Microwave Assisted Synthesis and Characterization of Silver Nanoparticles Using Citrullus Lanatus Leaf Extract and Its Anti-Inflammatory Activity Against Human Blood Cells

P. Anitha, P. Sakthivel

Abstract: ***The use of engineered nanomaterials has increased as a result of their positive impact on many sectors of the economy, including agriculture. In the current study, the plant extract of Citrullus Lanatus is used for the synthesis of silver nanoparticles. The leaf extract is mixed with $AgNO_3$, and then it is incubated. The extract is kept in microwave oven for exposure of heat, then it is dried and powdered. The synthesized dried powder is confirmed as nanoparticles by color transformation. The characterization of silver nanoparticles was studied by UV–Vis spectroscopy, FTIR, XRD& TEM. The silver nanoparticles synthesized were generally found in size 1-100 nm. The average size of synthesized silver nanoparticles is found to be 15.98 nm using XRD data by Scherrer's formula, which is approximately similar as the size obtained in TEM Analysis 16.32 nm. In totality, the AgNPs prepared are safe to be discharged in the environment and possibly utilized in processes of pollution remediation. AgNPs may also be efficiently utilized in Anti-inflammatory activity of Pharmaceutical research to obtain better result of plant as shown by our study. The Anti-inflammatory activity of silver nanoparticles was tested on human blood cells which confirms that the plant mediated synthesis of silver nanoparticles have a significant Anti-inflammatory effect on human blood cells.***

Keypads: Silver Nanoparticles, UV–Vis Spectroscopy, FTIR, TEM, XRD, Anti-Inflammatory, Human Blood Cells, etc.

I. INTRODUCTION

Nanotechnology is a branch of science that is related to nanomaterials, which helps to overcome the limitations of size. The interactions of Nanomaterials with plants have not been fully elucidated. There have been different and often conflicting reports on the absorption, translocation, accumulation, biotransformation, and toxicity of nanoparticles in various plant species. The effects of silver nanoparticles (AgNPs) are still under investigation [1],[2].The impact of AgNPs on higher plants appears to depend on the species and age of the plants; the size and concentration of the nanoparticles; the experimental conditions, such as temperature, duration and method of exposure [3]. Silver nanoparticles are of interest because of the unique properties (e.g., size and shape depending on optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components [4]. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles. The most popular chemical approaches, including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physiochemical reduction, and radiolysis are widely used for the synthesis of silver nanoparticles [5].

Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry, and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry) 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 [6].C. lanatus is an important cucurbit crop, accounting for 7% of the worldwide area devoted to vegetable production. Watermelon (Citrullus Lanatus) is a fruit crop of the family Cucurbitaceous, along with other cucurbit crops including the melon, cucumber, and zucchini. Watermelon thrives in the temperate regions of Africa, central Asia, and the Mediterranean. Fruit of Citrullus Lanatus consumed in the summer season and it give schilling effect and reduce thirst. The fruit contains so many active phytoconstituents and minerals. The seeds contain fatty acids and have good activities. Different literature revels medicinal the importance fruit as well as seeds [7].In the present investigation, Citrullus Lanatus 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

A. *Collection of leaf*

Fresh leaves of the Sample was collected from T.V Kovil, Trichy, Trichy, during the month of May and identified by Dr. John Britto, The Director, Rabinat Herbarium and Center for Molecular Systematic, St.Joseph's College (Campus),Tiruchirappalli-2, Tamil Nadu. India. (Plant authentication No: PN003).

B. *Preparation of leaf extract*

The fresh and young leaf sample was collected & washed thoroughly with sterile double distilled water (DDW).

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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.

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.

