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SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *ACALYPHA INDICA* LEAF EXTRACT AND ITS ANTI-INFLAMMATORY ACTIVITY AGAINST HUMAN BLOOD CELLS

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ABSTRACT

In the present study, bio synthesis of silver Nanoparticles using aqueous leaf extract of *Acalypha indica* and its anti-inflammatory activity against different micro-organisms were investigated. About 10 ml of aqueous extract of *A. indica* is added with 90 ml of $AgNo_3$ (1mM) solution, the resulting mixture was incubated at 37^0C under static condition. After a period of 30 minutes the colour of the mixture changes from yellow to brownish colour, which indicates the formation of AgNPs. Green synthesis of silver nanoparticles (AgNPs) was characterized by UV-visible spectroscopy, Transmission electron microscopy, Fourier transform infrared spectroscopy, and X-ray diffraction (XRD). The Ag-Nps are monitored with the help of UV-visible spectrophotometer at the range of wavelength of 267.95nm. The average size of Ag-Nps was found to be 16.86 nm and 16.6 nm determined by using XRD and TEManalysis respectively. The technique was employed to visualize the size of Ag-Nps. It is observed from the results that biologically synthesized Ag-Nps from *Acalypha indica* aqueous leaf extract showed effective anti-inflammatory activity against human blood cells when comparable with standard values.

KEYWORDS

Acalypha indica, Silver Nanoparticles, UV–Vis spectroscopy, FTIR, TEM, XRD and Human blood cells.

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INTRODUCTION

Indian greeneries are the chief and cheap source of medicinal plants and plant products. From centuries till date, these medicinal plants have been extensively utilized in Ayurveda. Recently, many such plants have been gaining importance due to their unique constituents and their versatile applicability in various developing fields of research and development. Nano biotechnology is

presently one of the most dynamic disciplines of research in contemporary material science whereby plants and different plant products are finding an imperative use in the synthesis of nanoparticles (NPs). In general, particles with a size less than 100 nm are referred to as Nanoparticles. Entirely novel and enhanced characteristics such as size, distribution and morphology have been revealed by these particles in comparison to the larger particles of the mass material that they have been prepared from[1]. NPs of noble metals like gold, silver and platinum are well recognized to have significant applications in electronics, magnetic, optoelectronics and information storage[2-5]. One such important member of the noble metal NPs are silver NPs (AgNPs). They are also broadly applied in shampoos, soaps, detergents, cosmetics, toothpastes, medical, pharmaceutical products and are hence directly encountered by human systems[6,7]. Earlier, the antifungal properties of silver and silver nitrate were well incorporated in the field of medical science. Also, the medicinal importance of innumerable plants and plant parts were known. But the plant-mediated silver Nano product is a relatively newer concept. Nano biotechnology and their derived products are unique not only in their treatment methodology but also due to their uniqueness in particle size, physical, chemical, biochemical properties and broad range of application as well. This current emerging field of Nano biotechnology is at the primary stage of development due to lack of implementation of innovative techniques in large industrial scale and yet has to be improved with the modern technologies. Hence, there is a need to design an economic, commercially feasible as well environmentally sustainable route of synthesis of AgNPs in order to meet its growing demand in diverse sectors.

There are so many ways to synthesize nanoparticles, but greater emphasis is given on "Bio based microwave synthesis". The physical characterization of synthesized AgNPs was done by scanning electron microscope (SEM) and UV- Vis Spectrophotometer. Some study also deals with the removal of NO_2 and SO_2 from atmosphere. The reason behind selection of these gases is their carcinogenic trait. These gases are found to cause cancer especially breast cancer[8], lungs cancer and cardiac disorders[9], mainly in the industrial surrounding localities. *Acalypha indica* Linn. is commonly known as copper leaf (Indian Nettle) belongs to the family Euphorbiaceae and is seen in many parts of Asia including India, Pakistan, Yemen, Sri Lanka and throughout Tropical Africa and South America (Ramachandran, 2008)[10].

