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RESEARCH ARTICLE

Sunlight-Assisted Fabrication of Silver Nanoparticles using *Terminalia neotaliala* Capuron Aqueous Bark Extract: Evaluation of *In vitro* Antioxidant and Anti-inflammatory Activities

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ABSTRACT

Objective(s): Sunlight-assisted rapid fabrication of silver nanoparticles (AgNPs) using aqueous bark extract of *Terminalia neotaliala* Capuron (TnB-AgNPs) and evaluation of *in vitro* antioxidant and anti-inflammatory activities.

Methods: Aqueous bark extract of *T. neotaliala* was used as a reducing agent in AgNPs formation under direct sunlight. Techniques like UV-Vis Spectroscopy, FTIR, XRD, DLS, HR-TEM with SAED were employed for the characterization studies. Further, antioxidant activity was determined by DPPH radical scavenging assay and anti-inflammatory activity by BSA anti-denaturation assay.

Results: The TnB-AgNPs formation was observed as a colour change from light-yellow to dark-brown with a λmax value of 414nm. FTIR spectra revealed different functional groups such as -OH, -CH, -C=O, -NH, -C-N groups associated with the presence of polyphenolic compounds in the bark extract. XRD confirmed the Face Centered Cubic crystalline nature with an average crystallite size of 26 nm. Z-average particle size was found to be 57nm with a zeta potential value of -40.9mV indicating excellent stability. HR-TEM studies depict spherical shape with average particle size of 34.81nm and the lattice planar spacing of 0.24nm. The TnB-AgNPs and bark extract showed % scavenging of 70.66±0.27% and 80.78±0.39 % (100 µg/mL) for antioxidant activity and % inhibition of 79.49±1.22% and 67.92±1.00% (500µg/mL) for anti-inflammatory activity at their highest concentration.

Conclusion: The present study demonstrates a rapid approach in producing small, spherical, stable, crystalline AgNPs using *T. neotaliala* bark extract under the influence of direct sunlight. The synthesized TnB-AgNPs showed moderate antioxidant and excellent anti-inflammatory activity indicating its potentiality in biomedical applications.

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INTRODUCTION

Nanoparticles are atomic aggregates, spherical or quasi-spherical in shape with diameter ranging from 1-100nm [1]. Recently, nanotechnological research is mainly concentrated on the synthesis and characterization of silver nanoparticles due to their remarkable physical, biological and pharmaceutical applications [2]. Among all the noble metals used for synthesis of nanoparticles, silver nanoparticles have received considerable attention due to their strong absorption in the visible region of the electromagnetic spectrum which can be easily monitored through a UV-Vis Spectrophotometer [3]. From thousands of years, silver is a well-known antimicrobial agent. In contrast, silver nanoparticles exhibit good catalytic activity, thermal stability and also various biological activities such as antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory and anticancer activities being

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employed in pharmaceutical products due to lesser toxicity to human cells [4]. There are several routes to synthesize silver nanoparticles such as physical, chemical and biological. The former two methods need sophisticated instrumentation, technical expertise and use of toxic reducing agents that circumscribes their usage [5].

Over a decade, the rise in environmental concerns has directed the focus of scientists to explore biosynthesis methods that are both economically feasible and eco-friendly [6]. The application of principles of green chemistry to the field of nanotechnology fosters the usage of environment friendly reducing and stabilizing agents [7]. So, plant-based fabrication of nanoparticles tends to be a straight forward approach compared to using other biological systems. Apart from the use of naturally available reducing agents from plants, it's also equally important to make the process eco-friendlier by using energy efficient techniques. The use of solar radiation from sunlight proves to be a cheap source in assistance of nanoparticle formation [8] as this is less time consuming, renewable and an energy efficient technique [2]. Moreover, the combination of using plant extracts as reducing agents with the assistance of sunlight makes the process quite impressive and recently is taking a step higher compared to the other methods. Such a method has been reported in producing AgNPs using plant extracts like *Allium ampeloprasum* [9], *Jasminum subtriplinerve* [10], *Zingiber officinale* [2], *Ocimum sanctum* [11], *Sida retusa* [12] *etc.*

The plant *Terminalia neotaliala* Capuron belongs to Combretaceae family. The synonym of this plant is *Terminalia mantaly* H. Perrier. It is used as an ornamental tree because of its conspicuously layered branches. It is commonly known as Madagascar almond, Umbrella tree or French mantaly and is native to Madagascar [13-15]. The bark of the tree is rich in tannins and is made use for dyeing purposes [15]. In Ivorian medicine, the bark and the leaves of this plant are used as a remedy to treat dysentery, diarrhoea, mouth and digestive candidiasis, postpartum care and bacterial infections [16, 17].