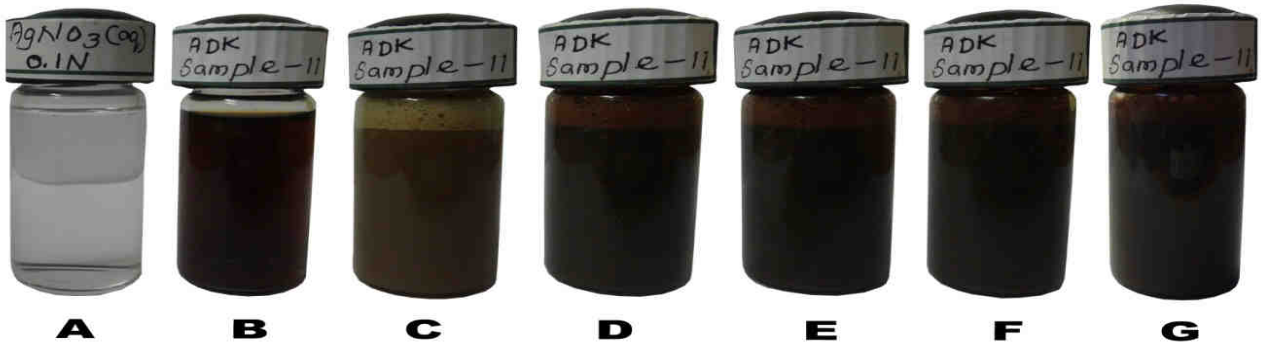

Fig 1. Optical photograph of Citrullus LanatusA- 0.1 N $AgNO_3$ solution B- Leaf extract C- Leaf extract + $AgNO_3$ D- Leaf extract + $AgNO_3$(After 30mins) E- Leaf extract + $AgNO_3$(After 1 hr) F- Leaf extract + $AgNO_3$(After 2 hrs) G- Leaf extract + $AgNO_3$(After 24 hrs)

D. *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.

E. *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.

F. *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.

G. *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.

III. ANTI-INFLAMMATORY ACTIVITY

A. *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 isohaline 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^{0}C for 30 minutes, centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectra photo metrically at 560nm. 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,

B. *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

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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

IV. RESULTS

UV-Visible Spectroscopy Analysis

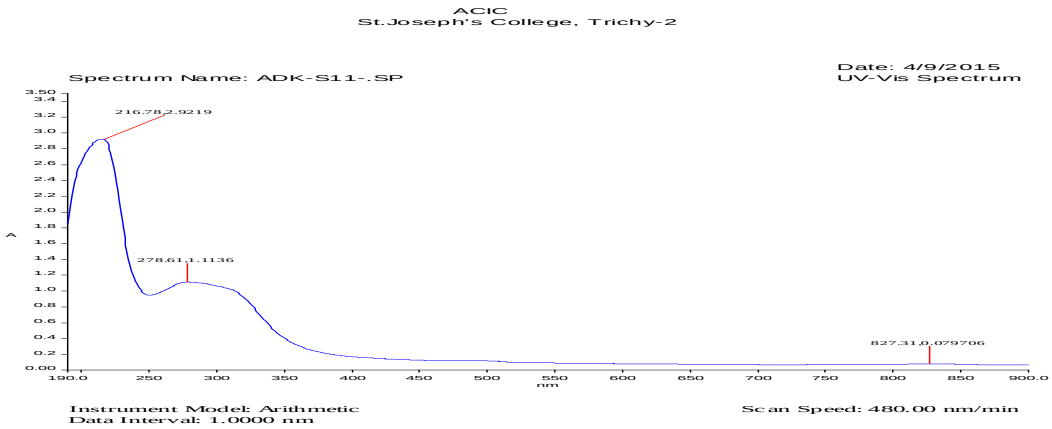

Fig.2-UV-Visible spectrum of synthesized silver nanoparticles using leaf extracts of Citrullus Lanatus .

UV-Vis spectroscopy analysis shows the absorbance band of silver nanoparticles synthesized using Citrullus Lanatus leaf extract at 216.78 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

