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Green Synthesis of Nano-Particles and its Application in Treatment of Rheumatoid Arthritis

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Abstract- This paper presents an overview of silver nanoparticles (Ag NPs) preparation by green synthesis approaches that have advantages over conventional methods involving chemical agents associated with environmental toxicity and also explains its application in the cure of Rheumatoid arthritis. We have used Night Jasmine (Nyctanthes arbor-tristis), one of the best medicinal plants to synthesis Ag NPs. The plant extract contain some biomolecules like proteins carbohydrates etc., which act as both reducing and capping agents forming stable and shape-controlled Ag NPs. The basic principle behind the synthesis is that the silver (Ag^+) of $AgNO_3$ is reduced to Ag^0 by the bio-molecules present in the extract. Rather than applying the conventional method of using micro waves to induce the reduction reaction, we have used direct sunlight as the inducer by which the reduction is faster than the former. The synthesized silver nanoparticles were characterized with UV-Visible spectroscopy, X-ray Diffraction (XRD), scanning electron microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). The results of all these analysis were shown. Our work mainly concerns about the cure for Rheumatoid arthritis so, we have checked the Nano-particles for the Anti-Arthritic activity and the results were positive. The procedure for Invitro analysis of anti-arthritic activity and its result are clearly shown.

I. INTRODUCTION

Silver Nano Particles: Nano-particles are the substances that are in the size between 1nm to 100nm. The most commonly used Nano-particles are Silver Nano-particles. This is due to their unique size-dependent optical, electrical and magnetic properties. It can be applied in wide range of sectors ranging from catalysis, optics, antimicrobials and biomaterial production. In our work we have synthesized the Silver Nano particles biologically with the help of a biological extract. Since the biological compound is used for the synthesis it is called as Green synthesis.

The basic principle behind the synthesis is the reduction of silver (Ag^+) of $AgNO_3$ into Ag^0. As the reduction takes place, the biomolecules aggregate with silver metal ions to form Nano-sized. The Nano-particles with Silver ion and biomolecules of the extract exhibits both the properties of silver and biomolecules. By studying the characteristics of these biomolecules the particles can be applied with respect to the property it exhibits. It was also shown that the average size of silver nanoparticles can be controlled by varying the concentration of silver nitrate and the volume of leaves extract. These silver particles are found to have the property of treating Rheumatoid arthritis which is explained in later part of the paper. Night Jasmine: We have used the leaves extract of Nyctanthes arbor-tristis (Night Jasmine) to synthesis the silver nanoparticles. Nyctanthes arbor-tristis, a night flowering sad tree of family Oleaceae (Nyctaginaceae) is well known in India and its neighboring countries as one of the most versatile medicinal plants having a wide spectrum of biological activities. Some of the medicinal properties are Anti-inflammatory, Antibacterial, Hepatoprotective and Immunopotential, Anti pyretic, Antioxidant and Anti-fungal activities. It is widely cultivated in tropical and subtropical regions all over the world. It is a terrestrial woody perennial having life span of 5-20 yrs. It is usually a shrub or a small tree. In India, it grows in the outer Himalayas and is found in tracts of Jammu and Kashmir, Nepal to east of Assam, Bengal and Tripura extended through the central region up to Godavari in the south. Flowering usually occurs from July to October. Nyctanthes prefers a secluded and semi-shady place to grow. This tree grows well in a wide variety of loamy soils and in soils found in average garden situations with pH 5.6-7.5.

Rheumatoid arthritis: Rheumatoid arthritis is a disorder in which the body's own immune system starts to attack self-tissues. The attack is not only directed at the joint but also at many other parts of the body. In rheumatoid arthritis, most damage occurs to the joint lining and cartilage which eventually results in erosion of two opposing bones. Rheumatoid arthritis often affects joints in the fingers, wrists, knees and elbows. The pain in the joints is due to the denaturation of a protein called Collagen, the extracellular matrix found all over the body. This disease in association with some environmental factors causes the denaturation of such protein resulting in the loosening of tissues ultimately causing pain at the joints. In the market, it is believed that Diclofenac sodium inhibits such denaturation of collagen and hence it is used as a drug against this disease. This is not the cure but it is believed that it can inhibit the protein denaturation. We used this Drug as Standard and the results were compared with that of the standard. We studied the protocol to find the anti-arthritic activity and conducted experiments with Synthesized Nano particles, Standard drug and Control. We believed that the Nano particle we synthesized inhibits the protein denaturation and can be used against Rheumatoid arthritis. The results were as we believed.

II. MATERIALS AND METHODS

1. OPTIMIZATION:

Optimization is done to find out the optimal concentration of silver nitrate and optimal volume of leaves extract at which maximum silver nanoparticles are synthesized. It was done by conducting experiments with varying volume of Leaves extract. The leaves extract can be prepared from the leaves of Night jasmine by either boiling or grinding.

