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Bio- Synthesis Of Silver Nanoparticles From Leaf Extracts Of Martynia Annua L. And Its Antimicrobial Activity.

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ABSTRACT*: In the present study Silver nanoparticles were synthesized from aqueous leafs extract of Martynia annua L . and characterized by UV-Vis spectroscopy, Fourier transform infrared spectroscopy (**FTIR**) , X-Ray Diffraction (XRD), Scanning electron microscopy (SEM) and Energy Dispersive X(EDX).Characterization by the above said instrument analysis confirmed the presence, size and stability of silver nanoparticles.After characterization, the silver nanoparticles were tested at 100µg^{-ml},200µg^{-ml},300µg^{-ml} and 400µg^{-ml} concentrations to check the bactericidal activity against clinical isolates of five bacterial pathogens. We observed that, if the concentration of leaf extracts synthesized nanoparticle increases, the zone of inhibition also get increased in all the test five clinical bacterial pathogens against streptomycin and result suggested the potential use of leaf extracts synthesized silver nanoparticles against other clinical pathogens*.

KEY WORDS: *Martynia annua leaf extrcts, silver nanoparticle synthesis, characterization, antibacterial activity against clinical pathogens.*

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I INTRODUCTION

Nanotechnology can be delineated as a research for the design, synthesis, and manipulation of structure of particles with dimension smaller than 10 nm. The idea of nanotechnology was first introduced by (Richard Feynman in 1959). The popular term" Nanotechnology" was coined by Norio Taniguchi, a Japanese professor of Tokyo Science University, in 1974 engineer materials at nanometer level. Different types of Nano-Materials are present in Quantum Dots, Nanocrystals, Nanoparticles, Metallic Nanoparticles, Dendrimers, Bucky balls, and Nanoutubes.Nanotechnology is an interdisciplinary field and is formed from the convergence of chemistry physics and biology.

Nanoparticles show completely new or improved properties such as size distribution and morphology of the particles etc. Novel application of nanoparticles and nonmaterial are emerging rapidly field (Kaviya et al.,2011). As motioned above, generally silver is used in the form of nirate to induce antimicrobial effect. But when silver nanoparticles are used there is huge increase in surface area available for the microbe to be exposed to. Though silver nanoparticles find use in many antibacterial applications the action of this metal on microbes is not fully known. Silver nanoparticles have long been known for their antibacterial,antifungal,antiviral and anti inflammatory properties. In medicines silver is used to prevent infection of burns and open wounds (Duran et al., 2005). Medical devices and implants are prepared with silver impregnated polymers (Ro et al., 1999).In textile industry silver embedded fabrics are now used in sporting equipment (Klaus et al 1999).Biological methods are ecofriendly and are cost effective.

The pure metals in nanoparticles form are applied in the field of diagnostics antimicrobial agents, drug delivery, textiles (clothing) electronics, bio sensing food industry paints, cosmetics, medical devices and treatment of several acute and chronic diseases like – malaria, hepatitis, cancer and aids due to various events occurring in the environment among them human activities of prime importance that result production of reactive oxygen species (ROS) showing negative impact in biological system. The progress in time evidenced development in technology that revealed the ability metals of nanoscale to perform specific functions better than the bulk form of metals. The noble metals like silver gold, platinum palladium copper, zinc and iron were used in synthesis of particles of nano size. The nanoparticles such as size shape, composition, crystalline nature and structure determine their applications. Nanoproticles involve algae, actinomycetes, bacteria, fungi and plants.