Recently, microwave heating has been explored as a promising technique for nanoparticle synthesis. In the present study, we first report the reduction of silver ions using aqueous *Acalypha indica* leaf extract under microwave irradiation for facile and fast phytosynthesis of silver nanoparticles (AgNPs). To the best of our knowledge, no reports pertaining to a microwave method by using *Acalypha indica* leaf extract are yet available. The anti-inflammatory property of silver nanoparticles was also investigated.

MATERIALS AND METHODS

Collection of leaf

Fresh leaf of *Acalypha indica* were collected from Perambalur, 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: PN008).

Preparation of leaf extract

The fresh and young leaf samples of *Acalypha indica* was collected and washed thoroughly with sterile double distilled water (DDW). Twenty gram 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 (Figure No.1).

Synthesis of silver nanoparticles

Silver nitrate was used as precursor in the synthesis of silver nanoparticles. 100 ml of *Acalypha indica* 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.

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 and the investigation was 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 is Tecnai T20 Making in FEI, Netherlands operating at 200KeV Tungsten Filament.

Anti-Inflammatory Activity

The human red blood cell (HRBC) membrane stabilization method

The method as prescribed 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 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

RESULTS

UV-visible spectroscopy analysis

UV-Vis spectroscopy analysis showed that absorbance peak of silver nanoparticles using *Acalypha indica* leaf extract at 267.95 nm, which

confirms the presence of poly-unsaturated and aromatic compound (Isoquinoline) (Advanced strategies in food analysis, UV/VIS spectrometry by Richard Koplík) (Figure No.2).

FT-IR measurement

The *Acalypha indica* related functional groups were identified using the peak assignments. A strong peak at 3900.32 $cm^{-1}$and 3765.75 cm^{-1} was assigned to the OH stretching in Phenol group; The sharp peak at 3414.55 cm^{-1} was assigned to OH ,H-bonded alcohol and phenols; The medium peak at 2926.05 cm^{-1} was assigned to C- H stretching in alkenes group; The medium peak at 1597.37 N-H bend stretching may be present in primary amides group; The strong peak at 1385.32 cm^{-1} was assigned N-O stretching may be present in Nitro group; The strong peak at 1107.01 $cm^{-1}$was assigned to C-0 stretching in alcohol and carboxylic acids, ester and ether; The medium peak at 819.47 cm^{-1} was assigned to C-Cl stretching of alkyl halides and the medium peak at 615.62 cm^{-1} was assigned to C-Br stretching in alkyl halides were observed (Figure No.3).

XRD measurement

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 value of the XRD peak at the diffraction angle: θ- Diffraction angle.

Applying XRD data in Scherrer's formula, the average size of particle is pproximately 16.86nm (Figure No.4).

TEM analysis

The figure shows the TEM image of 0.1N Silver nanoparticles in the leaf extract of *Acalypha indica*. The Average size of Silver nanoparticles using leaf extracts of Acalyphaindica was found to be 16.6 nm (Figure No.5).

Anti-Inflammatory Activity

Anti-inflammatory studies like human red blood cell (HRBC) (Table No.1) (Figure No.6), membrane stabilization, inhibition of albumin denaturation indicate the anti-inflammatory activity (Table No.2) (Figure No.7). The medical use of *Acalypha indica* has a good anti-inflammatory activity. As the concentration of the sample increases, the percentage of inhibition also increases.

DISCUSSION

Silver nanoparticles (AgNPs) appear yellowish brown in colour in aqueous medium as a result of surface Plasmon vibrations. As the different leaf extracts were added to aqueous silver nitrate solution, the colour of the solution changed from faint light yellowish brown to reddish brown and finally to colloidal brown indicating formation of AgNPs. Similar changes in colour have also been observed in previous studies and hence confirmed the completion of reaction between leaf extract and $AgNO_3$. The UV-vis spectra recorded after time intervals of 15 minutes, 30 minutes, 45 minutes, 60 minutes and 24 hrs from the initiation of reaction are shown. Absorption spectra of AgNPs formed in the reaction media has absorption maxima in the range of 425 to 475 nm due to surface Plasmon resonance of AgNPs. The UV-vis spectra recorded, implied that most rapid bioreduction was achieved using banana leaf extract as reducing agent followed by *tulsiandneem* leaf extracts. This was denoted by broadening of the peak which indicated the formation of polydispersed large nanoparticles due to slow reduction rates[11-15]. The UV-vis spectra also revealed that formation of AgNPs occurred rapidly within the first 15mins only and the AgNPs in solution remained stable even after 24 hrs of completion of reaction.

