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Research Article

BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES BY USING LAB LAB *PURPUREUS* FLOWER EXTRACTS AND ITS ANTI-MICROBIAL ACTIVITIES

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A B S T R A C T

In recent years, green synthesis of silver nanoparticles (AgNPs) has gained much interest from chemists and researchers. In this concern, Indian flora has yet to divulge innumerable sources of cost–effective non hazardous reducing and stabilizing compounds utilized in preparing AgNPs. This study investigates an efficient and sustainable route of AgNP preparation from 1mM aqueous $AgNO_3$ using flower extracts of Lablab *purpureus* from Fabaceae well adorted for their wide availability and medicinal property. The AgNPs were characterization by UV-visible (vis) spectrophotometer, scanning electron microscopy (SEM). Fourier transform infrared spectrometer (FT-IR) analysis was carried out to determine the nature of the capping agents in each of these flower extract. AgNPs obtained showed significantly higher antimicrobial activities against Staphylococcus aureus, and E.coli in comparition to both $AgNO_3$ plant extracts. In totality, the AgNPs prepared are safe to be discharged in the environment and possibility utilized in processes of pollution remediation. AgNPs may also be efficiently utilized in agricultural research to obtain better health of crop plants as shown by our study.

INTRODUCTION

An attempt was made to explore the ethno botanical, economical and biological importance of Phyllanthus acidus. The plant is used for 28 types of remedies like cathartic, emetic, coughs, hypertension, asthma, skin diseases etc and as a food in the raw form. In India, it is found in different states including Tamilnadu and South Indian states as home garden ornaments. Synthesis and characterization of nanoparticles is under exploration due to its wide medical applications and various research interests in nanotechnology. In the current study, the leaf extract of Phyllanthus acidus (Family: Euphorbiaceae) is used for the synthesis of silver nanoparticles. The leaf extract is mixed with $AgNO_3$ and incubated. The synthesized silver nanoparticles were confirmed by color transformation. It was characterized by UV, FTIR, XRD and TEM. The silver nanoparticles synthesized were generally found in size 1-100 nm. The results showed that the leaf extract is optimum for the synthesis of silver nanoparticles and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The average size of synthesized silver nanoparticles is found to be 27.9nm using XRD data by Scherrer's formula, which is approximately similar as the size obtained in TEM Analysis (28.6nm). 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 against human blood cells which confirms that the plant mediated synthesis of silver nanoparticles have a significant anti-inflammatory activity against human blood cells[1].

The field of Nanoscience has blossomed over the last twenty years and the need for nanotechnology will only increase, as miniaturization becomes more important in areas such as computing, sensors, and biomedical applications. Advances in this field largely depend on the ability to synthesize nanoparticles of various nano material, based on their sizes, and shapes, as well as their efficiency to assemble them into complex architectures (David D *et al*., 2005). Nanotechnology provides the ability to engineer the properties of materials by controlling their size, and this has driven research towards a multitude of potential uses for Nanomaterial. Phyllanthus acidus Skeels, an important medicinal plant belonging to the genus Phyllanthus (Euphorbiaceae), is widely cultivated in worldwide, and its extracts have been used for treating alcoholism. Phyllanthus acidus, known as the Otaheite gooseberry, Malay gooseberry, Tahitian gooseberry, country

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gooseberry, star gooseberry, starberry, West India gooseberry, damsel, grosella (in Puerto Rico), jimbilin (in Jamaica), damsel (in Grenada), karamay (in the Northern Philippines), cermai (in Indonesia and Malaysia), Goanbili (in Maldives) or simply gooseberry tree, is one of the trees with edible small yellow berries fruit in the Phyllanthaceae family. Despite its name, the plant does not resemble the gooseberry, except for the acidity of its fruits. It is mostly cultivated for ornamentation. The medicinal activities of Phyllanthus species are antipyretic, analgesic, anti-inflammatory, anti-hepatotoxic and antiviral [2-5]. Fruits of the two well-known species, P. acidus L. and P. emblica L. contain high contents of vitamin C and have been used for used for improving eyesight and memory. It prevents action against Diabetes and reliefs from cough [8]. Another species of the family, P. amarusis an important herbal medicine due to its effective antiviral activities especially towards the hepatitis B virus.Traditionally, P.acidus has been used in the treatment of several ailments including inflammatory and oxidative stress-related disorders such as gastric trouble [6-10].