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- Extraction:

The leaves extract was done by boiling method. 5gms of leaves were weighed and transferred into a flask. 50 ml of water was then added. The flask was then closed with a cotton plug and placed in water bath. As the result the water in the flask boils and makes the leaves to lose their texture and molecules. The leaves' molecules are extracted out. Then it was filtered to remove the leaves debris. The filtrate (Leaves extract) was collected and stored in refrigerator.

- Synthesis of Silver Nano particles:

50 ml of Silver nitrate stock solution with 0.17mg/ml concentration was prepared by adding 8.5 mg of Silver nitrate powder in 50 ml of DDW (Double Distilled Water). Two sets of combinations were made as follows: 10:3 and 10:5. The first number of ratio represents the volume of silver nitrate stock and the second number indicates the volume of leaves extract. All these mixtures are kept under sunlight for 20 minutes. Sunlight is the inducer here helping the reduction of silver nitrate to silver metal. The appearance of reddish brown color indicates the synthesis of nanoparticles. All the mixtures were observed under spectrophotometer to measure their absorbance at various wavelengths ranging from 400 nm to 500 nm. From the observation, the optimized volume of Silver nitrate stock and leaves extract were determined as 10:3 (10 ml of silver nitrate (0.17mg/ml) and 3 ml of leaves extract). The graph between absorbance and wavelength has been shown at the later part of the paper.

2. BULK PRODUCTION:

- Preparation of Leaves extract:

After optimization, the optimized combination was determined as 10:3. This value is scaled up for the bulk production. Accordingly the leaves were weighed and the leaves extract was prepared as stated in optimization. 500 ml of leaves extract was prepared and stored in the refrigerator.

- Synthesis of Silver Nano particles:

1000 ml Silver nitrate stock solution with 0.17mg/ml concentration was prepared by dissolving 170 mg of silver nitrate powder in 1000 ml of DDW. With this silver nitrate stock, 300 ml of the prepared extract was added and cotton plugged. The mixture was made to expose to Sunlight for 20 minutes. Reddish brown color appeared indicating the reduction of silver nitrate was successful. The mixture was kept undisturbed for 24 hours in dark place.

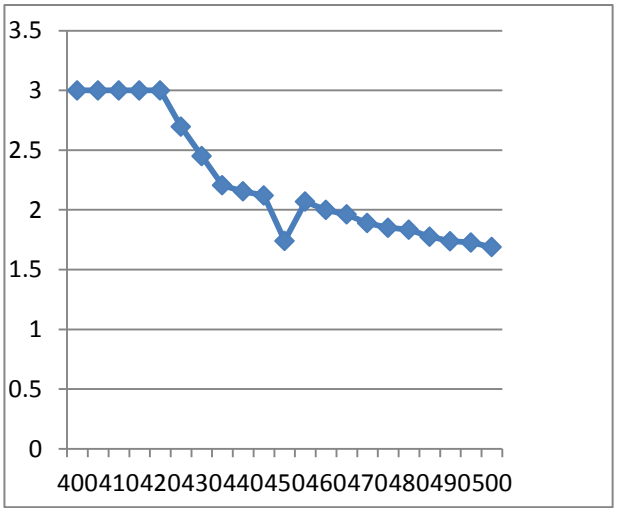
- Recovery of Nano particles:

After the incubation time, the mixture was well mixed and subjected to centrifugation. Centrifugation is the process of separating the particles from solution based on the density difference. Since the particles suspended in the solution are denser than the solution, it gets settled at the bottom which can be later isolated. This process was used to separate the Nanoparticles from the solution. The RPM was set at 6000 and spun for 20 minutes. After the process, the solution was decanted and the few volume of Acetone was added to the settled particles. Then it was vortexed and poured into a petri dish. The solution was kept in room temperature for evaporation. The evaporated particles were then scraped and stored in a vial covered by aluminum foil. Then the amount of nanoparticles synthesized was weighed and found to be 48 mg.

III. CHARACTERIZATION OF NANO PARTICLES SYNTHESIZED

- UV-Visible Spectroscopy analysis:

Synthesis of silver nanoparticles by reducing the silver ions with Nyctanthes arbor-tristis leaves extract may be easily absorbed by UV-Visible spectroscopy. The absorbance was measured in the wavelength range of 400-500nm. The following graph shows a peak at 455 nm indicating the synthesis of nanoparticles.



