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Microwave Assisted Synthesis and Characterization of Silver Nanoparticles Using Ipomoea staphylina Leaf Extract and Its Anti-Inflammatory Activity against Human Blood Cells

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Abstract: *We report for the first time, the green synthesis of silver nanoparticles using Ipomoea staphylina leafextract.0.1N of silver nitrate is mixed with leaf extract & incubated for a period of 30 minutes. After that, the colour of the mixture changes from pale yellow to dark brown which confirms the presence of silver nanoparticles. The synthesized AgNPs is characterized using UV-Visible spectroscopy (UV), Fourier transform infra-red spectroscopy (FTIR), Transmission electron microscope (TEM) and X-ray diffraction (XRD). The average size of synthesized silver nanoparticles using XRD data is found to be 14.27 nm by Scherrer's formula, which is approximately similar as the size obtained in TEM Analysis 14.08nm.Our finding supports the reported therapeutic use of herb Ipomoea staphylinain tribal medicine for the treatment of inflammation. The most active extracts can be subjected to isolation and used for the therapeutic as anti-inflammatory agents and also to undertake further pharmacological studies.*

Keywords: *Ipomoea staphylina leaf, Silver nanoparticles, Anti-inflammatory activity.*

I. Introduction

Nanotechnology is the most momentous branch of technology that deals with the improvement in the new materials in all fields in our day to day activities, especially in the field of medicine. Nano predominantly deals with the materials that are in the range from 1 to 100 nm. Recently researcher's major concerns were synthesizing of metal nanoparticles that possess high novel physical, chemical, biological, electrical and magnetic properties. The most important property of the nanoparticles is the large surface to volume ratio where all the surface atoms present in the particles actively participate in changing the property of the material [1]. The size, shape, and surface morphology of nanoparticles play a key role in controlling the property, nature of the materials so the materials can be used in the place where the possibility is highly questionable.

Two major approaches were used for the preparation of nanoparticles namely top-down and bottom-up. Various physical and chemical methods such as ball milling, chemical solution, co-precipitation, physical vapor deposition, chemical vapor deposition, sol-gel, micro-wave assisted, and electrochemical synthesis methods were available for the preparation of metal nanoparticles comes under the above mentioned approaches [2-8]. The chemicals used for the processes of nanoparticle synthesis were highly toxic, expensive and non-ecofriendly that limits their role, particularly in medical and food applications compared with other applications (electronics, mechanical, etc.,) where it can be used as such. Hence, researchers were progressively aiming at biological methods of synthesizing of metal nanoparticles to use in the medical application. The major advantage of biological methods over other methods are simple, less expensive, openly applicable without any binders, no need for toxic chemicals, no toxic byproducts and mostly it is environment friendly [9,10]. In the biological approach, the synthesis of nanoparticles has been evolved from various sources that were available enormously in the earth such as microorganisms and terrestrial plants. The active ingredients found in the sources were responsible for the reduction of metallic ions to nanoparticles in green methods [11-13].

Silver is used extremely because of its significant property and recently contributing its role towards the medical applications [14-16]. Silver nanoparticles (AgNPs) were reported to have an extraordinary anti-bacterial, anti-fungal and anti-inflammatory activity. Synthesizing AgNPs using biological methods helps the particles by direct usage for the medical treatment and other medicinal applications. All the parts of the source such as leaves, stem, flower, seeds, root and skin of the fruits were separately used earlier for the synthesis of AgNPs. Synthesized nanoparticles using green method were observed to contain the biomolecules of the plant extract on the surface that were found to be having high medical benefits that can be used as drugs, carrier for drugs, cosmetic, food and other pharmaceutical applications, etc. [17].

In one of the recent study, Ipomoea staphylina was taken to evaluate in vivo anti-inflammatory activity. The study revealed that the plant Ipomoea staphylina possesses a significant anti-inflammatory activity in carrageenan induced paw edema method, which supports the folkloric claim of the plant. Further studies are

needed to isolate the active constituents, elucidate structure and mechanism of action of these extracts. Traditional medical practices are an important part of the primary health care system in the developing world herbal medicines are comparatively safer than synthetic drugs. Plant-based traditional knowledge has become a recognized tool in search for new sources of drugs and neutraceuticals[18]

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance [19].