Green Synthesis and characterization of nanoparticles is under exploration due to its wide medical applications and various research interests in nanotechnology. In the current study, the plant extract of Perseaamericana (Avocado)(Family-Lauraceae) is used for the synthesis of silver nanoparticles (AgNPs). This study investigates an efficient and sustainable route of AgNPs preparation from 1 mM aqueous $AgNO_3$ using leaf extracts. The complete reduction of silver ions was observed after 12 hrs of reaction at 40^o C under shaking condition. The colour changes in reaction mixture (pale yellow to dark brown colour) was observed during the incubation period, because of the formation of silver nanoparticles (AgNPs) in the reaction mixture enables to produce particular colour due to their specific properties (Surface Plasmon Resonance). The formation of silver nanoparticles was confirmed by UV-Visible spectroscopy, Fourier Transform Infra-Red (FT-IR) spectroscopy analysis, X-Ray Diffraction (XRD) pattern, Transmission electron microscopy (TEM). The results showed that the leaf extract is optimum for the synthesis of silver nanoparticles and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The average size of synthesized silver nanoparticles is found to be 27.42 nm using XRD data by Scherrer's formula, which is approximately similar as the size obtained in TEM Analysis (27.58nm). In total, 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 [11].

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape and controlled dispersity. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanoparticles, generate a large amount of hazardous byproducts. Thus, there is a need for green method that includes a clean, non-toxic and environment friendly method of nanoparticles synthesis [12]. As a result, researchers in the field of nanoparticles synthesis and assembly have turned to biological system of inspiration [13]. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extra celluarly using plants or their extracts in a controlled manner according to their size, shape and dispersity [14]. The aqueous silver nitrate solution, after reacting with geranium leaf extract, led to rapid formation of highly stable, crystalline silver nanoparticles (16 to 40 nm) [15]. Various approaches available for the synthesis of silver NPs include chemical [16], electrochemical [17], radiation[18], photochemical methods [19] and Langmuir-Blodget and biological techniques [20-22].

MATERIALS AND METHODS

Preparation of flower extract

The fresh and young flower samples (Lab lab *purpureus)* was collected and washed thoroughly with sterile double distilled water (DDW). Twenty gram of sterilized flower 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 4^0C

Synthesis of silver nanoparticles

Silver nitrate was used as precursor in the synthesis of silver nanoparticles. 100 ml flower 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 500 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 + Lab lab *purpureus* flower 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.

SEM Analysis

Sample is dispersed with acetone and exposed in ultrasonic 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 SEM instruments using model is Tecnai T20 Making in FEI, Netherlands operating at 200KeV Tungsten Filament.

Antibacterial activity

Microorganisms and culture media

Bacterial cultures such as, *Staphylococcus aureus*, *E.coli,* were obtained from Eumic analytical Lab and Research Institute, Tiruchirappalli. Bacterial strains were maintained on Nutrient agar slants (Hi media) at 4˚C.

Inoculum preparation

Bacterial cultures were sub-cultured in liquid medium (Nutrient broth) at37˚C for 8h and further used for the test (10^5-10^6CFU /ml). These suspensions were prepared immediately before the test was carried out.

Preparation of culture media

Nutrient agar medium

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes:

Ingredients	:	Grams/Litre
Peptone	:	5gm
Beef extract	:	3gm
Agar	:	15gm
Sodium chloride	:	5gm
Yeast extract	:	1.5gm
p^H	:	7.0

After adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ib psi pressure (121˚C)for 15 minutes.