This is the first report on sunlight-assisted fabrication of AgNPs using *Terminalia neotaliala* Capuron aqueous bark extract. To the best of our knowledge, no other report has demonstrated the use of this method for synthesis of AgNPs using *T. neotaliala* bark extract. This process occurred within a short period of time, without the use of any external reducing agent, or heating and stirring. In addition, the synthesized AgNPs were characterized and evaluated for antioxidant activity by DPPH free radical scavenging assay and anti-inflammatory activity by BSA anti-denaturation assay.

MATERIALS AND METHODS

Silver Nitrate ($AgNO_3$) of Analytical grade and Bovine Serum Albumin (BSA) were procured from Hi-Media Laboratories Pvt. Ltd. DPPH (2,2-diphenyl-1-picrylhydrazyl) was procured from Sigma-Aldrich, Methanol and Ascorbic acid were procured from SD-fine-chem limited, Mumbai. Diclofenac sodium was purchased from a local medical shop. The plant selected for the study was *Terminalia neotaliala* Capuron, identified and authenticated (Acc. No. 19558) by Dr. K. Kotresha, Associate Professor, Karnatak College, Dharwad. Fresh bark of this plant was collected from the Botanical Garden of Karnatak University, Dharwad, Karnataka, India.

Preparation of Aqueous Bark Extract

The Bark of *T. neotaliala* Capuron was thoroughly washed with tap water followed by distilled water to remove dust and other adhering impurities. It was shade dried at room temperature for several days. The dried bark was cut into small pieces, milled to a coarse powder in an electric grinder and was stored in an air-tight container. The extract was prepared by soaking 5gms of the coarse bark powder in 100mL of distilled water for about an hour. Later, it was boiled at 60-70°C for 45mins. After cooling, the solution was filtered through No. 1 Whatman filter paper and stored at 4°C for future use.

Sunlight-Assisted Fabrication of TnB-AgNPs

For the synthesis of AgNPs, silver nitrate ($AgNO_3$) was used as a precursor. To 98ml of 1mM $AgNO_3$, 2ml of aqueous bark extract of *T. neotaliala* was added so as to make the final volume to 100mL. $AgNO_3$ solution and bark extract were maintained separately as control. The reaction mixtures were exposed to bright sunlight. To study the effect of sunlight on synthesis, the solution was incubated in dark conditions also. The effect of pH on nanoparticle formation was also studied by adjusting the pH to 8, 9 and 10. The particles were separated by repeated centrifugation at 13,000 rpm

for 30mins followed by redispersion in distilled water to remove uncoordinated bio-inorganic molecules. The obtained pellet was dried and used for further studies.

Characterization of TnB-AgNPs

The biosynthesized AgNPs were characterized using various analytical techniques

i. UV-Vis Spectroscopy
   UV-Vis Spectrophotometer (Jasco V-670) was used to confirm the formation of TnB-AgNPs by measuring the λmax in the range of 300 to 700nm.
ii. Fourier Transform Infrared Spectroscopy (FTIR)
   Fourier Transform Infrared Spectrophotometer (Nicolet, Thermofischer Scientific) was used to detect the functional groups of phytochemicals responsible for reduction and capping of TnB-AgNPs in the range of 4000 to 500cm^{-1}.
iii. X-Ray Diffraction (XRD)
   X-Ray diffractometer (Rigaku, Smart Lab) was used to determine the crystalline nature of powdered TnB-AgNPs in the range of 30-90° with the scanning rate of 10°/min at 40kV and 30mA.
iv. Particle Size and Zeta Potential
   Particle size analyser (Horiba SZ-100) was used to study the size, polydispersity index and zeta potential of TnB-AgNPs.
v. High Resolution - Transmission Electron Microscopy (HR-TEM) with Selected Area Electron Diffraction (SAED)
   High Resolution - Transmission Electron Microscope (Jeol/JEM 2100) operated at 200kV was used to know the exact shape and size of TnB-AgNPs. The crystalline nature of AgNPs was also analysed through SAED.