Martynia annua L. belongs to the family Martyniaceae. The plant is commonly known as Devil's claw (English) Bichu (Hindi) Kakanasika (Sanskrit) Vechchida (Gujarati) and tamil name buli nagam.It is a small berbaceous, erect,branched, glandular hairy annual herb growing up to 0.9- 1.2 m in height leaves are large simple opposite green in color broadly ovate to triangular ovate, glandular hairy 9-22 x 9-20 cm cordate at base branched and covered with glandular hairs. Flowers are drooping large pale mouve or lavender in short spikes at the end of branches. They are tubular shaped 4-6 cm long pink and dark purple blotched with yellow inside foxglove shaped ill-smelling and terminate in 5 spreading lobes with a prominent spot between each. The plant is used for the treatment in Ayurveda. The plant is known as kakanasika. This is being used in Indian traditional medicines for epilepsy, inflammation and tuberculosis. The leaves of *Martynia annua* are edible and used antiepileptic and antiseptics applied locally to tuberculosis glands of the neck the juice of the leaves as a gargle for sore throat and the leaf paste for wounds of domestic animal. The leaf is used to kill bugs.The leaf paste is antidote to venomous strings and applied to tuberculosis glands. The leaf juice is used in gargle treatment. The methanolic extract fo martynia annua leaves shows the presence of higher amount of terpenoid alkaloids glycosides steroids tannins, saponins and moderate amount of p-hydroxyl benzoic acid, synaptic acid an fatty acid such as plasmatic acid and stearic acid.

The present study explores the pharmacological potential of the plant. The chloroform, thyl acetate and methanol extract of *Martyina annua* leaves were found to have antibacterial activity. When evaluated against gram positive and gram negative bacteria. All the solvent extracts show antibacterial action respective to different bacteria. Chloroform extract shows higher antibacterial activity against proteus vulgaris, Bacillus subtitles and B. thuringensis. Ethyl acetate extract was potentially effective against salmonella paratyphoid, A, Salmonella paratyhoid B, Proteus mirabilis the methanol fraction of ethanol extract of Martynia annua leaves shows significant wound healing effect by stimulating of wound contraction as well as epithelializtion.

Moreover, phytochemical studies demonstrated that. The methanol fraction mainly contains flavanoid lute olin responsible for enhacement of wound healing process due to the free radical scavenging mechanism. I t si imperative that more clinical and pharmacological trials are needed to investigate the unexploited potential of this plant.

In the present study synthesis of silver nanoparticles using the leaves extract of the plant *Martynia annua*. To characterize the silver nanoparticles by UV-VIS, FTIR, SEM, EDAX and XRD and to find out the antimicrobial activity of the synthesized silver nanoparticles against clinical pathogens.

II MATERIALS AND METHODS:

MATERIALS AND METHODS:

PLANT COLLECTION:

Fresh leafs *Martyina Annua* were collected at Bharathidasan University campus at Trichy, Tamil Nadu India waste places and road sides. Plant was authentificated from (Dr.S.Joh britle) St. Joseph College (Campus) Tiruchirappali.

PREPARATION OF LEF EXTRACT

The fresh leafs was washed with running tap water for 20 mints. and dry in shade at room temperature for one week. Then the leaves are cut into small pieces and make into fine powder.20g of leafs extract are weighed and dissolved in 200ml distilled water in a 500ml Erlenmeyer flask and boil for 30mins . The extracts was filtered with Whatman No.1filter paper and stored in an airtight container under dark condition until for further use.

PREPARATION OF SILVER NANOPARTICLES

1mM of silver nitrate (AgNO3) was prepared in 1000ml beaker (0.1698 g $AgNO_3$ is added to 1000ml of distilled water). The 100ml seaweed extract were mixed with 900ml silver nitrate solution (1:9) ratio. and kept under dark condition. Colour change of the solution from white with pale yellow indicated that the silver nanoparticles get synthesized and then the solution was centrifuged at 7,000rpm, 20^oC for 15mints. Then the supernatant was collected from the tube and it was kept for evaporation (to sediment the particles) until it gets fully evaporated. Collect the pellet which is kept in hot air oven at 40^0C for twenty four hours. Further, the synthesized sample was used for characterization and antimicrobial activity studies.

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, and 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 deionised water was used as the blank.

FOURIER TRANSFORM INFRAED SPECTROSCOY (FTIR)

FTIR analysis were carried out to identify the possible bio molecules responsible for reduction of the Ag^+ ions and capping of the bio reduced silver nanoparticles synthesized.