FTIR analyses carried out to characterize the AgNPs obtained from each type of plant extract (curve A, banana; curve B, neem and curve C, tulsi) in all three AgNP solutions, prominent bands of absorbance were observed at around 1,025, 1,074, 1,320, 1,381, 1,610 and 2,263 cm−1. The observed peaks denote -C-OC-, ether linkages, -C-O-, germinal methyls, -C=C- groups or from aromatic rings and alkyne bonds, respectively. These bands denote stretching vibrational bands responsible for compounds like flavonoids and terpenoids and so may be held responsible for efficient capping and stabilization of obtained AgNPs. The TEM images obtained by the reaction of 5% of each type of leaf

extract and 1 mM silver nitrate solution separately. A mixture of plates (triangles, pentagons and hexagons) and spheres was obtained, though mainly spherical shape was predominant. A similar trend is also observed in the SEM images. It is clear that the triangles, pentagons and hexagons are plate structures with sizes of up to 200 nm[16-18].

Table No.1: Anti-inflammatory activity of human red blood cell (HRBC) by using AgNPs of *Acalypha indica*

S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean±S.E.M
1	100	45.09 ± 0.56
2	200	57.28 ± 0.48
3	400	61.93 ± 0.73
4	600	70.39 ± 1.81
5	800	84.15 ± 1.63

Table No.2: Anti-inflammatory activity of Albumin denaturation method by using AgNPs of *Acalypha indica*

S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean±S.E.M
1	100	42.31 ± 0.87
2	200	51.48 ± 0.29
3	400	58.73 ± 0.92
4	600	69.91 ± 1.63
5	800	80.18 ± 1.28

Figure No.1: Optical photograph of *Acalypha indica* 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)