Antioxidant activity of TnB-AgNPs

The antioxidant activity was determined by DPPH free radical scavenging assay following the protocol previously reported by Blois M. 1958 [18] with minor modifications. TnB-AgNPs/ bark extract at concentrations of 20, 40, 60, 80, 100 µg/mL were mixed 0.1mM DPPH in methanol. The tubes were vortexed and incubated in the dark for 30mins. Absorbance was measured at 517nm using UV-Vis Spectrophotometer. Ascorbic acid was used as a standard drug and DPPH without sample as control. The free radical scavenging % was calculated using the following equation

% DPPH scavenging = [(Acontrol-Asample)/Acontrol] x 100

Where, Acontrol represents absorbance of the control and Asample represents absorbance of the sample

Anti-inflammatory activity of TnB-AgNPs

The anti-inflammatory activity was determined by BSA protein anti-denaturation assay following the protocol previously reported by Grant *et al.,* 1970 [19] and Aware C. *et al.,* 2017 [20] with minor modifications. TnB-AgNPs/ bark extract at concentrations of 100, 200, 300, 400, 500 µg/mL were mixed with 1% BSA in 50mM tris buffer (pH 6.5). The tubes were incubated at room temperature for 20mins followed by heating in a water bath at 64°C for 5-10 mins till the solution got turbid. After cooling, absorbance was measured at 660nm using UV-Vis Spectrophotometer. Diclofenac Sodium was used as a standard and BSA without sample as control. The % inhibition of BSA denaturation was calculated using the following equation.

% Inhibition of BSA denaturation = [(Acontrol-Asample)/Acontrol] x 100

Where, Acontrol represents absorbance of the control and Asample represents absorbance of the sample.

RESULTS AND DISCUSSION

UV-Vis Spectroscopy analysis

The primary indication of sunlight-assisted fabrication of AgNPs using aqueous bark extract of *T. neotaliala* was noticed as a rapid change in colour from light yellow to dark brown within 5 minutes of sunlight exposure (Fig. 1A). A similar colour inference and reaction time of 5 mins for synthesis of AgNPs using *Azadirachta indica* leaf extract was reported by Mankad M *et al.,* (2020) [21]. After the change in colour, the pH of the reaction mixture was found to be 4.9 with an SPR band at 426nm. It is known that acidic pH hinders the formation of nanoparticles [22]. With further adjusting the pH to 8, 9 and 10, the intensity of absorbance increased with mild blue shift in SPR band. From the fig.1B, it can be observed that the peak at pH 8 showed hike in absorbance with a λmax value of 414nm compared to other pH. So, pH 8 was considered to be optimum for the synthesis of nanoparticles. Similar results were seen in earlier reports [23]. The change in colour and presence of absorption

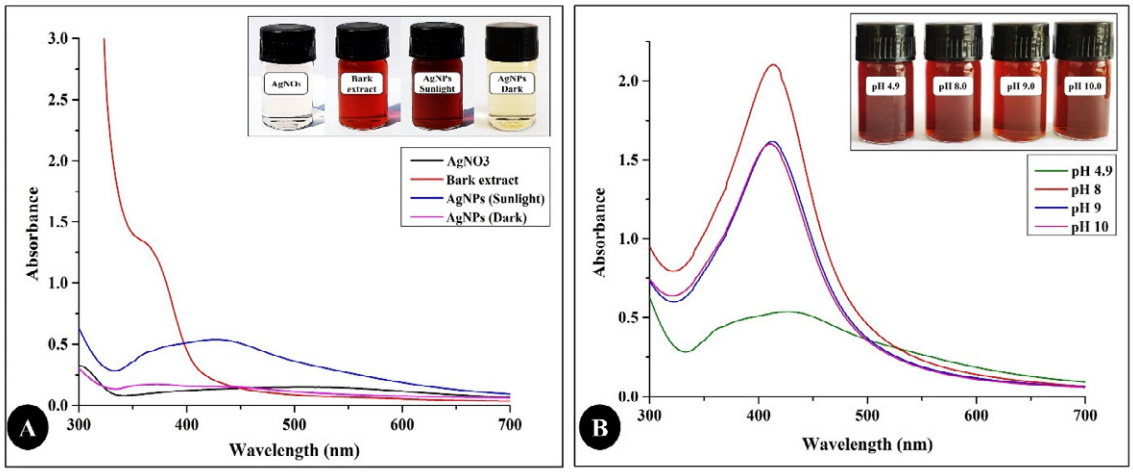

Fig. 1. (A) UV-Vis spectra of $AgNO_3$, bark extract and TnB-AgNPs exposed to sunlight and TnB-AgNPs in Dark (B) UV-Vis spectra of TnB-AgNPs at pH 4.9, 8, 9 and 10