Nutrient broth

The nutrient broth was prepared by the same composition without agar. At the adding all the ingredients into the distilld water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 ib psi pressure (121^0C) for 15 minutes.

Preparation of plant material

Flowers, of the plant materials taken for this study were shade dried individually at room temperature and then powdered by using electric, blender. About 10gm of fresh plant materials (flower) were extracted with 100ml of distilled water 91:10. They were kept for seven days at room temperature (31˚C) for complete extraction. After seven days. The extracts were filtered through what man no.1 filter paper. This extract was collected in both and kept in refrigerator.

Continuous hot extraction using soxhlet apparatus

When concentrated preparations are manufactures, there is first extraction followed by evaporation. In the continuous both the operations i.e., extraction and evaporation are combined in the apparatus used for this purpose. To execute continuous not extraction a soxhlet apparatus is used soxhlet continuous extractor. The apparatus is used for the extraction on coarse drug powder placed in a thimble made of filter paper is inserted into the wide tube of the extractor. The solvent which is taken is taken in the flask is heated, the vapors arise from the solvent get in to the condenser through a side tube and the liquid condensed from the vapors drips into the thimble. The solvent liquid level slowly rises and during this period the dried flower materials gets extracted of its soluble constituents. When the level of the liquid reaches the top of the siphon it gets siphoned into flask. The suction effect of the siphoning assists permeation of the solvent through the drug.

Again a portion of the solvent from the solution vaporized leaving the constituents in the flask itself and the process mentioned above is repeated. The same process is repeated again and again until all the solutes are extracted. This kind of continuous not percolation (soxhlation) is undertaken when the active constituents are not readily soluble in the cold and are thermo labile e.g., grainder Oleoresin is extracted with ethanol.

Assay of antimicrobial activity

Microbial inoculum preparation

The nutrient broth were prepared, then identified bacterial colonies were inoculated into the broth culture were used for antimicrobial activity.

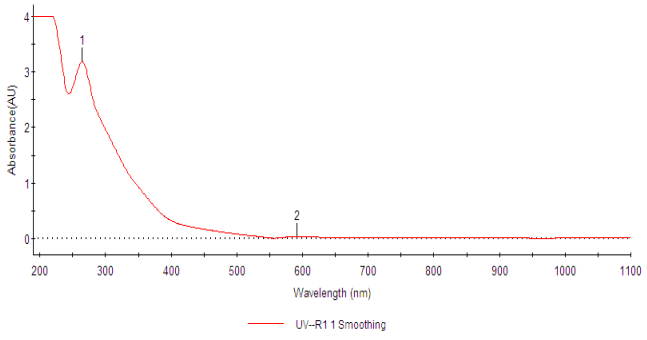
Kirby bauer agar well diffusion assay

The nutrient agar medium was prepared and sterilized by autoclaving at 121˚C 15 lbs pressure for 15 minutes then aseptically poured the medium into the sterile petriplates and allowed to solidify the Bacterial broth culture was swabbed on each petriplates using a sterile buds. Then wells were made by well cutter. The organic solvent extracts of flower were added to each well aseptically.

This procedure was repeated for each Petri plates then the petriplates were incubated at 37˚C for 24 hrs. After incubation the plates were observed for the zone of inhibition.