Ipomoea staphylina Linn family Convolvulaceae commonly called as onnan kodi, Ipomoea staphylina is Perennial, large straggling or climbing, woody, glabrous, shrubs Common, Gregarious in heavy masses on thickets, trees etc, Plains to 1200 m [20]. The plant ipomoea staphylina Linn has been used in different systems of traditional medication for the treatment of diseases and ailments of human beings. It has been reported as the anti-inflammatory [21]. For this all reasons we take a plant to bring out and official manner by the thorough investigation on this plant such as phytochemical [22,23]. In the present investigation, Ipomoea staphylina leaf extract was used for the synthesis of AgNPs for the first time using its leaf extract. To confirm the formation of nanoparticles, different characterization techniques have been used.

II. Materials And Methods:

Collection of leaf

Fresh leaves of samples was collected from TV Kovil, Trichy, during the month of May and identified by Dr.JohnBritto, The Director, Rabinat Herbarium and Center for Molecular Systematics, St.Joseph's College (Campus), Trichirappalli-2, Tamil Nadu, India. (Plant authentication No: PN005).

Preparation of leaf extract

The fresh and young leaf sample was collected & washed thoroughly with sterile double distilled water (DDW). Twenty grams of sterilized leaf samples were taken and cut into small pieces. Finely cut leaves were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile DDW. After that, the mixture was boiled for 5 minutes and then filtered. The extract was stored in 4 ^{0}C.

Synthesis of silver nanoparticles

Silver nitrate was used as precursor in the synthesis of silver nanoparticles**.** 100 ml of leaf extract was added to 100 ml of 0.1N $AgNO_3$ aqueous solution in conical flask of 250 ml content at room temperature. The flask was thereafter put into shaker (100 rpm) at 50^0 C and reaction was carried out for a period of 12 hrs. Then the mixture is kept in microwave oven for exposure of heat. The mixture was completely dried after a period of 20 minutes and hence nanoparticles in form of powders were obtained.

Fig 1. Optical photograph of Ipomoea staphylina A- 0.1 N $AgNO_3$ solution B- Leaf extract C- Leaf extract + $AgNO_3$ D- Leaf extract + $AgNO_3$(After 30mins) E- Leaf extract + $AgNO_3$(After 1 hr) F- Leaf extract + $AgNO_3$(After 2 hrs) G- Leaf extract + $AgNO_3$(After 24 hrs)

UV-visible spectroscopy analysis

The colour change in reaction mixture (metal ion solution + leaf extract) was recorded through visual observation. The bio reduction of silver ions in aqueous solution was monitored by periodic sampling of solid and subsequently measuring UV-visible spectra of the solid sample. UV-visible spectra of sample were monitored as a function of time of reaction on the UV-visible spectroscopy & the investigation is carried out using PERKIN ELMER (Lambda 35 model) spectrometer in the range of 190 nm to 1100 nm.

FT-IR measurement

The Fourier transform infrared (FTIR) investigation is carried out using PERKIN ELMER (Spectrum RXI) spectrometer in the range of 400 cm^{-1} to 4000 cm^{-1}. The functional groups were identified using the peak assignments.

XRD measurement

The sample was drop- coated onto Nickel plate by just dropping a small amount of sample on the plate frequently, allowed to dry and finally thick coat of sample was prepared. The particle size and nature of the silver nanoparticle was determined using X-ray diffraction (XRD). This was carried out using Rigaku miniflex-3 model with 30kv, 30mA with Cukα radians at 2θ angle.

TEM analysis

Sample is dispersed with acetone and exposed in ultrasonics for 5 minutes. Take a drop of a solution from the sample and drop it on the grid, leave until it dries. After drying the sample is inserted into TEM instruments using model Tecnai T20Making in FEI, Netherlands operating at 200KeV Tungsten Filament.