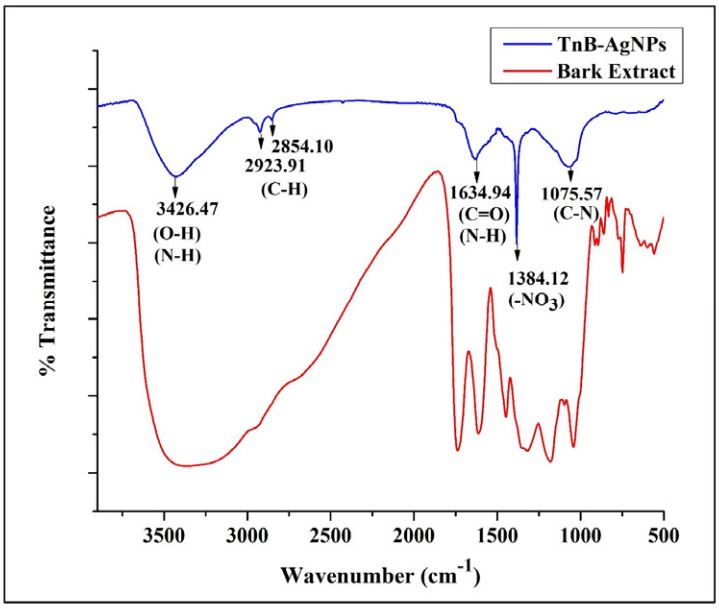

Fig. 2. FTIR spectra of *T. neotaliala* aqueous bark extract mediated AgNPs

peak in the range of 400-450nm clearly indicates the formation of AgNPs [3]. The solution kept in the dark showed only a slight change in colour after 30mins. The colour of the control solution exposed to sunlight showed no signs of colour change (Fig. 1A). This shows that the synthesis of AgNPs requires both bark extract and sunlight exposure in reducing Ag^+ ions to Ag^0. Moreover, previous researches have shown the synthesis of AgNPs using plants which required hours or days of time for the reaction to complete such as 24 hrs for AgNPs synthesis using leaf extract of *Cannabis sativa* [24] and *Aloe vera* [25], 48 hrs using aerial parts of *Acacia cyanophylla* [26], and 72 hrs using banana peel extract [27]. But with this technique, the reaction time is significantly reduced showing its efficiency in nanoparticle formation at the preliminary level.

FTIR analysis

The functional groups of phytoconstituents present in the *T. neotaliala* aqueous bark extract responsible for reduction and capping of TnB-AgNPs were determined using FTIR analysis. The

Table 1. FTIR spectra interpretation of *T. neotaliala* aqueous bark extract mediated AgNPs

Sl. No.	Wavenumber (cm^{-1})	Peak intensity	Possible functional groups assigned
1	3426.47	Medium	H-bonded OH of alcohols or phenols/ carboxylic acid/ NH stretch of primary and secondary amines and amides
2	2923.91	Strong	Asymmetric stretching vibration of C-H groups
3	2854.10	Strong	Symmetric stretching vibration of C-H groups
4	1634.94	Strong	C=O of amides/ NH bend of primary and secondary amines and amides
5	1384.12	Strong	Residual NO_3
6	1075.57	Medium	C-N of amines