RESULT AND DISCUSSION

UV-Visible Spectroscopy Analysis

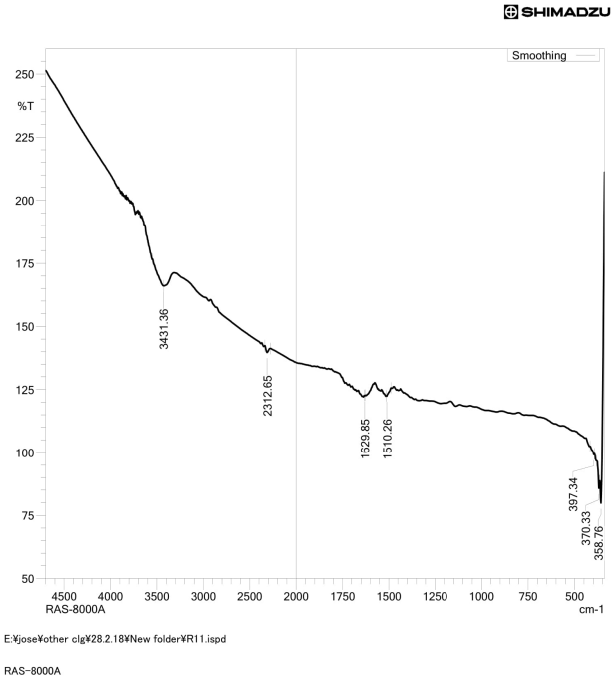


UV-Visible spectrum of synthesized silver nanoparticles using Lab lab *purpureus* flower extract

UV-Visible spectroscopy analysis showed the absorbance band of silver nanoparticles synthesized using fabaceae flower extract at 263.80nm to 591.30nm which conforms the presence of poly-unsaturated and aromatic compound (Isoguinoline) (Advanced strategies in food analysis, UV-VIS spectrometry

International Journal of medicine and Pharmaceutical Research by Richartkoplik

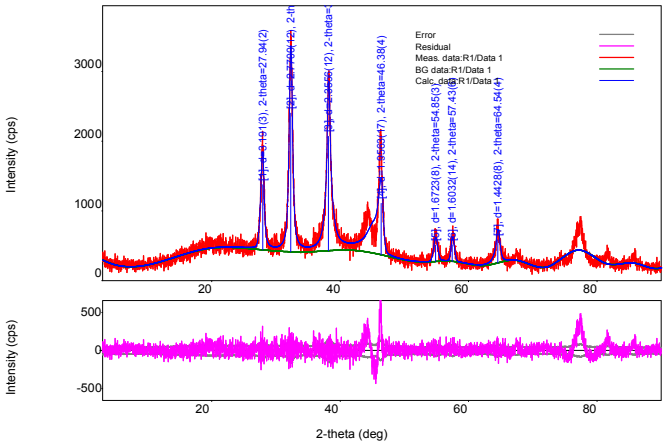
FT-IR measurment



FT-IR Spectrum of synthesized silver nanoparticles using Lablab *purpureus* flower extract

The fabaceae related functional groups were identified using the peak assignments. A strong peak at 3431.36 cm $^{-1}$ was assigned to the OH stretching in alcohol group, the strong peak at 2312.65 cm^{-1} was assigned to O=C=O stretching Carbon-dioxide group, medium peak at 1629.85cm^{-1} was assigned to C-O Stretching Amide group, a strong peak at 1510.26 cm^{-1} assigned to N-O stretching nitro compound groups are observed

XRD measurement



XRD Spectrum of synthesized silver nanoparticles using Lab lab *purpureus* flower extract

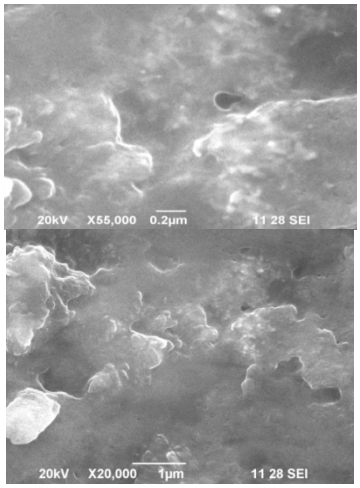
Determination crystalline size

Gauss value

Particalsize (D) = 14.8316nm

Surface area (S) = 59.60457 m^2 /g

SEM Analysis



SEM image of synthesized silver nanoparticles using Lab lab *purpureus* flower extract

