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Sunlight Mediated Synthesis of Silver Nanoparticles using *Terminalia neotaliala* Capuron Fruit Extract: Characterization and *In vitro* Anti-inflammatory Activity

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ABSTRACT

In the present study, a green, cost-effective and robust approach for the synthesis of silver nanoparticles (AgNPs) using naturally available *Terminalia neotaliala* Capuron fruits and freely available solar energy with no expenses on external conventional energy was employed. This approach favoured the formation of AgNPs within 5 mins of sunlight exposure with a colour change from colourless to reddish brown. The characterization done using analytical techniques such as UV-Vis spectroscopy, FTIR, XRD, DLS, Zeta Potential, AFM, HR-TEM and SAED revealed that the AgNPs had a characteristic absorption peak at 412 nm and were spherical, crystalline with FCC structure, sizes in between 10 to 50 nm and stable (-41.7 mV) which further confirmed their formation. As protein denaturation is one of the main causes of Inflammation, we further tested the effect of synthesized AgNPs on inhibition of BSA protein denaturation. Results depict maximum % inhibition of 73.68 % at its highest concentration i.e., 500 μg/mL in comparison with the diclofenac sodium, an anti-inflammatory drug (97.16 %) which revealed the potential of AgNPs to act as an anti-inflammatory agent.

Keywords: Green Synthesis; Photocatalyzed; HR-TEM; BSA Anti-denaturation; *Terminalia mantaly* H. Perrier

1 INTRODUCTION

In material sciences, nanotechnological field is the most dynamic area of research and synthesis of metal nanoparticles is picking up significantly throughout the world [1]. Metal nanoparticles have a size between 1–100 nm with high surface area to volume ratio [2–4]. Numerous approaches such as physical and chemical like hydrothermal, solvo thermal, microwaves etc. have been used in the synthesis of metal nanoparticles. However, these methods have turned out to be costly as well as harmful to the environment [5, 6]. To address these problems, in recent years, synthesis of nanoparticles via biological methods has received enormous attention. This method is safe, clean, cost-effective, eco-friendly and it involves naturally available biomaterials such as plant extracts with no maintenance of cultures or use of harsh chemicals [5, 7]. Among the various metal nanoparticles, silver nanoparticles (AgNPs) are of great interest due to their wide range of applications such as antibacterial, anti-inflammatory, anticancer, biosensing, catalytic, imaging, drug delivery and optoelectronics etc [8, 9].

Recently, sunlight assisted synthesis of AgNPs using plant extracts is in limelight as it is safe, renewable and economical [10]. As we know that solar energy is important for the plants to carryout photosynthesis, during which the light energy is converted to chemical energy through electron transfer reactions [11]. Therefore, it is hypothesized that in the presence of sunlight, the phytochemicals get photosensitized and transfer electrons that reduce silver ions to silver nanoparticles. The phytochemicals also form a capping on the surface of AgNPs and stabilize them [12]. In this regard, extracts from plants such as *Polyalthia longifolia* [8], *Nigella arvensis* [5], *Premna integrifolia* [13], *Annona squamosa* [14], *Centella asiatica* [12] have been used for sunlight mediated synthesis of AgNPs.

Terminalia neotaliala Capuron is an evergreen tree native to Madagascar and belongs to the family Combretaceae [15, 16]. The synonym of this plant is *Terminalia mantaly* H.

Perrier. Madagascar almond or French mantaly or Umbrella tree are its vernacular names [17, 18]. It is generally used as an ornamental, for shelter purposes and in dyeing industries due to rich tannin content [19–21]. The leaf and bark of this plant are used to treat various ailments such as diarrhoea, dysentery, malaria, diabetes etc. [16, 22]. The fruits of *T. neotaliala* have not been so far explored. Hence, fruits of this plant were selected.