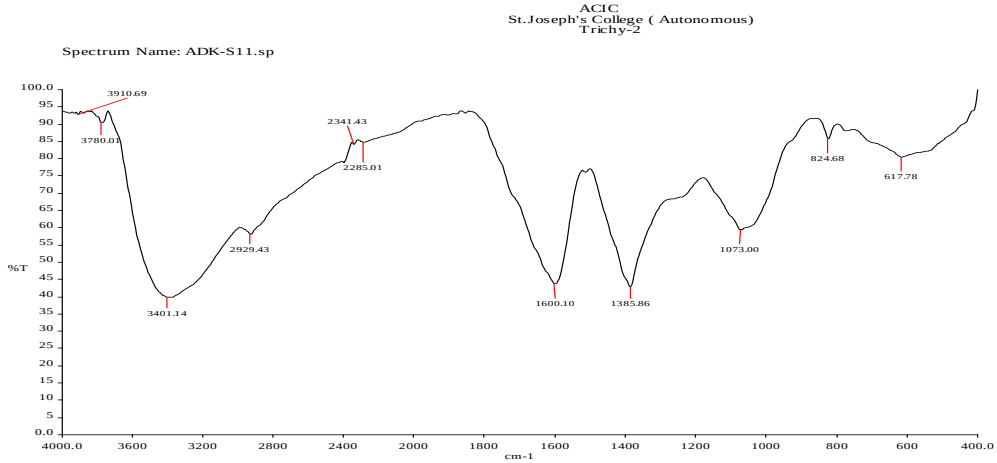

Fig.3.FT-IR spectrum of synthesized silver nanoparticles using leaf extracts of Citrullus Lanatus .

The Citrullus Lanatus related functional groups were identified using the peak assignments. A strong peak at 3910.69 cm^{-1} and 3780.01 cm^{-1} was assigned to strong OH stretching in Phenol group; The sharp and bend peak at 3401.14 cm^{-1} was assigned to OH; H-bonded alcohol and phenols or medium N-H stretching may be present primary, secondary amines and amides group;The medium peak at 2929.43 cm^{-1} was assigned medium C-H stretching in alkenes; weak peak at 2341.43 cm^{-1} was assigned to –C (triple bond) C- stretching in alkenes group; The peak at 2285.01 cm^{-1} was assigned C-H stretching in alkenes; The medium peak at 1600.10cm^{-1} was assigned to C-C stretch (in-ring) in aromatic group; The strong peak at 1385.86 cm^{-1} was assigned to -N=O stretching in Nitro group; The medium peak at 1073.00 cm^{-1} was assigned to C-N stretching in aliphatic amine group; The medium peak at 824.68 cm^{-1} was assigned to C-Cl stretching in alkyl halide group and The medium peak at 617.78 $cm^{-1}$was assigned to C-Br stretching in alkyl halides are observed.

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XRD Measurement

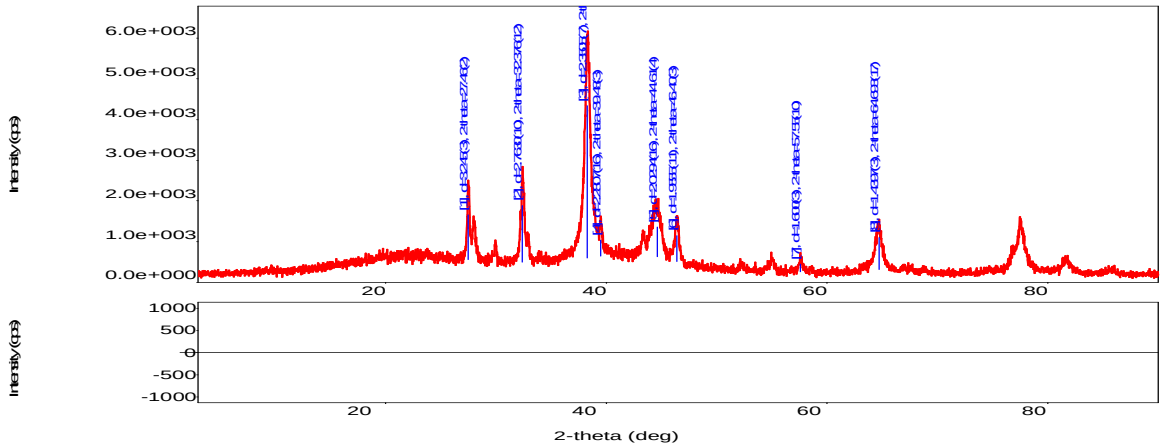

Fig.4. XRD spectrum of synthesized silver nanoparticles using leaf extracts of Citrullus Lanatus .