Anti-Inflammatory Activity

The human red blood cell (HRBC) membrane stabilization method

The method as prescribed (Gopalkrishnan et al., 2009; Sakat et al., 2010) was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of extracts were prepared in mg/ml using distilled water and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37^0C for 30 minutes, centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 Jg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and the mean value of the three was considered. The percentage (%) of HRBC membrane stabilization or protection was calculated using the following formula,

Percentage of Protection (%) = (100- OD of drug treated sample/OD of Control) X 100

Albumin Denaturation Method

The method as prescribed (Sakat et al., 2010) was followed with some modifications. The reaction mixture consists of test extracts and 1% solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes. After cooling the sample the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percent inhibition of protein denaturation was calculated as follows,

Percentage of inhibition (%) = (OD of Control- OD of Sample/ OD of Control) X 100

III. Results

UV-visible spectroscopy analysis

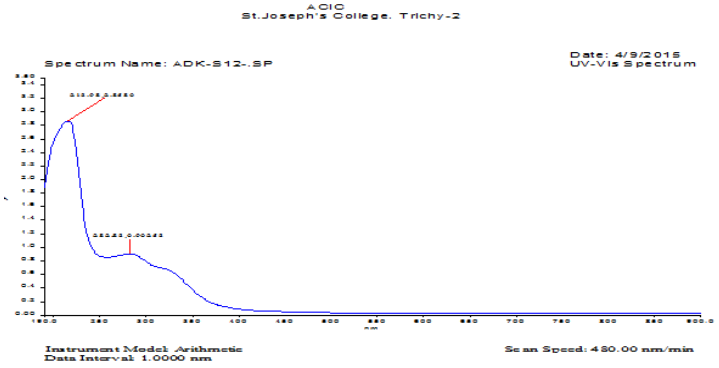

Fig.2-UV-Visible spectrum of synthesized silver nanoparticles using leaf extracts of Ipomoea staphylina.

UV-Vis spectroscopy analysis shows the absorbance band of silver nanoparticles synthesized using Ipomoea staphylina leaf extract at 213.98 nm, which confirms the presence of poly-unsaturated and aromatic compound (Isoquinoline) (Advanced strategies in food analysis ,UV/VIS spectrometry by Richard Koplík)

FT-IR measurement

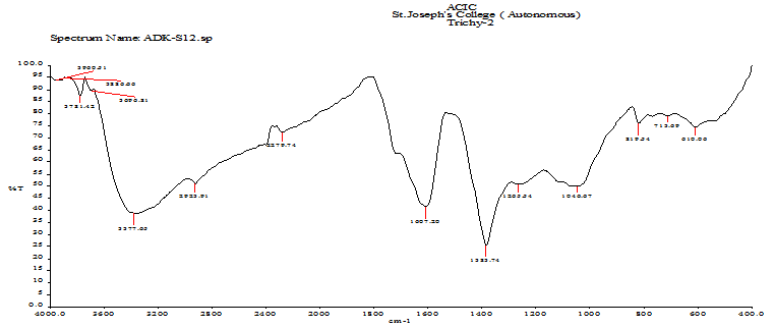

Fig.3.FT-IR spectrum of synthesized silver nanoparticles using leaf extracts of Ipomoea staphylina.

The Ipomoea staphylina related functional groups were identified using the peak assignments. A strong peak at 396.51 cm^{-1},3886.66 cm^{-1},3781.42 $cm^{-1}$and 3696.81cm^{-1} was assigned to the OH stretching in Phenol group; The sharp and bend peak at 3377.65 cm^{-1} was assigned to medium N-H stretching may be present primary, secondary amines and amides group; The medium peak at 2923.91 cm^{-1} was assigned medium C-H stretching in alkenes; alkyl group peak at 2279.24 cm^{-1} was assigned C-H stretching; The medium peak at 1607 cm^{-1} was assigned to N-H bend in primary amine group; The variable peak at 1383.74 $cm^{-1}$was assigned to C-H bending in alkenes; The medium peak at 1265.54 cm^{-1} was assigned to C-H wag in alkyl halides; The medium peak at 1046.67 cm^{-1} was assigned to C-N stretching in aliphatic amine group; The medium peak at 819.54 cm^{-1} was assigned to C-Cl stretching in alkyl halide group; The medium peak at 713.69 cm^{-1} was assigned to C-H stretching in rock alkanes and the medium peak at 610.06 $cm^{-1}$was assigned to C-Br stretching in alkyl halides are observed.