In the present study, we have concentrated on developing a greener way for the synthesis of AgNPs using *T. neotaliala* fruit extract. This is the first report stating the use of fruit extract of *T. neotaliala* and the use of sunlight for favouring the AgNPs formation. We have tried to make the procedure simple, quick and cost effective. The synthesized AgNPs were characterized and assessed for its *in vitro* anti-inflammatory potential via inhibition of BSA protein denaturation assay.

Figure 1: Fruits of *Terminalia neotaliala* Capuron

2 MATERIALS AND METHODS

2.1 Chemicals

All the chemicals used for the present study were of analytical grade (AR) and used as received without further purification. Silver Nitrate ($AgNO_3$), Bovine Serum Albumin (BSA) and Tris Buffer were procured from HiMedia Laboratories Pvt. Ltd. Diclofenac sodium was purchased from a local medical store.

2.2 Collection and Preparation of Aqueous Fruit Extract

The fruits of *T. neotaliala* were collected from the Botanical Garden of Karnatak University, Dharwad, Karnataka, India (Figure 1). The fruits were thoroughly washed under tap water followed by distilled water to get rid of the adhered impurities. The fruits were then air dried completely on a blotting paper to remove the residual moisture. 10 grams of the fresh fruits were added to a conical flask containing 100 mL distilled water and boiled at 60–70°C on a water bath for about 45 mins. The extract was then cooled down to room temperature and filtered through Whatman No. 1 filter paper. The prepared extract was stored at 4°C in a refrigerator for further work in this regard.

2.3 Synthesis of AgNPs using T. neotaliala Aqueous Fruit Extract

For the synthesis of AgNPs, 5 mL of aqueous fruit extract of *T. neotaliala* was added to 95 mL of 1mM $AgNO_3$. The experiment was carried out both in bright sunlight and in dark conditions. The fruit extract and silver nitrate were maintained separately as control. The change in colour of the reaction mixture and the time required was noted down. The synthesized AgNPs were separated by repeated centrifugation at 13,000 RPM for 30 mins. The pellet obtained was dispersed in distilled water to get rid of the bio-inorganic impurities and oven dried.

2.4 Characterization of Synthesized AgNPs

The UV-Visible Double Beam Spectrophotometer (Jasco V670) was used to analyse Surface Plasmon Resonance (SPR) of AgNPs by measuring the λ_{max} in the range of 300–600 nm. The Fourier Transform Infrared Spectrophotometer (FTIR Nicolet 6700, Thermo Fischer Scientific) was used to identify the probable functional groups of phytochemicals present in the extract involved in reduction, capping and stabilization of AgNPs in the range of 500 to 4000 cm^{-1}. X-ray Diffractometer (XRD Rigaku, Smart Lab SE) was used to study the crystalline nature of AgNPs in the range of 30-90°. Particle Size Analyser (Horiba SZ-100) was used to determine the size, polydispersity index and zeta potential of the AgNPs. Atomic Force Microscope (AFM Nanosurf Easyscan 2) was used to study the surface morphology such as size, shape and roughness of the AgNPs. High Resolution Transmission Electron Microscope (HR-TEM Jeol/JEM 2100) was used to study the actual size and shape of the AgNPs. Selected Area Electron Diffraction (SAED) conjoined with HR-TEM was further used to confirm the crystalline nature of AgNPs.

2.5 In vitro Anti-inflammatory Activity

2.5.1. Inhibition of BSA Protein Denaturation

The Inhibition of BSA protein denaturation assay was used to assess the anti-inflammatory activity of *T. neotaliala* fruit extract mediated AgNPs [23]. 1% BSA in 50 mM Tris buffer (pH 6.5) was used for this. 1 mL of different concentrations i.e., 100, 200, 300, 400, 500 μg/mL of AgNPs were mixed with BSA solution and incubated at room temperature for 20 mins followed by heating in a water bath at 64°C for 5-10 mins till turbidity developed. Once it was cooled, the absorbance was measured at 660 nm spectrophotometrically. Diclofenac sodium was used as standard and BSA as control.

The % inhibition of BSA protein denaturation was calculated using the following equation

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.6 Statistical Analysis

The experiment was performed in triplicates. All the values obtained are expressed as mean ± standard deviation.