- Fourier Transform Infrared Spectroscopy:

Synthesized silver nanoparticles were measured by using JASCO FT/IR with 4000-500cm-1 of spectral range in KBr pellet. FTIR analysis was used for the characterization of the extract and the resulting silver nanoparticles. Powder samples for the FTIR was prepared similarly as for powder diffraction measurements. The FT-IR spectra of plant extracts taken before and after synthesis of nanoparticles were analyzed which discussed for the possible functional groups for the formation of nanoparticles. FTIR measurements were carried out to identify the possible biomolecules in the leaves extract responsible for the reduction of ions and also the capping agents responsible for the stability of the biogenic nanoparticle solution. The results of FTIR analysis of this study show different stretches of bonds shown at different peaks; 3324.68—N–H stretch, 2333.45—O–H, 1651.73—C=C stretch, and 1046.19—C=O stretch.

- SEM analysis:

SEM analysis shows an image of high-density Ag NPs synthesized by Nyctanthes arbor-tristis leaves extract and it is shown in the figure below. It was shown that relatively spherical and uniform Ag NPs were formed with diameter ranging from 46nm to 80nm. The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the Ag NPs. The nanoparticles were not in direct contact even in the aggregated condition, indicating stabilization of the nanoparticles by capping agent .The larger

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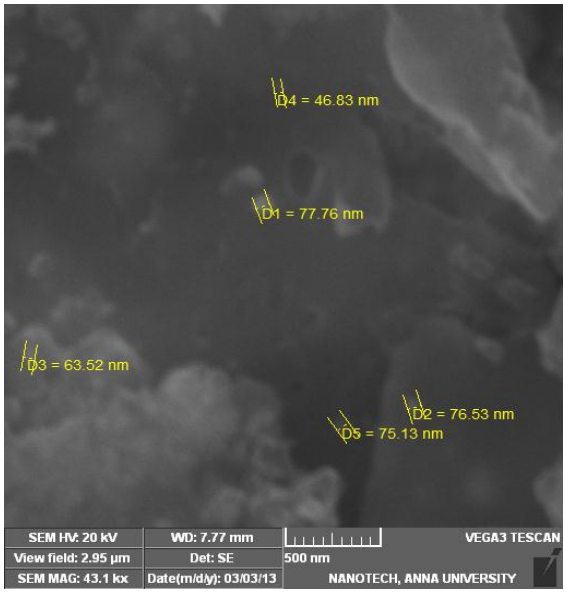
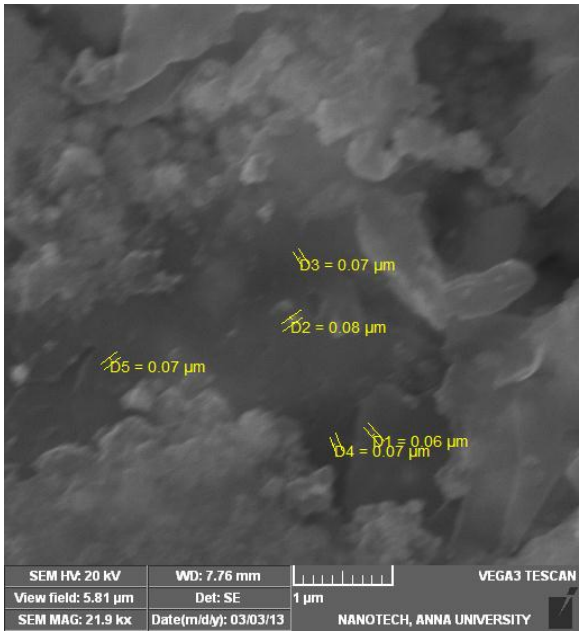
silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements.

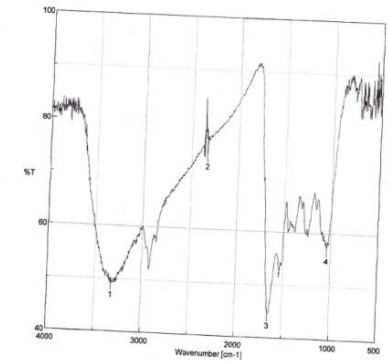
S. NO	2 θ Value	Plane	Element	Phase
1	25.4840	100	Ag	Cubic
2	31.9000	110	Ag	Hexagonal
3	37.7614	111	Ag	Cubic

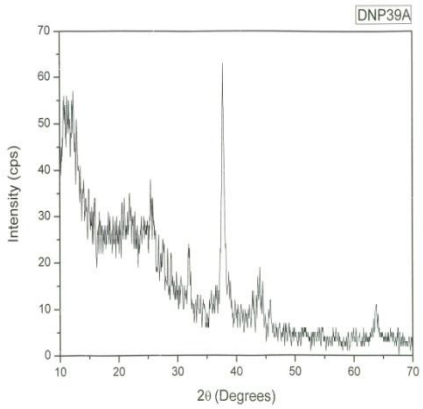


- XRD Analysis:

The structure of the biosynthesized silver nanoparticles was further demonstrated and confirmed by the characteristic peaks observed in the XRD image. The XRD pattern showed three intense peaks in the whole spectrum of 2θ value ranging from 10 to 70. Average size of the particles synthesized was 15nm with size range 10 to 50nm with cubic and hexagonal shape. The typical XRD pattern (Figure) revealed that the sample contains a mixed phase (cubic and hexagonal) structures of silver nanoparticles.