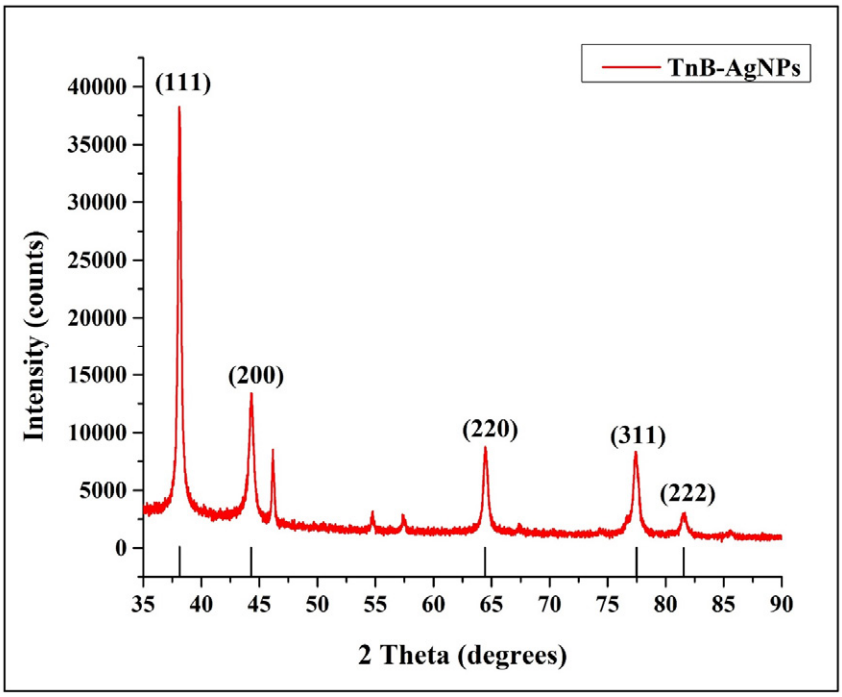

Fig. 3. XRD spectra of *T. neotaliala* aqueous bark extract mediated AgNPs

FTIR spectra of bark extract and TnB-AgNPs are shown in the fig. 2. Characteristic peaks at 3426.47, 2923.91, 2854.10, 1634.94, 1384.12, 1075.57cm^{-1} were obtained for TnB-AgNPs respectively. The minor shifts in peak position between the spectra clearly indicate the involvement of bark extract in nanoparticle formation. The values obtained for TnB-AgNPs assigned to their respective functional groups are shown in Table 1. The Preliminary phytochemical studies on aqueous bark extract of *T. neotaliala* reported the presence of phenols, flavonoids, tannins, alkaloids, anthraquinones, triterpenes, steroids, glucosides, and saponins [28]. The FTIR groups obtained from the surface of nanoparticles may pertain to the phytochemicals mentioned above showing their direct involvement in reduction and capping of AgNPs.

XRD analysis

The XRD analysis of TnB-AgNPs elucidate their crystalline nature as shown in fig. 3 with peaks at 2θ value of 38.09°, 44.29°, 64.43°, 77.38° and 81.44° respectively. These peaks can be indexed to (111), (200), (220), (311) and (222) planes of Face Centered Cubic crystal structure of metallic silver and is in good agreement with ICDD card No. 00-004-0783. The results are in accordance with the previous reports [29]. Compared to the other four planes, very strong reflection at (111) signifies the growth path of nanocrystals [30]. The average crystallite size calculated using Debye-Scherrer's equation was found to be 26.41nm (Table 2). A few unassigned peaks at 46.17°, 54.77°, 57.40°, 67.36° were also observed which may be due to the phytoconstituents from the bark extract that act

Table 2. XRD profile of *T. neotaliala* aqueous bark extract mediated AgNPs

Position 2θ (°)	Height (counts)	FWHM (°)	d-spacing (Å)	Planes (hkl)	Particle Size (nm)
38.0940	1532.5	0.2878	2.3608	(111)	30.50
44.2953	449.7	0.4346	2.0432	(200)	20.61
64.4327	344.8	0.3389	1.4448	(220)	28.93
77.3838	338.6	0.3865	1.2321	(311)	27.05
81.4430	82.1	0.4463	1.1807	(222)	24.53
Average crystallite size					**26.41**