3 RESULTS AND DISCUSSION

3.1 Visual Observation and UV-Vis Spectroscopic Analysis

The synthesis of AgNPs was carried out using the *T. neotaliala* fruit extract under the influence of sunlight. Within 5 mins, there was a change in colour of the reaction mixture from colourless to reddish brown on exposure to bright sunlight, which indicated the formation of AgNPs. The solution kept in dark conditions showed no signs of colour change. This revealed that the reaction was completely photocatalytic in nature. The change in colour of the reaction mixture is associated with a well-defined absorption peak in the visible region of the electromagnetic spectrum in the range of 400-440 nm [6, 24, 25]. UV-Vis Spectroscopy is a very reliable technique to monitor the formation of AgNPs [26]. From the UV-Vis spectral analysis, an absorption peak was observed at 433 nm (pH 4.9). This peak was broad which suggested that the AgNPs formed were bigger in size which possibly may be because of acidic pH of the reaction mixture [27]. So, the pH was further adjusted to 8, 9 and 10. The absorption peak at pH 8 had the highest absorbance and was narrow as compared to peak at pH 9 and 10 (Figure 2). And the peak shifted from 433 nm to 412 nm. From this, it was confirmed that pH 8 was optimum for the synthesis of small sized nanoparticles. Such results were reported by Cyril *et al.*, 2020 [28]. From the results, we can notice that sunlight acted as a catalyst in the formation of AgNPs which eventually speeds up the process as compared to synthesis in dark conditions.

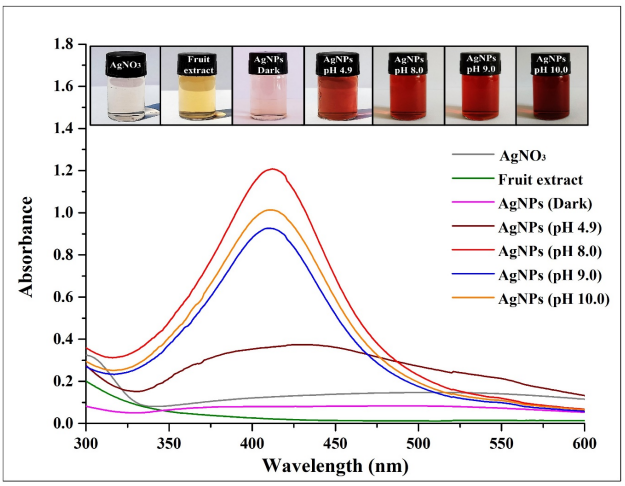

Figure 2: UV-Vis spectra of *T. neotaliala* fruit extract mediated AgNPs

3.2 FTIR Spectral Analysis

The possible interactions between the silver ions and phytochemicals from the fruit extract in reduction, capping and stabilization were studied using FTIR spectroscopy. The FTIR analysis of AgNPs showed characteristic peaks at 3396.47, 2923.55, 2850.27, 1635.34, 1553.85, 1384.34, 1070.51 and 615.03 cm^{-1} (Figure 3). The peak at 3396.47 cm^{-1} corresponds to O-H stretch of phenols & alcohols and N-H stretch of primary & secondary amines and amides. The peaks at 2923.55 and 2850.27 cm^{-1} can be attributed to asymmetric and symmetric -CH stretching vibration of alkanes. A peak at 1635.34 cm^{-1} was due to C=O of amides and N-H bend in primary & secondary amines and amides. The peak at 1553.85 cm^{-1} corresponds to N-H bend in primary and secondary amines and amides. A peak at 1384.34 cm^{-1} was enhanced possibly due to the amount of NO_3^- remaining in the sample. The peak at 1070.51 cm^{-1} may be associated with C-N stretch of amines and C-O of alcohols, esters and carboxylic acids. Peak at 615.03 cm^{-1} can be assigned to C-Cl stretching of alkyl halides. With this, it is clear that the phytochemicals from the fruit extract have played a role in the formation of AgNPs. The O-H groups may be linked to phenols and flavonoids and the N-H and C=O to peptide linkages found in proteins [29–31].

