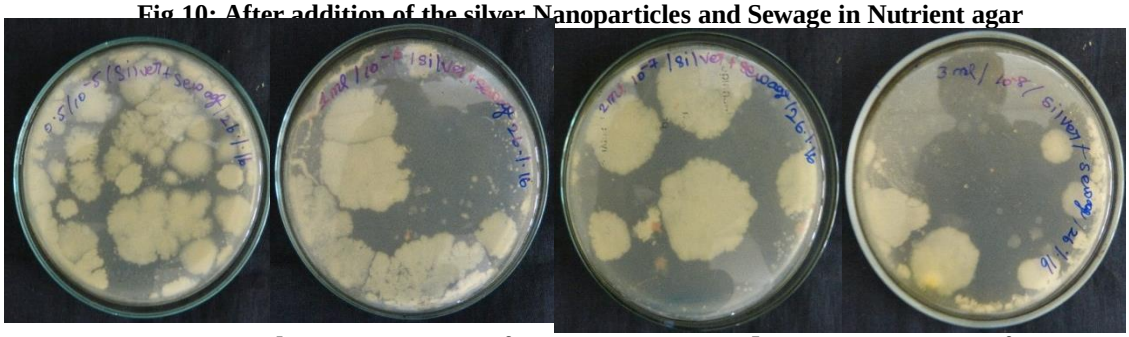


Fig 10: After addition of the silver Nanoparticles and Sewage in Nutrient agar



10^{-5} 10^{-6} 10^{-7} 10^{-8}

Application of Silver Nanoparticles Sewage Treatment

Silver nanoparticles have been used as antimicrobial compounds for coliform found in waste water (Jain and Pradeep, 2005). In the present study, the CFU of bacteria before treating with silver nanoparticles were in sewage water (table 2). The CFU of bacteria was reduced to 17 x 105 /ml and 35 x 105 /ml in case of silver nanoparticles from *silver nanoparticles* and leaf extracts of *Hygrophila ariculata* respectively at a concentration of 3ml and a time interval of 24hrs (Table 3). This effect is found to be concentration and time dependent.

Antioxidant Activity

DPPH Assay

DPPH is a more stable and well-known free radical based on the reduction of accepting hydrogen or electron from donors. The DPPH reducing ability of the AgNPs was assessed by observing colour change and the control does not show any colour change. The DPPH scavenging assay exhibited effective inhibition activity of AgNPs when compared with the standard, Tri Hhcl (Fig. 9). The DPPH activity of the AgNPs was found to increase in a dose dependent manner. However, the AgNPs exhibited more inhibition with 90 % scavenging activity of DPPH. When adding AgNPs in the DPPH solution, color change was occur which is due to the scavenging of DPPH due to donation of hydrogen atom to stable the DPPH molecule which is responsible for the absorbance of 517 nm (Molyneux 2004; Kanipandian et al. 2014). The antioxidant potential of AgNPs could be attributed to functional groups adhered to them which were originated from the leaf extract.

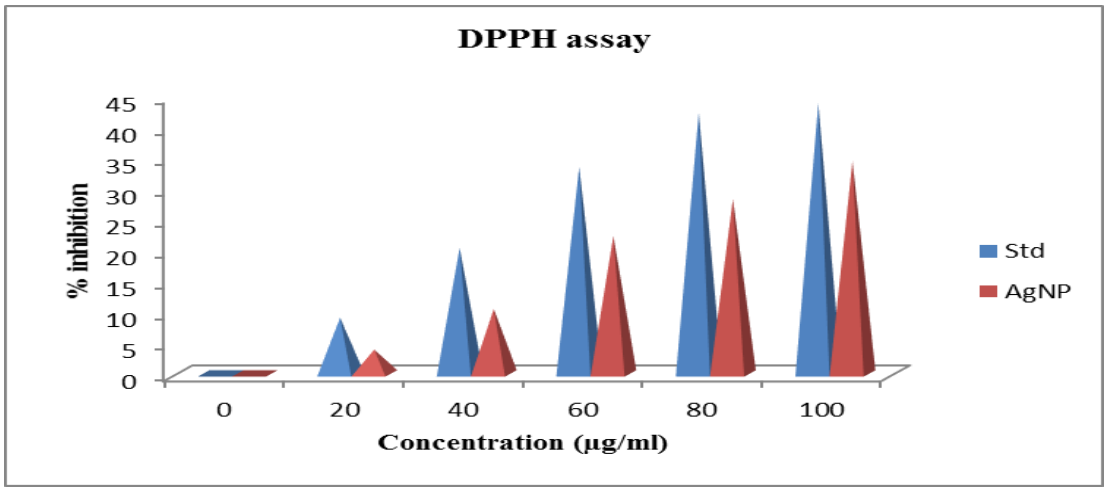


Fig 11: Anti Oxidant Activity by DPPH Assay Method

Addition of the aqueous leaf extract of *Hygrophila auriculata*, to 1mm solution of silver nitrate led to the appearance of yellowish brown colour as a resultant of formation of silver nanoparticles in the solution because of phytochemicals used to form silver nanoparticles from leaf extract. The rate of formation was literally raid comparable to the chemical method of synthesis, the pale yellow colour appears immediately after the addition of the aqueous plant extract, and the reaction was completely in about 2hrs. This makes the investigation highly significant for rapid synthesis of silver nanoparticles.