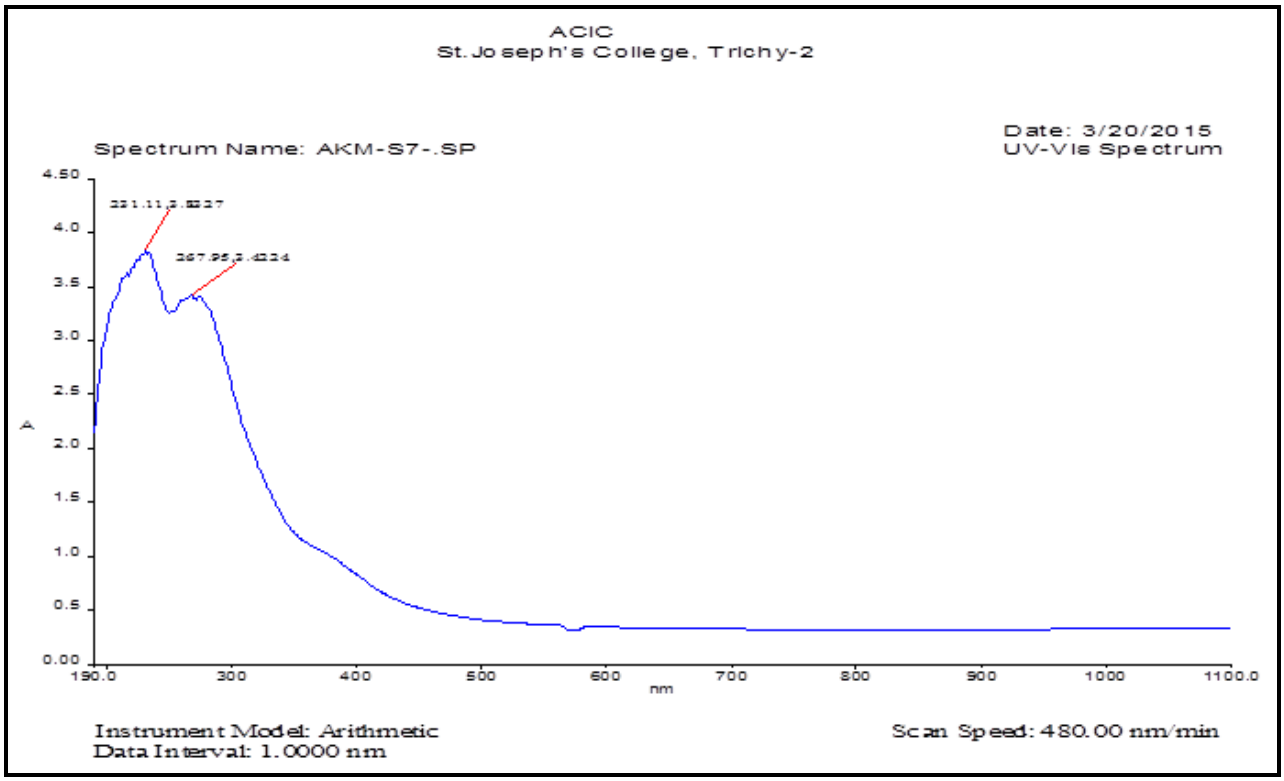

Figure No.2: UV-Visible spectrum of synthesized silver nanoparticles using leaf extracts of *Acalypha indica*