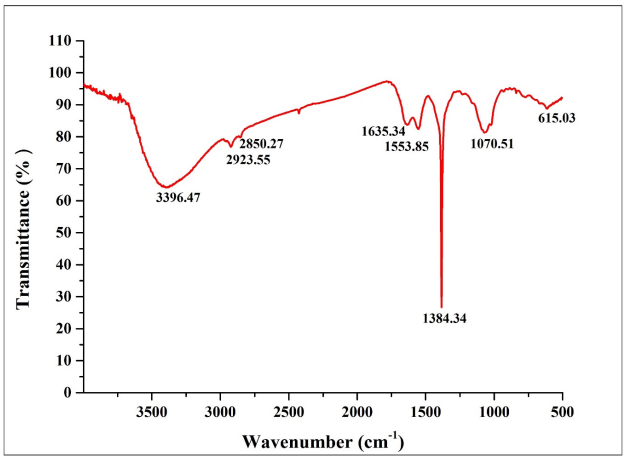

Figure 3: FTIR spectrum of *T. neotaliala* fruit extract mediated AgNPs

3.3 XRD Spectral Analysis

X-ray diffraction studies determine the crystalline structure of the AgNPs. The XRD studies of AgNPs shows characteristic diffraction peaks at 38.11°, 44.31°, 64.48°, 77.41°, 81.51° respectively which can be indexed to (111), (200), (220), (311) and (222) planes of face centered cubic silver as confirmed from the standard ICDD pattern (00-004-0783) (Figure 4). The average crystallite size was 20.86 calculated using Debye-Scherrer's equation ($d=k\lambda/\beta\cos\theta$). The extra peaks may be due to the crystallization of phytochemicals on the surface of AgNPs [32, 33].

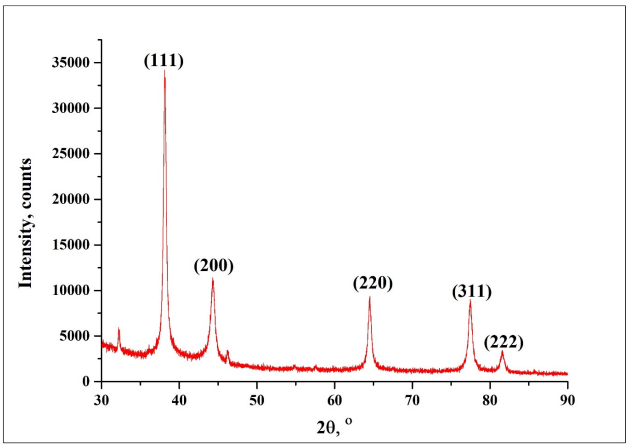

Figure 4: XRD spectrum of *T. neotaliala* fruit extract mediated AgNPs

3.4 Particle Size and Zeta Potential Analysis

The particle size measured via the phenomenon of Dynamic Light Scattering (DLS) was found to be 61.4 nm with a PDI value of 0.498 (Figure 5A). The particle size obtained was large in comparison with the particle size from TEM studies, which indicates that in DLS the particle size is measured along with the capped molecules and solvent layer around the AgNPs [7, 34]. The polydispersity index explains regarding the spread of particle size distribution. The value ranges from 0.1 to 1, <0.1 indicates mono dispersity, whereas > 0.1 implies polydispersity [35].

Zeta potential gives an idea regarding the surface charge of the AgNPs which in turn reflects its stability. The zeta potential of AgNPs was found to be -41.7 mV (Figure 5B). The zeta potential more than +30 or less than -30 mV indicates good stability of the nanoparticles [36]. On the other hand, the negative charge suggests that negative ions are present around the nanoparticles, as a result of which they repel and hinder agglomeration [37].