X-Ray diffraction analysis (XRD)

The X-ray diffraction pattern indicated the crystalline structure of silver nanoparticles.The XRD spectrum confirmed the presence of silver nanoparticles. The diffracted intensities were recorded from 2Ɵ angle.

SEM (Scanning Electron Microscope) analysis

The silver nanoparticles were also characterized by scanning electron microscopy (SEM). The direct electron microscopic visualization allows measuring the size and shape of biocapped 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 can be measured by an energy –dispersive spectrometer.

Antibacterial activity studies

Silver nanoparticles synthesized from *Martyina Annua* leafs were screened for antibacterial activity against clinical pathogenic bacteria namely *Escherichia coli, Pseudomonas aeruginosa, Rhodococcus rhodnii, Streptococcus aureus* and *Proteus mirabilis*

Media preparation:

Nutrient broth (Peptone – 5g, Beef extract – 1.5g, Yeast extract – 1.5g, NaCl – 5g and Distilled water – 600ml; pH adjusted to 7.2) was prepared. After sterilization of the medium,the bacterial culture was inoculated in the nutrient broth. The inoculated broth has been incubated for 24 hours at 37°C in incubator.Nutrient agar (Peptone – 5g/l, Yeast extract – 1.5g/l and Beef extract – 1.5g/l, pH of 7.2) was prepared,sterilized and poured on to the sterilized petriplates.

Preparation of inoculums:

Bacterial inoculums were prepared by transferring a loop full of bacterial culture from fresh culture plates to tubes containing 10 mL of Nutrient Broth (Hi-media) and incubated for 24 hours at 37^oC. The tubes were shaken occasionally to aerate and promote growth. These cell suspensions were diluted with sterile Nutrient Broth to provide initial concentration cell counts of about 2×10^3 CFU^{-mL} at 600nm O.D at Spectrometer. After the solidification of the media in the petriplate, bacterial cultures were inoculated by swap method.

Disc diffusion method

The antibacterial activity of crystalline bio molecule capped synthesised silver nanoparticles from *Lobophora variegata* was determined by disc diffusion method. Discs of 6mm diameters were prepared from Whatmann No.1 Filter paper and kept in the hot air oven at 160°C for 1 hour. The nutrient agar plates were prepared and inoculated with test bacterial organisms by spreading the bacterial inoculums on the surface of the media. The discs were impregnated with different concentrations ranging of $100\mu g^{-ml}$, $200\mu g^{-ml}$, $300\mu g^{-ml}$ and $400\mu g^{-ml}$. A negative control was prepared by taking 1mM Silver nitrate dissolved in 1ml distilled water and positive control Streptomycin used as positive controls ($100\ \mu g^{-mL}$).

Evaluation of antibacterial activity

The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the area in which bacterial growth was inhibited around the disc and measured the diameter zone of inhibition (in mm).

III RESULT AND DISCUSSION:

Synthesis of silver nanoparticles:

Biological and biomimetic approaches for the synthesis of nanomaterials are being explored. Cell mass or extracellular components from microorganisms, such as *Klebsiella pneumonia*, *Bacillus licheniformis*, *Fusarium oxysporum*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Aspergillus clavatus*, and *Penicillium brevicompactum*. The unexploited plant resources for the synthesis of silver nanoparticles, various plant leaf extracts such as *Helianthus annus*, *Basella alba*, *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolour* and *Zea mays*,*Jatropha curcas* and *Aloe vera*.

Development of reliable and eco- friendly process for synthesis synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. The principle of preparation of silver nanoparticles by using microorganism is a bioreducation process the silver irons are reduced by the extracellular reductase enzymes produced by the microorganisms to silver metal in nanometer range. Synthesis of advanced materials energy storage devices, electronic and optical displays chemical and biosensors as well as biomedical devices, recognizing the important of nanomaterials in key future technology.