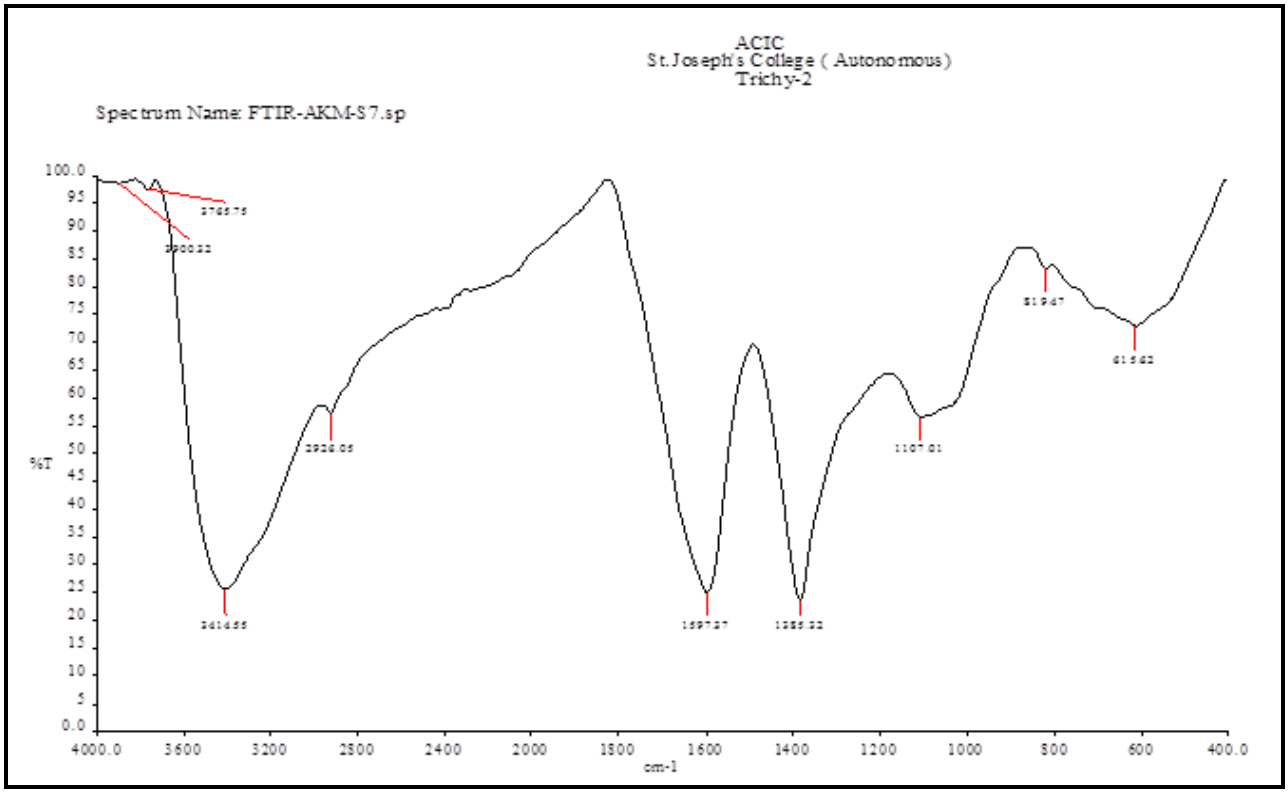

Figure No.3: FT-IR spectrum of synthesized silver nanoparticles using leaf extracts of *Acalypha indica*