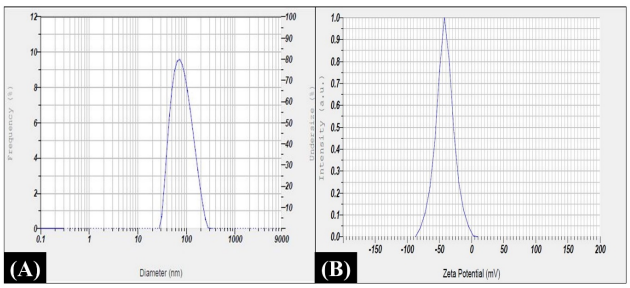

Figure 5: (A) Particle size and (B) Zeta Potential of *T. neotaliala* fruit extract mediated AgNPs

3.5 AFM Analysis

AFM is a powerful tool to study the surface morphology of the AgNPs. The AFM studies revealed the spherical shape of the AgNPs with random distribution. Here the particle size ranged from 25 to 87 nm (Figure 6) and was large compared to TEM studies. This could be due to agglomeration that might have occurred while preparing the slide [38]. These results correlate well with sizes obtained for AgNPs synthesized using soyabean seed extract [39].

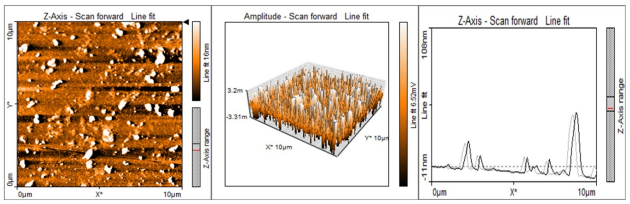

Figure 6: AFM of *T. neotaliala* fruit extract mediated AgNPs

3.6 HR-TEM and SAED Analysis

HR-TEM studies reveal the microscopic structure of the AgNPs. The HR-TEM images depict spherical shape of the AgNPs (Figure 7A). The histogram shows broad particle size distribution ranging from 10 to 50 nm with an average particle size of 29.04 ± 9.30 nm (n= 40) (Figure 7B). The HR-TEM image at a magnification of 2 nm clearly shows the lattice fringe with d-spacing of 0.24 nm (Figure 7C). This corresponds to (111) plane suggesting that the growth of AgNPs was oriented at this plane.

The SAED pattern shows five bright circular rings in the form of tiny spots which corresponds to (111), (200), (220), (311) and (222) planes of FCC structure of silver suggesting crystalline nature of the AgNPs (Figure 7D). This data correlates with the XRD data. Such similar SAED pattern was obtained in studies on AgNPs synthesis using *Sida retusa* leaves [40].

3.7 In vitro Anti-inflammatory Activity via Inhibition of BSA Protein Denaturation

The *in vitro* anti-inflammatory activity determined via inhibition of protein denaturation assay showed activity in a dose-dependent manner (Table 1). The lowest activity was 29.37%, 12.08 % and 42.33% at a concentration of 100 μg/mL and the highest activity recorded was 73.68%, 65.33% and 97.16% at a concentration of 500 μg/mL for AgNPs, fruit extract and diclofenac sodium (Figure 8A). The IC50 values obtained for AgNPs, fruit extract and diclofenac sodium were 296.01, 415.23 and 121.99 μg/mL respectively (Figure 8B).

Inflammation is the response from the body to stimuli of external agents such as infection, injury or destruction characterized by redness, swelling and pain in that area [41]. Denaturation of tissue proteins is one of the widely known causes of inflammation. A quest for compounds that can inhibit protein denaturation would be worthwhile in treating inflammation [42]. Extensive research in nanotechnological field has led to search of nanoparticles that not only reduce inflammation but also prevent amplifying of its associated