Note: FWHM- Full width half maximum

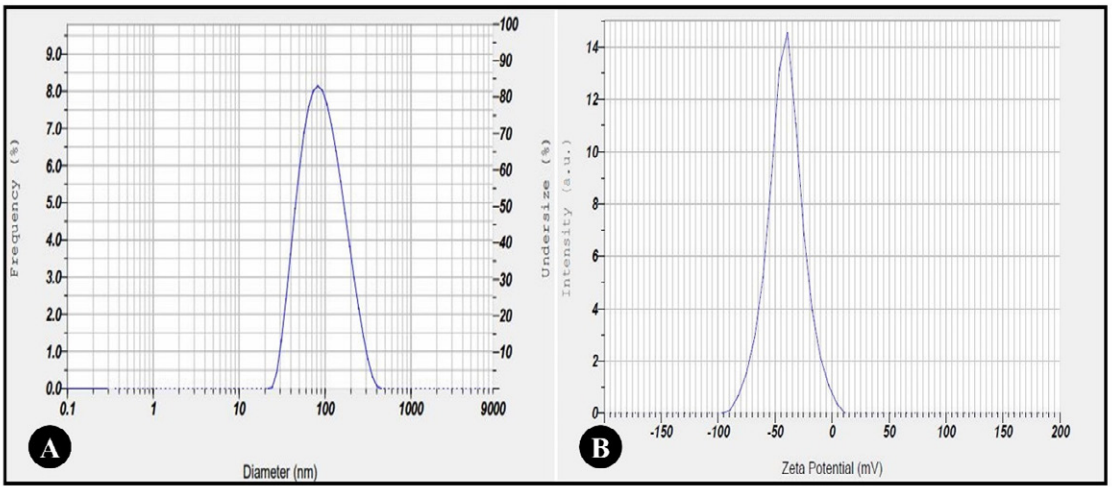

Fig. 4. (A) Particle size and (B) Zeta potential of *T. neotaliala* aqueous bark extract mediated AgNPs

as capping agents in stabilizing the nanoparticles [31,32].

Particle size and Zeta potential analysis

Particle size analyser was employed to measure the z-average particle size via dynamic light scattering and zeta potential of the nanoparticles. The z-average particle size of TnB-AgNPs was found to be 57.0nm (Fig. 4A) with a polydispersity index of 0.691. The size of AgNPs obtained was quite larger than TEM results which indicate that DLS measures the particle size along with the capping molecules and solvent layer [33]. The zeta potential value of -40.9mV (Fig. 4B) indicates greater stability of nanoparticles which may be due to very strong repulsive forces that exists between the nanoparticles, thereby decreasing the aggregation of the nanoparticles [34].

HR-TEM with SAED analysis

The Transmission electron microscopic analysis provided information regarding the size, shape and distribution of TnB-AgNPs. TEM image (Fig. 5A) reflects spherical shape of the AgNPs. The Histogram shows particle size distribution ranging from 10 to 70nm with an average size of 34.81±11.04, n=60 (Fig. 5B). AgNPs synthesized using *Physalis angulata* leaf extract showed size ranging from 11 to 96nm with average particle size of 35nm, which was similar to data obtained in the present study [35]. Images depict well dispersed nature of the nanoparticles and highly crystalline as seen from the lattice fringes (Fig. 5C) and selected area electron diffraction pattern (Fig. 5D).

Antioxidant activity of TnB-AgNPs

The antioxidant capacity of TnB-AgNPs was determined using DPPH free radical scavenging assay. The change in colour of DPPH from violet to yellow indicates a change in its free radical form (diphenylpicryhydrazyl) to non-free radical form (diphenylpicrylhydrazine). TnB-AgNPs exhibited antioxidant activity in a dose-dependent manner (Fig. 6). Maximum scavenging activity of 70.66 ±