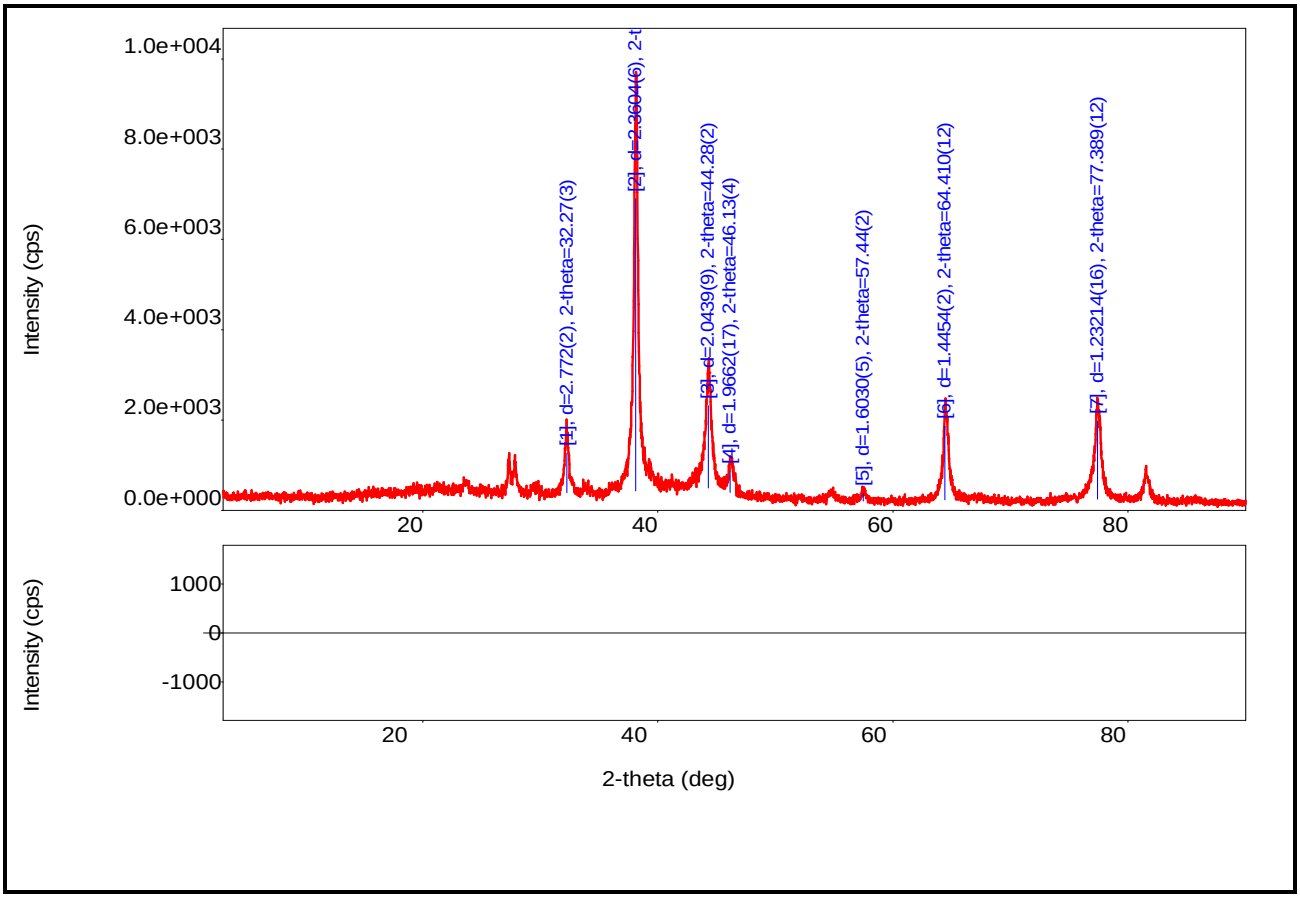

Figure No.4: XRD spectrum of synthesized silver nanoparticles using leaf extracts of *Acalypha indica*

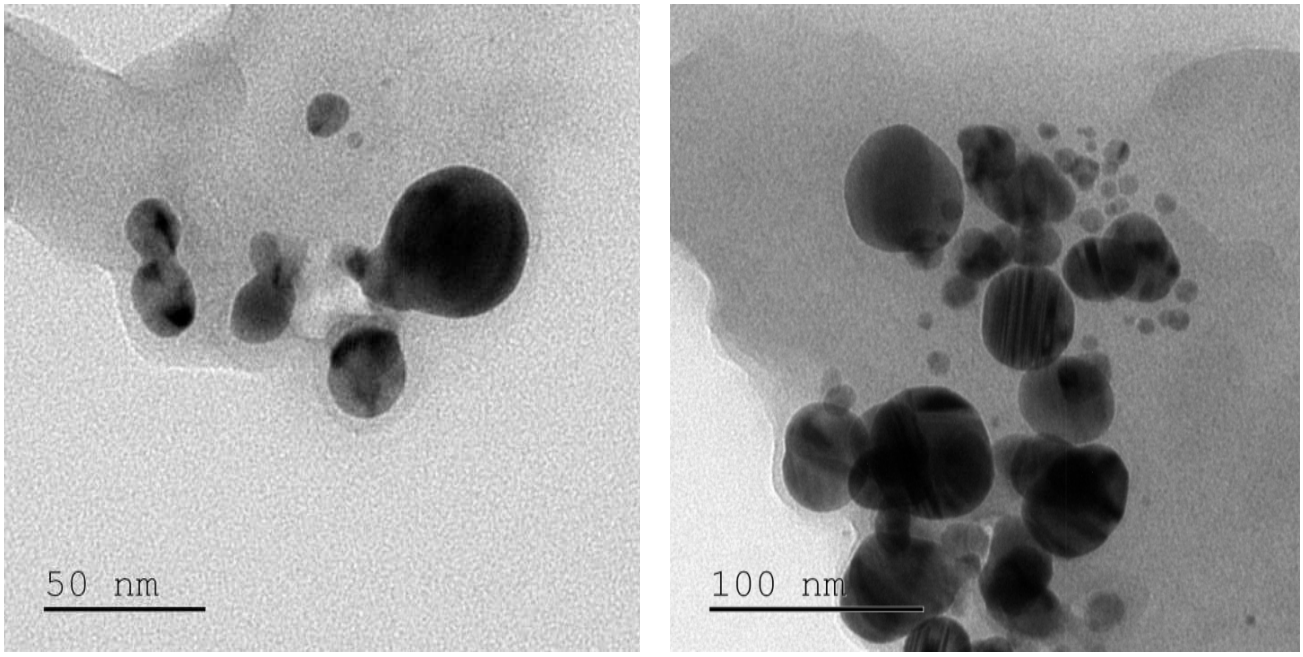

Figure No.5: TEM image of synthesized silver nanoparticles using leaf extracts of *Acalypha indica*