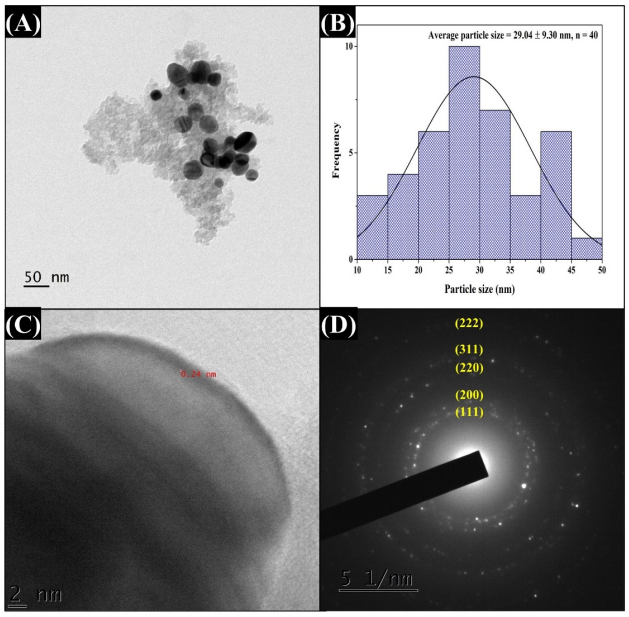

Figure 7: HR-TEM and SAED pattern of *T. neotaliala* fruit extract mediated AgNPs

diseases such as rheumatoid arthritis [43]. The selection of BSA anti-denaturation assay in the present study was to eliminate the use of live animals in the initial stages of drugs screening [44]. This *in vitro* assay is widely used, rapid, sensitive and reliable to determine the capacity of various compounds to inhibit protein denaturation [41]. From the results, it can be observed that the AgNPs could inhibit the protein denaturation and the activity was comparable to the standard. With this, the AgNPs exhibited marked anti-inflammatory activity against the denaturation of BSA protein. Such results were shown by I. Gnanasundaram and K. Balakrishnan, 2017 [42].

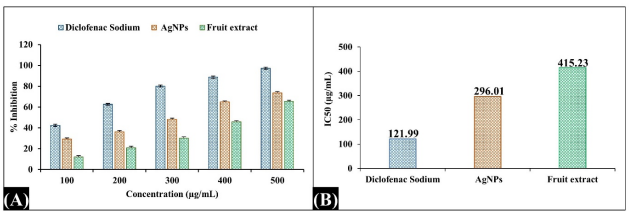

Figure 8: Anti-inflammatory activity of *T. neotaliala* fruit extract mediated AgNPs

4 CONCLUSION

The present study is the very first report demonstrating a novel, quick and eco-friendly procedure for the synthesis of AgNPs using *T. neotaliala* fruit extract. This route of AgNPs synthesis using sunlight proved out to be very efficient with no external energy or instrumental requirements.

Table 1: % Inhibition and IC50 values of *T. neotaliala* fruit extract mediated AgNPs on inhibition of BSA protein denaturation

Sl. No.	Concentration (μg/mL)	% Inhibition of BSA protein denaturation		
		Diclofenac Sodium	AgNPs	Fruit Extract
1	100	42.33 ± 1.05	29.37 ± 1.05	12.08 ± 1.28
2	200	62.54 ± 1.02	36.17 ± 1.19	20.97 ± 1.36
3	300	80.11 ± 1.08	48.16 ± 1.09	30.19 ± 1.12
4	400	88.67 ± 1.04	64.94 ± 1.00	45.77 ± 1.11
5	500	97.16 ± 1.14	73.68 ± 1.25	65.33 ± 1.15
	IC50	121.99	296.01	415.23

The experiment was conducted in triplicates and data obtained is expressed as mean ± standard deviation

The formed AgNPs were spherical and within the range of nanoscale. The phytochemicals from the fruit extract such as polyphenols may be involved in the reduction whereas proteins helped in the stabilization of AgNPs by forming a layer around them. Comparison of standard anti-inflammatory drug diclofenac sodium and AgNPs in inhibition of BSA protein denaturation, revealed the potential of AgNPs to act as an anti-inflammatory agent. Future *in vivo* studies might be a scope for its possibility in biomedical applications.

5 CONFLICT OF INTEREST

The authors declare no conflict of interest

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