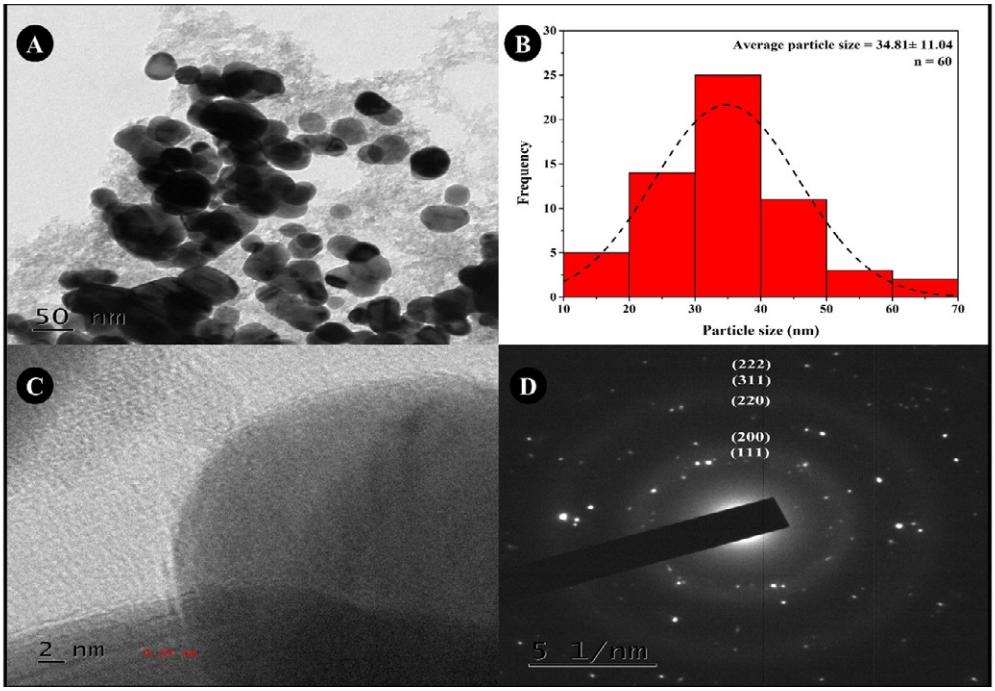

Fig. 5. HR-TEM and SAED pattern of TnB-AgNPs

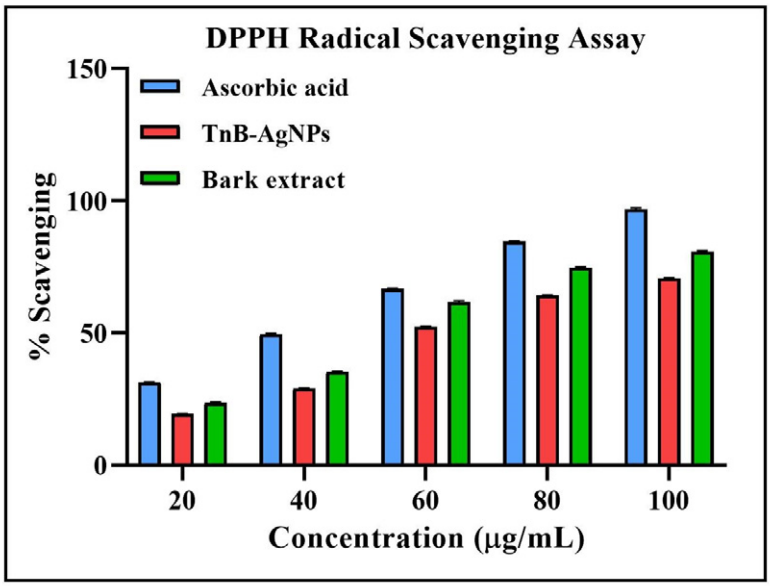

Fig 6. Antioxidant activity of Ascorbic acid, TnB-AgNPs and Bark extract at various concentrations

0.27 % was recorded at its highest concentration 100µg/mL with an IC50 value of 64.13 µg/mL. The IC50 value obtained for bark extract and standard Ascorbic acid were 53.15 and 40.99 µg/mL respectively (Table 3). Bark extract showed slightly higher activity than TnB-AgNPs. Such results were reported in previous literature [31]. Thus, antioxidant activity of TnB-AgNPs could be due to the phytochemicals bound on their surface. Generally, the antioxidant activity is correlated

Table 3. IC50 values of Ascorbic acid, TnB-AgNPs and Bark extract for antioxidant activity

Sl. No.	Concentration (µg/mL)	% Scavenging		
		Ascorbic acid	TnB-AgNPs	Bark Extract
1	20	31.28 ± 0.11	19.48 ± 0.13	23.67 ± 0.18
2	40	49.55 ± 0.31	29.05 ± 0.18	35.42 ± 0.10
3	60	66.59 ± 0.22	52.38 ± 0.20	61.61 ± 0.59
4	80	84.68 ± 0.12	64.20 ± 0.11	74.77 ± 0.39
5	100	96.88 ± 0.37	70.66 ± 0.27	80.78 ± 0.39
	IC50	**40.99**	**64.13**	**53.15**