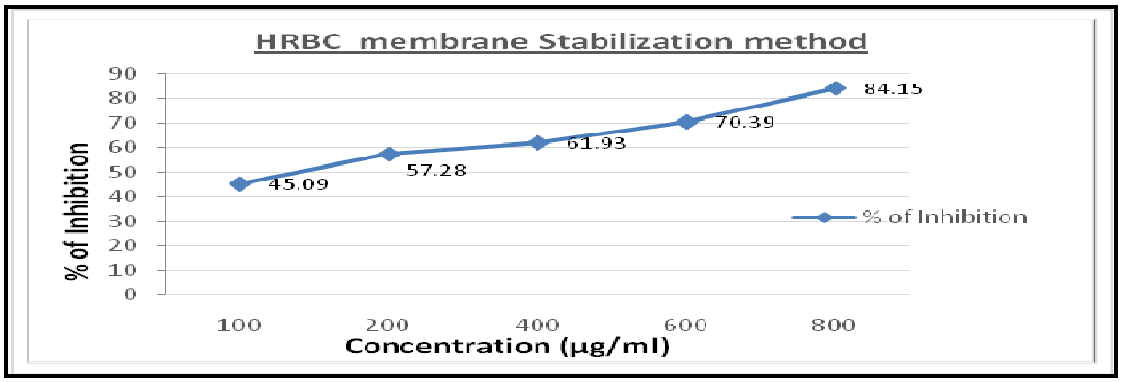

Figure No.6: Graphical representation of Anti-inflammatory activity of human red blood cell (HRBC) by using AgNPs of *Acalypha indica*

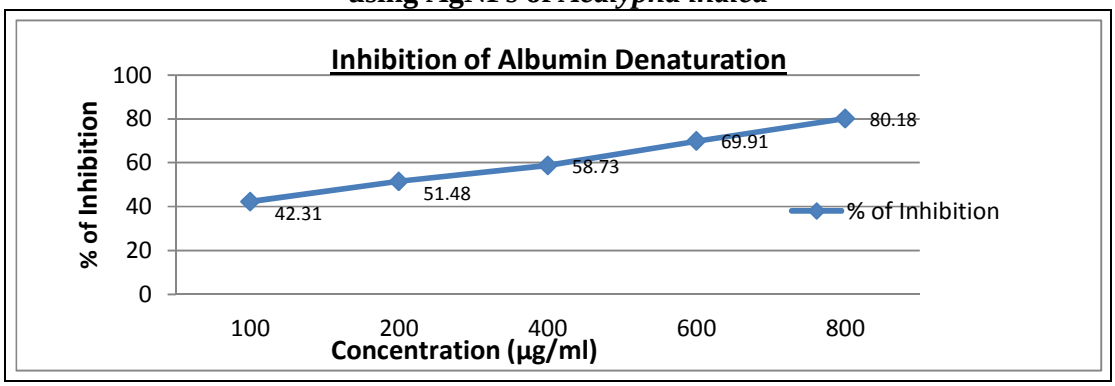

Figure No.7: Graphical representation of Anti-inflammatory activity of Albumin denaturation method by using AgNPs of *Acalypha indica*

CONCLUSION

Silver nanoparticles (AgNPs) were successfully obtained from bioreduction of silver nitrate solutions using *Acalypha indica* extracts. Owing to varying properties of these three plant species, AgNPs obtained from the smallest being yield using *Acalypha indica* extracts. AgNPs have been appropriately characterized using UV-Vis spectroscopy, FTIR, XRD and TEM analysis. FTIR analysis revealed the efficient capping and stabilization properties of these AgNPs. The average size of synthesized silver nanoparticles using leaf extracts of *Acalypha indica* by XRD and TEM analysis was Similar and found to be 16.86 nm and 16.6 nm respectively. The leaf extract of *Acalpha indica* has a good anti- inflammatory activity. Hence, due to their benign, stable nature and anti-inflammatory property, these AgNPs may be well utilized in industrial and remedial purposes. However, plant uptake and utilization of AgNPs requires 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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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Vanden Wildenberg W. Roadmap report on nanoparticles. *W and W Espanasl,* Barcelona, Spain.
2. Gratzel M. Photo electrochemical cells, *Nature*, 414, Nov-15, 2001, 338-344.
3. Okuda M, Kobayashi Y, Suzuki K, Sonoda K, Kondoh T, Wagawa A, Kondo A, Yoshimura H.