Synthesis of nanoparticles using microorganisms involves elaborate process of maintaining cell cultures, intracellular synthesis and multiple purification steps. In this regard using "green" methods in the synthesis of zinc oxide nanoparticles has increasingly become a topic of interests as conventional chemical methods are expensive and re-quire the use of chemical compounds/organic solvents as reducing agents (Cynthia Mason et al., 2012).Plants have a lot of phytochemicals in their parts: they are applied in various fields. The biochemicals may play an important role in the nanoparticles synthesis (T.Santhoshkumar, et al.,2011) Solanumtrilobatum Linn is an important medicinal plant of the family solanaceae. The leaves contain rich amount of calcium, iron, phosphorus, carbohydrates fat, crude fiber, and minerals (M.Jawhar, et al.,2004). This plant has strong immune stimulatory effect due to the presence of alkaloids and carbo hydrates. Due to these antioxidant and antibiotic properties this was used to synthesize silver nanoparticles.

In this present investigation the medically important plant is used for the synthesis of medically valued silver nanoparticles. The morphological, crystalline, and biochemical characters of green synthesized silver ananoparticles were analyzed by UV-VIS spectrophotometer,Scanning Electron Microscope, X-ray Diffraction pattern, and Fourier Transform Infrared spectroscopy. Finally the medical property of the silver nanoparticle was characterized using antibacterial assay. Many plants are used for synthesizing nanoparticles including *Cinnamomum camphora*, (Huang et al., 2007) Preparation and synthesis of silver nanoparticles form the callus and in-vitro derived plant roots of white flowered variety of *Clitora ternatea*.Few papers reported the biosynthesis of zinc oxide nanoparticles using plant extracts such as *Aloe barbadensis* M.(Sangeetha.,2011).*Parthenium hysterophorus* (Rajiv P et, al 2014) and *Poncirus trifoliate* plant dried fruits (Nagajyothi.,et al.,2013) and milky latex of *Calotropisprocera* (Singh R,et al 2011).

Martynia annua extract used to produce silver nanparticles in this experiment Ag +ions were reduced to Ag nanoparticles when plant extract was mixed with AgNO3 solution in 1:8 ration reduction was followed by on immediate change in yellowish to brown color in the aqueaous solution of the plant extract due to surface Plasmon vibration in silver nanoparticle.

Plant was mixed with $AgNO_3$ solution. The mixture was kept at room temperature for 24 hours. The appearance of yellowish brown color in the reaction vessel indicated formation of AgNo3 AgNo3 exhibit this yellow-brown color in aqueous solution due to excitation of surface Plasmon resonance in the $AgNO_3$.Primary conformation of the AgNo3 was carried out by UV-Visble spectrophotometric analysis. The nanoparticles showed maximum absorbance beak at 380 nm on UV-Vis spectra which is shown in Fig 1. The UV-Vis spectra recorded from the Martyina annua reaction vessel at the strong surface Plasmon resonance centered at 380 nm clearly showed.

FTIR spectrum:

The FTIR spectrum of extracellular biosynthesized AgNo3 was shown in Fig FTIR measurement of the freeze – dried samples were carried out to identify the possible interactions between silver and bio-molecules which may be responsible for synthesis and stabilizations of silver nano particles with capping agent available in the *Martyina annua* broth.

The amide linkages between amino acid resdues in protein give rise to well known signatures in the infrared region of the electromagnetic spectrum. The FTIR spectrum revealed five bands at 3429.8 cm present the O-H stretch H- bonded vibration of alcohols, and Phenols.

Scanning Electron Microscope (SEM) Analysis:

The SEM analysis was used to determine the structure of the reaction products that were formed Fig6 the silver nanoparticles observed were of spherical shape of the scanning electron microscope the nanoparticles were analyzed in the different magnifiers.

EDAS:

In the present study, for the conformation of AgNo3, EDAX spectroscopy analysis was performed which confirmed the presence of elemental silver by the sharp signals.