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 15.98 nm

TEM Analysis

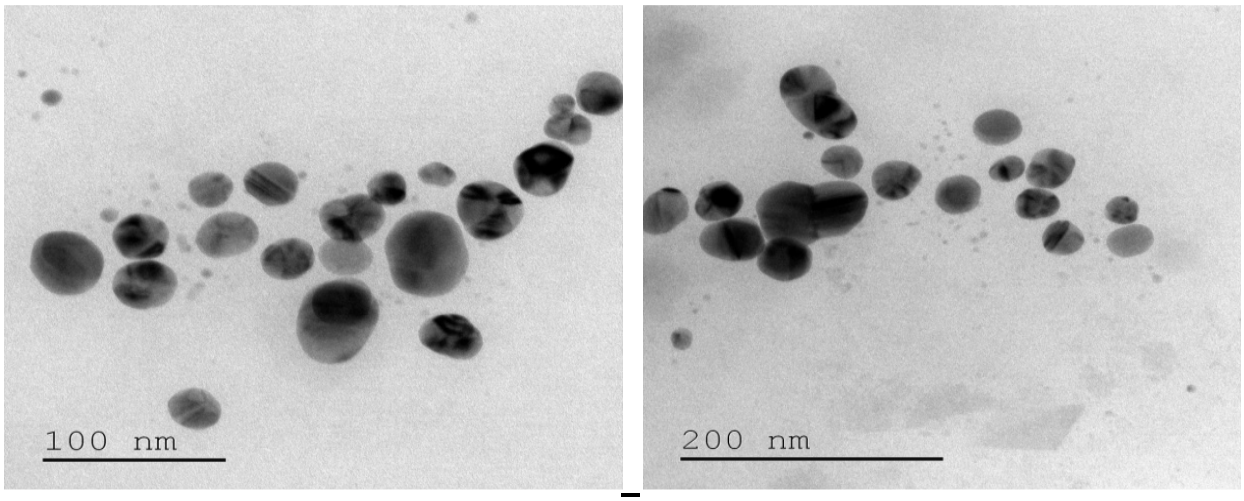

Fig.5.TEM image of synthesized silver nanoparticles using leaf extracts of Citrullus Lanatus.

The figure shows the TEM image obtained by the reaction of Citrullus Lanatus leaf extract and 0.1N silver nitrate solution separately. The Average size of Citrullus Lanatus AgNPs by TEM Analysis is found to be 16.32 nm.

Anti-inflammatory activity:

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

S. No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M
1	100	38.43 ± 0.91
2	200	42.61 ± 0.63
3	400	47.59 ± 0.21
4	600	54.19 ± 0.49
5	800	57.37 ± 0.52

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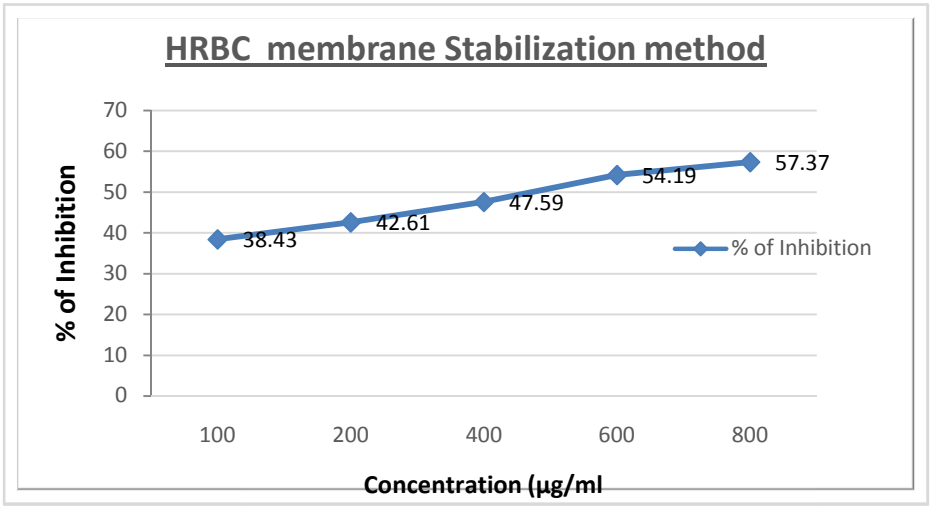

Fig.6.Graphical representation of Anti-inflammatory activity of human red blood cell (HRBC) by using AgNPs of Citrullus Lanatus .