XRD measurement

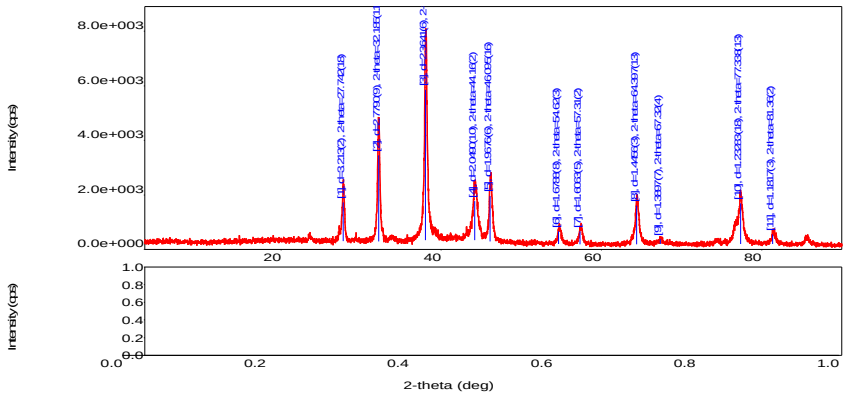

Fig.4. XRD spectrum of synthesized silver nanoparticles using leaf extracts Ipomoea staphylina.

Determination of crystalline size

Average crystallite size of silver was calculated using the Scherrer's formula,

$D = k\lambda / \beta\cos\theta$

D- Average crystallite size: K- Constant: λ- X- ray Wavelength: β- Angular FWHM of the XRD peak at the diffraction angle: θ- Diffraction angle.

By using XRD data in Scherrer's formula, the average size of the particle is approximately found to be 14.27 nm.

TEM analysis

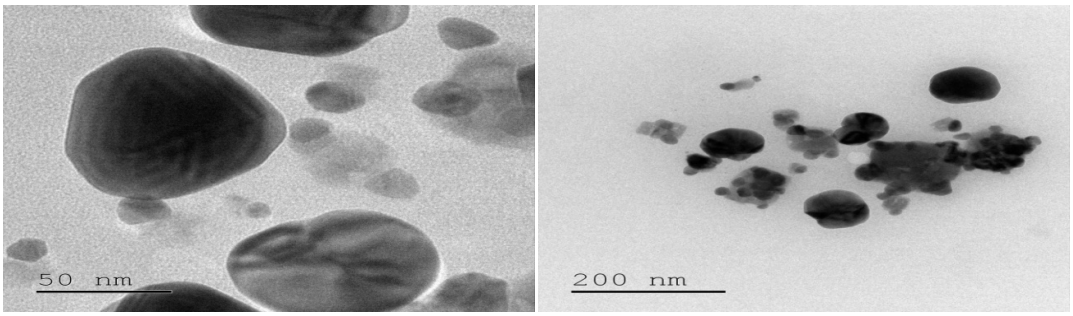

Fig.5.TEM image of synthesized silver nanoparticles using leaf extracts of Ipomoea staphylina.

The figure shows the TEM image obtained by the reaction of Ipomoea staphylina leaf extract and 0.1N silver nitrate solution separately. The Average size of Ipomoea staphylina AgNPs by TEM Analysis is found to be 14.08 nm.

Table-1.Anti-inflammatory activity of human red blood cell (HRBC) by using AgNPs of Ipomoea staphylina.