Addition of crude extract of *Hygrophila auriculata* 100ml and 1mm solution of silver nitrate led to the appearance of reddish brown color as resultant of formation of silver nanoparticles in the solution. The UV-VIS absorption spectrum recorded for the solution shows the characteristic surface plasmon resonance band for silver nanoparticles in the range of 440nm. The bio reduced suspension can reduces the formation of silver nanoparticles until it reaches 540nm.

FITR analyses of silver nanoparticles

Infrared spectroscopy can effectively be used for the charterisation of nanoparticles.the advantages of using FTIR over conventional method have been thoroughly reviewed. Ftir analysis was to characterize the nature of capping ligands that stabilizes the silver nanoparticles formed by bio reduction process.

SEM Analysis

The scanning electron microscopic studies were helped to visualize synthesised particles. The size formed indicated as silver nanoparticles.

EADX Analysis

In the analysis by Energy Dispersive Spectroscopy (EDS) of the AgNPs, the presence of elemental metal signals was confirmed. EDX analysis gives quantitative and qualitative status of elements that may be involved in formation of nanoparticles. Synthesized nanoparticle using *Hygrophila auriculata* plant leaf extracts and confirms the formation of silver nanoparticles,C,H,O.Spectrum also indicates some unidentified peaks in image which may be due to copper grid used for EDX analysis. EDAX results also show higher counts at 5.9 keV due to silver nanoparticles. Generally metallic silver nanocrystals show typical optical absorption peak approximately at 3 keV due to surface Plasmon resonance**.**

XRD Analysis

The crystalline nature of silver nanoparticles was confirmed from X-ray diffraction (XRD) analysis. The typical powder XRD patterns of the prepared particles originating from biosynthesis of plant extract showed diffraction peaks at 2Ø = 38.2, 44.40, 64.60, 77.50, and 81.70.

Antimicrobial Activity of Silver Nanoparticles

Research is underway to use nanotechnology in water purification for safe drinking. Nanoparticles especially silver are expected to play a crucial role in water purification since they have been used against coliforms found in sewage. In the present study, the treatment of the sewage sample with the nanoparticles significantly decreased the bacterial load. This decrease was a function of incubation time and the concentration of nanoparticles. Antibacterial effect of silver nanoparticles and ultrasonic irradiation from waste water and observed a

reduction in the number of colonies to very few of treatment of silver nanoparticles.

Anti Oxidant Activity of Silver Nanoparticles

DPPH assay is one of the most widely used methods for screening of antioxidant activity of plant Extracts. For obtained protein capped silver nanoparticles, *in-vitro* antioxidant activity against 1.1-Diphenyl-1-picrylhydrazyl (DPPH) free radical was determined. The hydrogen atom or electron donation ability of the Silver nanoparticles and the pure *Hygrophila auriculata* plant aqueous leaf extract were measured from the bleaching of a purple-colored methanol solution of DPPH. Because DPPH and peroxyl radicals have similar electronic structures (the unpaired electron is delocalized over both N atoms of hydrazyl and both O atoms of peroxyl), the reaction rate of DPPH and antioxidants give better approximation for scavenging activities with lipid peroxyl radicals.

CONCLUSION:

The field of nanotechnology is new and upcoming research area in the Indian scenario. Much has already been studied in synthesis of nanomaterials through physical and chemical processes. But synthesis of nanomaterials by biological agents offer several advantages like establishment of green chemistry lead to a pollution free production of nanomaterials in contrast to energy dependent, pollution based physical and chemical methods.

Nanotechnology has dynamically as an important field of modern research with potential effects in electronic and medicine. The development of reliable green process for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research. Among the various known synthesis methods, the use of plants for synthesis of AGNPs are rapid low cost, eco-friendly, safe for human therapeutic uses and a single step methods for biosynthesis process. The silver nanoparticles synthesized by *Hygrophila auriculata* plant leaf extract were characterized by UV-VIS spectrophotometer, FTIR, SEM, EDAX and XRD analysis.

We have found that the silver nanoparticles synthesized in our study effectively resistant to the growth and multiplication of pathogenic bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Proteus mirabilis.*LC-MS *characterization to be done for the synthesized nanoparticle.*Application of silver nanoparticles in sewage treatment showed a significant decrease in the bacterial load and this effect was found to be concentration dependent**.** Free radical scavenging activity of *Hygrophila auriculata* plant and synthesis of synthesis of silver nanoparticles measured by 1, 1-diphenyl-2-picryl hydrazyl (DPPH). The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm.

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