IV. INVITRO METHOD TO DETERMINE ANTI-ARTHRITIC ACTIVITY

- Preparation of Solutions required:

❖ Bovine serum albumin(5% W/V aqueous solution):

0.5 mg of Bovine serum albumin was dissolved in 10 ml of Distilled water and mixed well till complete dissolution was achieved.

❖ Test Stock solution:

The synthesized nanoparticles were dissolved in Double Distilled water to make the varying concentrations ranging from 4mg/ml to 10 mg/ml.

❖ Standard stock solution:

The commercial drug was purchased in the name of Voveron 50 mg. Varying concentration of solutions were prepared. Since it is a commercial product which has some dosage limitations (50mg), the equivalent concentrations of test solutions were calculated and prepared.

❖ Phosphate buffer:

8g of NaCl, 0.2g of KCl, 1.44 g of Na2HPO4 and 0.24g of KH2PO4 were dissolved in 800 ml of Distilled water. Then the solution was adjusted to pH 6.3 and then made up to 1000ml Distilled water.

- Standard Protocol:

❖ Test control solution (0.5ml) was made by mixing 0.45ml of Bovine serum albumin (5% W/V aqueous solution) and 0.05ml of Distilled water.

❖ Test solution (0.5ml) was prepared by adding 0.45ml of Bovine serum albumin and 0.05 ml of Nano particle

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solution. Varying concentrations (10mg/ml, 8mg/ml, 6mg/ml and 4mg/ml) of Nano particle solutions were taken.

- Test Product control (0.5ml) was prepared by mixing 0.45 ml of distilled water and 0.05 ml of Nano particle solution. Varying concentrations (10 mg/ml, 8mg/ml, 6mg/ml and 4mg/ml) of Nano particle solutions were taken.
- Standard solution (0.5ml) was made by adding 0.45ml of Bovine serum albumin and 0.05 ml of Diclofenac sodium solution. Varying concentrations (57mg/ml, 46mg/ml, 34mg/ml and 23mg/ml) of Diclofenac sodium solutions were taken.
- Standard product control (0.5ml) was prepared by mixing 0.45 ml of distilled water and 0.05 ml of Diclofenac sodium solution. Varying concentrations (57mg/ml, 46mg/ml, 34mg/ml and 23mg/ml) of Diclofenac sodium solutions were taken.
- All the above solutions were adjusted to pH 6.3 using 1N HCL.
- All the samples were incubated at 37˚C for 20 minutes and the temperature was increased to 57˚C and incubated for 3 minutes.
- After cooling, 2.5 ml of Phosphate buffer was added to the above solutions.
- The absorbance was measured using UV-Visible spectrophotometer at 416 nm.
- The control represents 100% protein denaturation. The results were compared with the Standard Diclofenac sodium.
- The percentage inhibition of protein denaturation can be calculated as, Percentage Inhibition= [100-(((OD of test solution – OD of product control)/ (OD of test control))* 100)]

V. RESULT AND DISCUSSION

Anti-arthritic effect of silver nanoparticles synthesised from the leaves extract of Nyctanthes arbor-tristis was studied significantly by using *in-vitro* inhibition of protein denaturation model. The effect of standard drug and silver nanoparticles on inhibition of protein denaturation is shown in table 2. Silver nanoparticles at different concentrations provided significant protection against denaturation of proteins. Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. Obtained data stated that *Silver nanoparticles* from leaf extract of *Nyctanthes arbor-tristis c*ould be used as potent anti-arthritic agent.

Table 1 Absorbance values of Nanoparticle solution and Standard solution OD of Test control= 0.395

Nano particle concentration (mg/ml)	OD of Nano particle solution at 416nm	OD of Test product control at 416nm	Standard concentration (mg/ml)	OD of Standard solutions at 416nm	OD of Standard product control at 416nm
4	0.107	0.033	23	0.973	0.910
6	0.073	0.016	34	0.521	0.470
8	0.055	0.011	46	0.229	0.187
10	0.048	0.007	57	0.165	0.126

Table 2 Effect of *Silver nanoparticles* on inhibition of protein denaturation

Percentage inhibition for Nano particle solution (%)	Percentage inhibition for Standard(Diclofenac sodium) (%)
81.20	84.06
85.56	87.09
88.89	89.37
89.62	90.13

VI. CONCLUSION

From the results obtained in the present study, it may be concluded that Silver nanoparticles from leaf extract of Nyctanthes arbor-tristis possess significant anti-arthritic activity. Hence it could be beneficial for further work as active anti-arthritic agent.

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