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

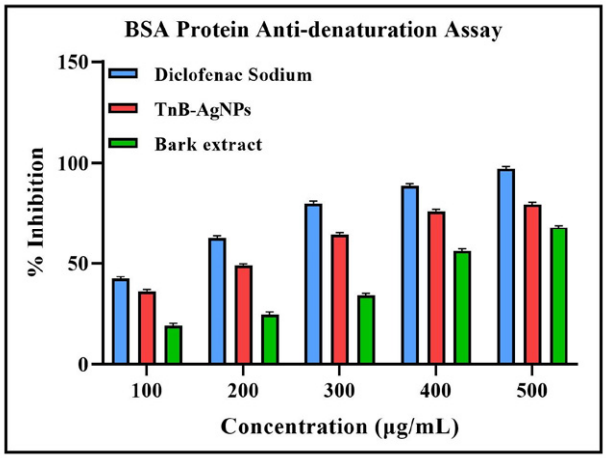

Fig. 7. Anti-inflammatory activity of Diclofenac sodium, TnB-AgNPs and Bark extract at varying concentrations

with the polyphenolic content present in plants and their mechanism of action is due to the ability to donate hydrogen atoms and scavenge free radicals [36]. So, it is suggested that the plant polyphenols not only act as reducing and capping agents during the formation of AgNPs but they also enhance the antioxidant activity of the nanoparticles [32].

Anti-inflammatory activity of TnB-AgNPs

The anti-inflammatory activity of TnB-AgNPs was determined using BSA protein anti-denaturation assay. This assay seeks to eliminate the use of live specimens in the drug development process. Denaturation of proteins results in the production of auto-antigens that cause serious inflammatory conditions such as rheumatoid arthritis [37]. The mechanism behind denaturation involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [19]. In this assay, the capacity of compounds to stabilize (prevent denaturation) BSA at pathological pH (6.2-6.5) was tested at various concentrations in terms of % inhibition. The % inhibition of BSA denaturation of TnB-AgNPs, bark extract and Diclofenac at various concentrations is shown in the Fig. 7. The IC50 values of TnB-AgNPs, bark extract and Diclofenac are 205, 374.42 and 121.99 µg/mL respectively (Table 4). The results depict significant anti-inflammatory activity of TnB-AgNPs in a dose-dependent manner with a maximum % inhibition of 79.49±1.22 at 500 µg/mL. The activity of TnB-AgNPs was almost near to the standard Diclofenac, whereas bark extract showed lesser activity. Considering the results obtained, AgNPs synthesized using *Terminalia neotaliala* bark extract are capable in stabilizing BSA.

The previous reports on sunlight mediated synthesis of AgNPs have mainly focused on testing the antibacterial potential against various pathogens, whereas our study has focused on antioxidant and anti-inflammatory activity. So, there aren't any reports on the same lines which corroborate with our present data.

CONCLUSION

We conclude that sunlight-assisted fabrication of AgNPs using aqueous bark extract of *Terminalia neotaliala* is a very simple, rapid, and efficient

Table 4. IC50 values of Diclofenac sodium, TnB-AgNPs and Bark extract for anti-inflammatory activity

Sl. No.	Concentration (µg/mL)	% Inhibition of protein denaturation		
		Diclofenac sodium	TnB-AgNPs	Bark Extract
1	100	42.33±1.05	35.87±1.00	19.09±1.17
2	200	62.54±1.02	48.73±0.97	24.56±1.33
3	300	80.11±1.08	64.22±0.84	34.08±1.01
4	400	88.67±1.04	76.08±1.04	56.21±1.03
5	500	97.16±1.14	79.49±1.22	67.92±1.00
	IC50	**121.99**	**205**	**374.42**

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

method. Sunlight offers a cheap source of energy that not only reduces the reaction time but also forms nanoparticles that are almost spherical in shape, small in size ranging from 10-70nm and crystalline in nature as confirmed from various characterization studies. The polyphenolic content from the bark extract might be involved in reducing, capping and stabilizing the nanoparticles which further improved its antioxidant and anti-inflammatory activity. Over the years, this technique can replace the conventional temperature mediated synthesis as this is less time consuming. Additionally, the use of plant extract makes it even more sustainable and is less toxic to humans and their surrounding environment.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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