X-Ray Diffraction (XRD) Analysis:

The powdered sample was used by a CuKal-X Ray Diffract meter for confirming the presence of silver nitrate and analyzing the structure. The graph showed main peaks main peaks corresponding to 2values of 37.5, 46.5 and 77.8 correspond to (111)(200)(311) in the multi-plot shown in Fig 6. The location of the peaks was compared to literature values and the presence of silver nitrate particles was confirmed. Similar results were reported earlier in silver nanoparticles.

Antimicrobial activity:

In the present study the assay for antimicrobial activity was done against escherchia coli, salmonella typhimurium, Vibrio cholaera Pseudomonas aerugines and pseudomonas mirabilis with various concentration (100,200,300 and 400 /ml) of green synthesized silver nanoparticles of Martynia annua and the result are showed in fig.7.The silver nanoparticles showed good activity against all tested organisms at all concentration. The highest zone of inhibition was found against *Vibrio choleare* (19mm at 400) and minimum level of antibacterial activity was observed against *pseudomonas aeruginosa* (10mm at 100). The above observation clearly indicated that the synthesized silver nanoparticles have the potential to kill the bacteria effectively.

IV SUMMARY AND CONCLUSION

The biological synthesis of silver nanoparticles using Martynia annua leaves extract provides environmental friendly and efficient method for synthesis of nanoparticles. The synthesized nanoparticles were of spherical shape and the estimated size. The size was bigger as the nanoparticles were surrounded by a thin layer of metabolites. Which were found from the characterization using UV-vis spectrophotometer,FTIR, EDAS,SEM and XRD techniques. All the above analyses proved that the concentration of plant extract to metal iron ration plays in important role into the shape determination of the nanoparticles.

The synthesized silver nanoparticles exhibited good anti bacterial activity against clinical isolates of five bacterial pathongens. The present study offers the ability of *Martynia annua L.* leaf extract to reduce silver ions to synthesis silver nanoparticles which can be used for several applications like anti-bacterial,antifungal activities and further LC-MS characterization is to be done for organic compound capped in it. From the technological point of view these silver nanoparticles have potential application in the biomedical field and this simple procedure has several advantages such as cost- effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