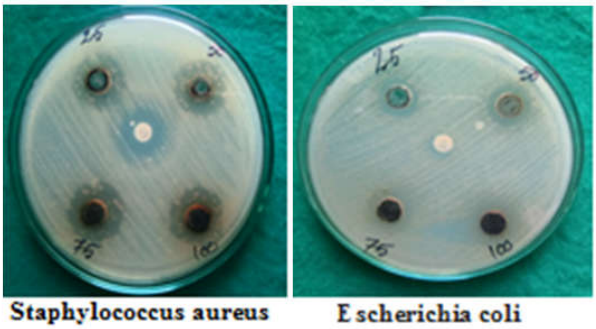
SEM analysis shows uniformly distributed silver nanoparticles on the surface of the cells. SEM analysis reveals individual polydispersed AgNPs as well as number of aggregates, which were irregular in shape. The size of silver nanoparticles was found to be 5-30 nm, with an average size 14.5nm. The larger silver particles may be due to the aggregation of the smaller ones

Anti-Microbial activity

In the present, isolated ethyl acetate fraction of fabaceae maxima flowers exhibited significant anti- microbial activity when compared with standard drug. It is evident from the data presented in table 1 that the sample possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 25mg/ml as 16mm, 12mm, for 50mg/ml showing 18mm and 14mm for 100mg/ml as 22mm, 19mm, against staphylococcus aureus, E.coli respectively when compared with standard drug Gentamicin. I showing 20mm, and 22mm zone of inhibition respectively. Then it is evident from the data presented in table 2 that the sample possesses anti-bacterial activity. The above result shows that the activity of the compound isolated from fabaceae maxima flower shows significant antibacterial activities

Lab lab *purpureus* flower Extracts	Extract 100 µl added and Zone of inhibition (mm/ml)				
	25 µl	50 µl	75 µl	100 µl	Control
Staphylococcus aureus	16	18	20	22	22
Escherichia coli	12	14	16	19	20

Anti-bacterial activity of the compound isolated from Lab lab *purpureus* flower in different strains



Graphical representation of anti- bacterial activity of the compound isolated from Lab lab purpureus flowers (standard: Gentamicin, concentration 1mg/ml)

CONCLUSION

Based on the result of the above study on the Lablab *purpureus*, we conclude that the compound isolated from Lab lab *purpureus* flowers shows superior anti- bacterial activity against the following microorganisms such as Staphylococcus aureus. Also it justifies the claimed uses of flower parts of the Lab lab *purpureus* in the traditional system of medicine to treat various infections disease caused by the microbes. Antimicrobial activities are aggravated by increasing the quantity of this compound, which can be used as an alternative for antibiotics. Therefore, it is necessary to characterization their active compounds and should be investigated for better understanding of its safety, efficacy and properties.