Table-3. Anti-inflammatory activity of Albumin denaturation methodbyusing AgNPs of Citrullus Lanatus.

S. No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M
1	100	35.29 ± 0.54
2	200	40.47 ± 0.29
3	400	43.33 ± 0.34
4	600	51.29 ± 0.37
5	800	56.78 ± 0.18

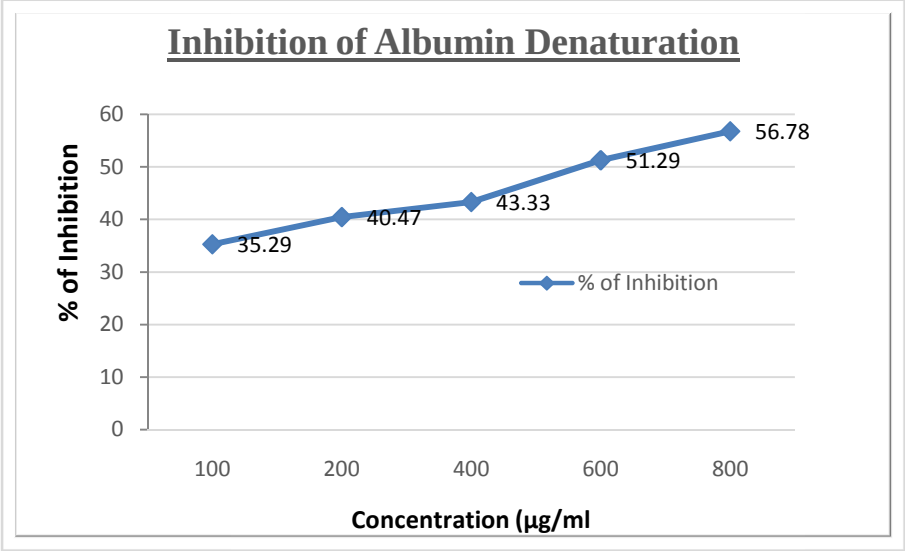

Fig.7.Graphical representation of Anti-inflammatory activity of Albumin denaturation methodby using AgNPs of Citrullus Lanatus .

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

V. DISCUSSION

Silver nanoparticles (AgNPs) appears yellowish brown in colour in aqueous medium as a result of surface Plasmon vibrations [8]. As the different leaf extracts were added to aqueous silver nitrate solution, the colour of the solution changed from faint yellow to brown then to reddish brown and finally to colloidal brown indicating AgNP formation. Similar changes in colour have also been observed in previous studies [9-13] and hence confirmed the completion of reaction between leaf extract and $AgNO_3$.

VI. CONCLUSION

Silver nanoparticles (AgNPs) were successfully synthesized from bio-reduction of silver nitrate solutions using Citrullus Lanatus leaf extracts. Owing to varying properties of these three plant species, AgNPs obtained from them also varied in size, the smallest being yield using Citrullus Lanatus.

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AgNPs have been appropriately characterized using UV-vis spectroscopy, FTIR,SEM, and TEM analysis. Results denoted Citrullus Lanatusextract to be a better reducing agent. By applying XRD data in Scherrer's formula, the average size of silver nanoparticles is found to be 15.98 nm, which is approximately similar as the size obtained in TEM analysis 16.32 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 Citrullus Lanatus have a good anti-inflammatory activity. As the concentration of the sample increases, the percentage of inhibition also increases. Besides, they also aided in plant germination and growth by sequestering nutrients for them and could hence be implemented for agricultural purposes. Hence, due to their benign and stable nature anti-inflammatory of AgNPs may be well utilized in industrial and remedial purposes. However, plant uptake and utilization of AgNPs require more detailed research on many issues like uptake potential of various species, process of uptake and translocation and the activities of the AgNPs at the cellular and molecular levels.

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