Self-organized inorganic nanoparticle arrays on protein lattices, *Nano Lett*, 5(5), 2005, 991-993.
4. Dai J, Bruening M L. Catalytic nanoparticles formed by reduction of metal ions in multilayered polyelectrolyte films, *Nano Lett*, 2(5), 2002, 497-501.
5. Murray C B, Sun S, Doyle H, Betley T. Monodisperse 3D transitionmetal (Co, Ni, Fe) nanoparticles, *MRS Bull*, 26(5), 2001, 985-991.
6. Bhattacharya R, Murkherjee P. Biological properties of "naked" metal nanoparticles, *Adv Drug Deliv Rev*, 60(11), 2008, 1289-1306.
7. Bhumkar D R, Joshi H M, Sastry M, Pokharkar V B. Chitosan reduced gold nanoparticles as novel carriers for transmucosal delivery of insulin, *Pharm Res*, 24(8), 2007, 1415-1426.
8. Goldberg M, Dan C, Nancy R and France L. 2010, Environmental Health Perspective, http://www.sciencedaily.com/releases/2010/10/101006104003.htm).
9. Nelson B P. Heart Disease and Lung Cancer from Air, Suite 101 Magazine, 2009. (http://www.prlog.org/10281223-the-heart-disease-lung-cancer-risks-from-nitrogen-dioxide-no2-air-pollution.html).
10. Ramachandran J. Herbs of Siddha Medicine/ the First 3D Book on Herbs, *Murugan P Patthipagam, Chennai, India*, 2008, 156.
11. Krishnaraj C, Jagan E G, Rajasekar S, Selvakumar P, Kalaichelvan P T, Mohan N. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens, *Colloids Surf B, Biointerfaces*, 76(1), 2010, 50-56.
12. Shukla V K, Pandey S, Pandey A C. Green synthesis of silver nanoparticles using neem leaf (*Azadirachta indica*) extract, *In Proceedings of International Conference on Advanced Nanomaterials and Nanotechnology, ICANN-2009, Guwahati, Assam (India),* 2010, 9-11 December 2009.
13. Namratha N, Monica P V. Synthesis of silver nanoparticles using *Azadirachta indica* (Neem) extract and usage in water purification, *Asian J Pharm Tech*, 3(4), 2013, 170-174.
14. Lalitha A, Subbaiya R, Ponmurugan P. Green synthesis of silver nanoparticles from leaf extract *Azhadirachta indica* and to study its anti-bacterial and antioxidant property, *Int J Curr Microbiol App Sci*, 2(6), 2013, 228-235.
15. Singhal G, Bhavesh R, Kasariya K, Sharma A R, Singh R P. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity, *J Nanoparticle Res*, 13(7), 2011, 2981-2988.
16. Philip D, Unni C. Extra cellular biosynthesis of gold and silver nanoparticles using Krishna tulsi (*Ocimum sanctum*) leaf, *Phys E*, 43(7), 2011, 1318-1322.
17. Huang Q, Li D, Sun Y, Lu Y, Su X, Yang H, Wang Y, Wang W, Shao N, Hong J, Chen C. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf, *Nanotechnol*, 18(10), 2007, 105104-105115.
18. Banerjee *et al.* Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants, synthesis, characterization, antimicrobial property and toxicity analysis, *Bioresources and Bioprocessing*, 1(3), 2014, 1-10.

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