References

1. P.Anitha, P.Sakthivel, Synthesis and Characterization of silver nanoparticles using Phyllanthus acidus leaf extract and its anti-inflammatory activity against human blood cells. *Int. J. Med. Pharm. Res*., 2016, 4(2): 95-96
2. Unander DW, Webster DW, Blumberg BS. Uses and bioassays in Phyllanthus (Euphorbiaceae) IV. Clustering of antiviral uses and other effects. *J Ethnopharmacol,* 1995; 45: 1-18.
3. Chang CC, Lien YC, Liu KCSC, Lee SS. Lignans from Phyllanthus urinaria. *Phytochemistry*, 2003; 63: 825-33.
4. Zhang YJ, Nagao T, Tanaka T, Yang CR, Okabe H, Kouno I.. Anti-proliferative activity of the main constituents from Phyllanthusemblica. *Biol Pharm Bull*., 2004; 27: 251-255.
5. Sousa M, Ousingsawat J, Seitz R, Puntheeranurak S, Regalado A, Schmidt A, *et al*. An extract from the medicinal plant Phyllanthus acidus Iisolated compounds induce airway chloride secretion: a potential treatment for cystic fibrosis. *MolPharmacol*., 2007; 71: 366-76.
6. Unander DW, Webster DW, Blumberg BS. Record of usage or assays in Phyllanthus (Euphorbiaceae) I. Subgenera Isocladus, Kirganelia, Cicca and Emblica. *J Ethnopharmacol.,* 1990; 30: 233-64.
7. Unander DW, Webster DW, Blumberg BS. Uses and bioassays in Phyllanthus (Euphorbiaceae): acompilation II. The subgenus Phyllanthus. *J Ethnopharmacol*., 1991; 34: 97-133.
8. Ott M, Thyagarajan SP, Gupta S. Phyllanthus amarus suppresses hepatitis B virus by interrupting interactions between HBV enhancer I and cellular transcription factors. *Eur J Clin Invest*., 1997; 27: 908-15.
9. Rai V, Khatoon S, Bisht SS, Mehrotra S. Effect of cadmium on growth, ultramorphology of leaf and secondary metabolites of Phyllanthus amarusSchum and Thonn. *Chemosphere*, 2005; 61: 1644- 50.
10. Jules, Janick, Paull, Robert E. 2008. The Encyclopedia Q5 of Fruit & Nuts.*CABI Publishing* p.373.
11. P.Anitha, P.Sakthivel, Synthesis and Characterization of Silver Nanoparticles Using Persea americana (Avocado) and its Anti-inflammatory Effects on Human Blood Cells, *Int. J. Pharm. Sci. Rev. Res*., 35(2), November – December 2015; Article No. 32, Pages: 173-177.
12. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S.R. Sainkar, M.I. Khan, R. Parishcha, P.V. Ajaykumar, M. Alam, R. Kumar, M. Sastry, Fungus-Mediated Synthesis of Silver Nanoparticles and Their Immobilization in the Mycelial Matrix: A Novel Biological Approach to Nanoparticle Synthesis, *Nano Letters,* 1(10), 2001, 515–519.
13. K.Simkiss, K.M. Wilbur, "Bio mineralization," Cell Biology and Mineral Deposition, Academic Press New York (1989).
14. V. Kumar, S.K. Yadav, J. Chem. Technol., Syzygiumcumini leaf and seed extract mediated biosynthesis of silver nanoparticles and their characterization, Volume 85, Issue 10 Pages, 1299–1422.
15. S.S. Shankar, A. Ahmad, R. Pasricha, M. Sastry, Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes *J. Mater. Chem*., 13, 2003, 1822-1826.
16. Sun Y, Yin Y, Mayers BT, Herricks T, Xia Y, Uniform form silver nanowires synthesis by reducing AgNO3 with ethylene glycol in presence of seeds and poly(vinyl pyrrolidone). *Chem Mater*, 14, 2002, 4736–4745.
17. Yin B, Ma H, Wang S, Chen S, Electrochemical synthesis of silver nanoparticles under protection of poly (Nvinylpyrrolidone). *J PhysChem* B, 107, 2003, 8898–8904.
18. Dimitrijevic NM, Bartels DM, Jonah CD, Takahashi K, Rajh T, Radiolytically induced formation and optical absorption spectra of colloidal silver nanoparticles in supercritical ethane. *J PhysChem* B, 105, 2001, 954–959.
19. Callegari A, Tonti D, Chergui M. Photochemically grown silver nanoparticles with wavelength-controlled size and shape. *Nano Lett,* 3, 2003, 1565–1568.
20. Zhang L, Shen YH, Xie AJ, Li SK, Jin BK, Zhang QF, One-step synthesis of monodisperse silver nanoparticles beneath vitamin E Langmuir monolayers. *J.Phys,Chem,* B 110, 2006, 6615–6620.
21. Swami A, Selvakannan PR, Pasricha R, Sastry M. One-step synthesis of ordered two dimensional assemblies of silver nanoparticles by the spontaneous reduction of silver ions by pentadecyl phenol Langmuir monolayers. *J.Phys,Chem* B, 108, 2004, 19269.
22. P. Anitha, P. Sakthivel, Microwave Assisted Synthesis and Characterization of Silver Nanoparticles Using Citrullus lanatus Leaf Extract and Its Anti-Inflammatory Activity Against Human Blood Cells, Volume-3 Issue-3, April 2016.

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