S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M
1	100	21.83 ± 0.41
2	200	25.18 ± 0.29
3	400	32.29 ± 0.73
4	600	37.43 ± 0.38
5	800	43.16 ± 0.91

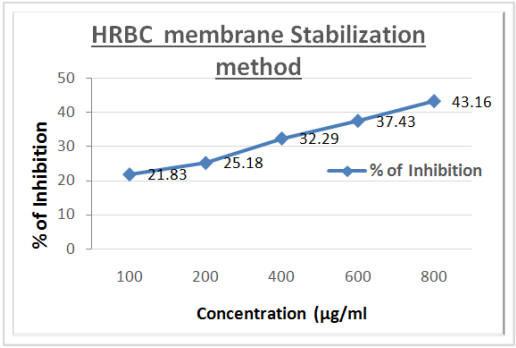

Fig.6.Graphical representation of Anti-inflammatory activity of human red blood cell (HRBC) by using AgNPs of Ipomoea staphylina.

Table-3.Anti-inflammatory activity of Albumin denaturation method by using AgNPs of Ipomoea staphylina.

S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M
1	100	21.65 ± 0.59
2	200	24.34 ± 0.73
3	400	30.23 ± 0.42
4	600	35.48 ± 0.57
5	800	40.61 ± 0.39

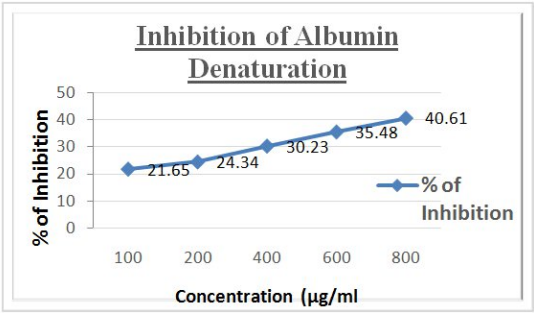

Fig.7. Graphical representation of Anti-inflammatory activity of Albumin denaturation method by using AgNPs of Ipomoea staphylina.

Anti-inflammatory study like human red blood cell (HRBC), membrane stabilization, inhibition of albumin denaturation indicated that anti-inflammatory activity .The medical use of Ipomoea staphylina has a good anti-inflammatory activity. As the concentration of the sample increases, the percentage of inhibition also increases.

IV. Discussion

In one of the recent investigation, Ipomoea staphylina plant was used for the synthesis of AgNPs for the first time using its leaf extract. To confirm the formation of nanoparticles, different characterization techniques were been used. The methanolic and aqueous extract of Ipomoea staphylina exhibited significant anti-inflammatory activity against carrageenan-induced rat paw edema [24].The plant extracts showed potent COX inhibition that clearly suggests the extracts contains potent anti-inflammatory components that could be of great therapeutic use [25]. The three different lines shows the activity inhibition by three different samples namely water, ethanol and n-butanol extracts respectively out of which ethanol extract posed the most significant inhibition activity [26].

V. Conclusion

In conclusion, the bio-reduction of aqueous silver ions by the leaf extract of the Ipomoea staphylina has been demonstrated. The reduction of the metal ions through leaf extract leading to the formation of silver nanoparticles and the synthesized nanoparticles are quite stable in solution. The sizes of silver nanoparticles were determined by using XRD &TEM analysis. Transmission Electron Micrograph of Silver nanoparticles finds the size of the nanoparticles. By applying XRD data in Scherrer's formula, the average size of silver nanoparticles is found to be 14.27 nm, which is approximately similar as the size obtained in TEM analysis 14.08 nm**.** In addition to that anti-inflammatory studies like human red blood cell (HRBC), membrane stabilization, and inhibition of albumin denaturation indicate the anti-inflammatory activity. The medical uses of Ipomoea staphylina have a good anti-inflammatory activity. As the concentration of the sample increases, the percentage of inhibition also increases. Thus, the prepared AgNPs from the leaf extract of Ipomoea staphylina can be directly used as a carrier for drugs. The synthetic methods based on naturally occurring biomaterials provide an alternative means for obtaining the nanoparticles. Use of plants in synthesis of nanoparticles is quite novel leading to truly 'green Environment' route. This green environment approach towards the synthesis of nanoparticles has many advantages such as process scaling up, economic viability and safe way to produce nanoparticles.

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