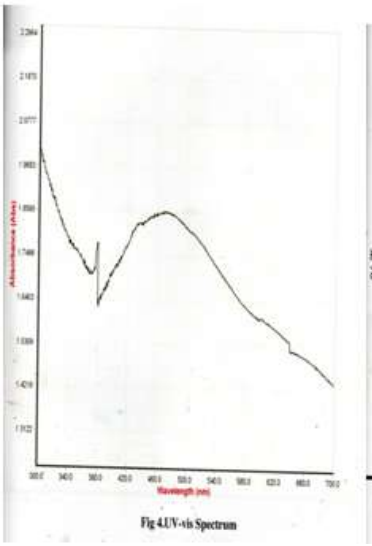

Fig 4.UV-vis Spectrum

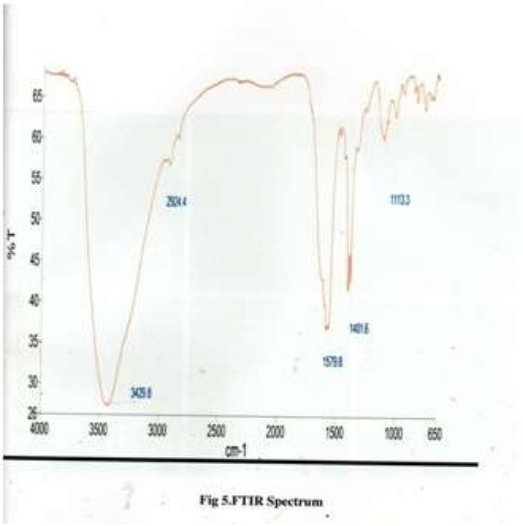

Fig 5.FTIR Spectrum

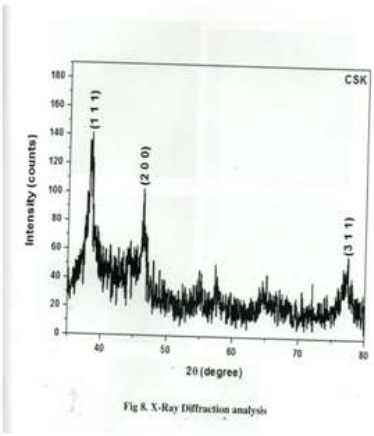

Fig 8. X-Ray Diffraction analysis

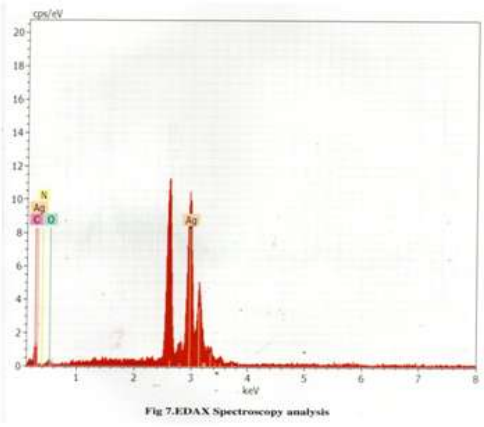

Fig 7.EDAX Spectroscopy analysis

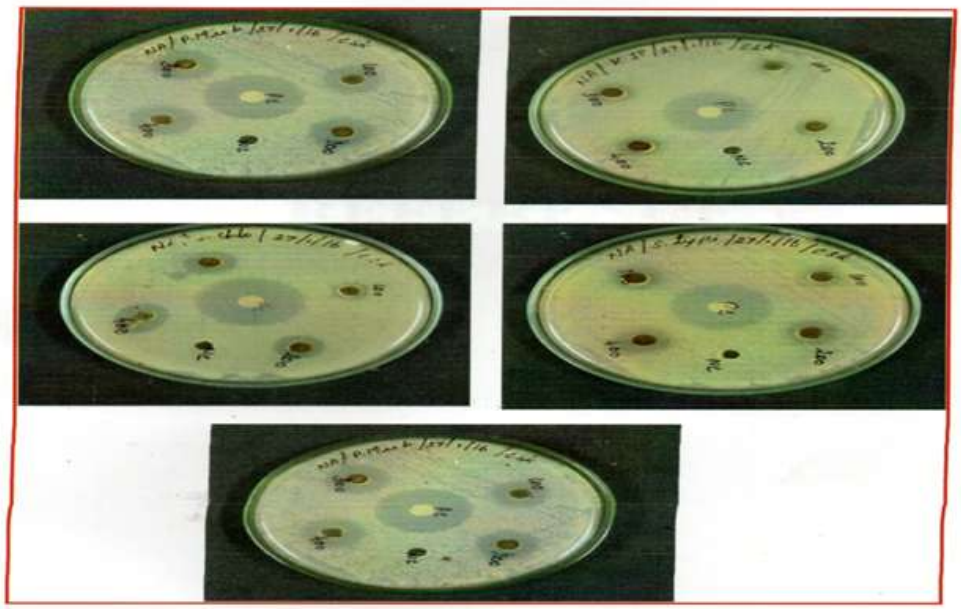

Fig 9.Antimicrobial activity of Martynia annua silver nanoparticles

Table 1: Antimicrobial activity of *Martynia annua* silver Nanoparticles

Sample concentration (µg/ml)	Diameter of Zone inhibition (mm)				
	E.coli	*Sallmonella tyhpymurium*	*Vibrio choleare*	*Psudonmonas aeruginosa*	*Psudomononas mirabilis*
100	14	11	12	10	14
200	16	13	14	13	15
300	17	15	18	15	16
400	18	18	19	17	18

Table 2: FTIR Spectroscopy analysis

FREQUENCY cm	BOND	FUNCTIONAL GROUP
3429.8	O-H stretch – bond	Alcohols phenols
2924.4	C-H stretch	Alkanes
1579.8	C-C stretch (in-ring)	Aromatics
1401.6	C-H bend	Alkanes
1113.3	C-N stretch	Aliphatic amines

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