J. Water Environ. Nanotechnol., 7(3): 306-316 Summer 2022

ORIGINAL RESEARCH PAPER

Facile synthesis of silver nanoparticles, anti-inflammatory, antibacterial and photocatalytic activities using *Pogostemon speciosus* Benth. An endemic medicinal plant

Sathiya Sheela. D*, P. Viswanathan, K. Kalimuthu, A.Vanitha

PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore – 641 018, Tamil Nadu, India.

Received: 2022-04-12 Accepted: 2022-06-22 Published: 2022-07-01

ABSTRACT

The development of the biologically enthused green synthesis of silver nanoparticles (SNPs) has concerned significant global awareness about medical science and disease treatment. This paper discusses the green synthesis of SNPs using organic green sources; here we report a facile bottom-up 'green' route for the synthesis of SNPs using aqueous leaves extract of Pogostemon speciosus (Benth.) and evaluate its in-vitro anti-inflammatory, antibacterial and photocatalytic activities. The nanoparticles were investigated for the preparation of denaturation particles with PSLASNPs and the evaluation of anti-inflammatory activity with Protein denaturation and HRBC stabilization assays. Later, these PSLASNPs were studied for their potential role in antibacterial activity by well diffusion method, and Photocatalytic activity on degradation of dyes was demonstrated by using dyes Crystal violet, Coomassie blue, and Congo red. At 1000 µg/ml, the PSLASNPs have the greatest prevention of protein denaturation (71.92±1.37%), whilst the stabilization of the HRBC membrane exhibited significant anti-inflammatory action (64.39±1.61 %). The PSLASNPs showed the best antibacterial activity at the concentration of 10 µg/ml against Bacillus subtilis (8.2 mm), followed by *Pseudomonas stuberia* (6.2 mm) and *Escherichia coli* (6.4 mm), *Staphylococcus aureus* (5.3 mm), *Staphylococcus gallinarium* (4.5 mm) respectively at the same concentrations. Crystal violet, Coomassie blue, and Congo red were used for Photocatalytic activity on the breakdown of dyes. After 35 minutes, the degradation process was determined to be complete by the transformation of the reaction mixture's color to colorless. As a result, the PSLASNPs have anti-inflammatory, antibacterial, and photocatalytic activities.

Keywords: *Green synthesis, Protein denaturation assay, HRBC stabilization assay, Antibacterial activity, Dye degradation.*

How to cite this article
Sheela. D S., Viswanathan P. , Kalimuthu K., Vanitha A. Facile synthesis of silver nanoparticles, anti-inflammatory, antibacterial and photocatalytic activities using Pogostemon speciosus Benth. An endemic medicinal plant. J. Water Environ. Nanotechnol., 2022; 7(3): 306-316.
DOI: 10.22090/jwent.2022.03.006

INTRODUCTION

According to the current situation, more than ten thousand medicinal plant species are used by the people of India, of which 60% occur in high-altitude regions. Many diseases can be treated by medicinal plants using various technological methods, among them nanotechnology holds great potential by using components ranging in size from 1-100nm [1]. In recent years, silver nanoparticles (SNPs) have greatly focused the researcher's attention because of their important application as antimicrobial, catalytic, textile fabrics, and plastics to eliminate micro-organisms [2,3]. Nanoparticles, especially silver nanomaterials, are being biosynthesized from plant extracts or organic sources because of their multitude of properties and bioactive reducing metabolites. Traditionally, plants have been preferred sources of nanoparticles for synthesis, compared to bacteria and algae, plants are less sensitive to metal toxicity, providing a greener way to synthesize silver nanoparticles [4].

* Corresponding Author Email: *manavaisheela1357@gmail.com*

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In this case, silver nanoparticles are synthesized more effectively using green leaves than in other plants because of their origin for photosynthesis and their availability of more Hp ions for reducing silver nitrate creation [5]. Nanoparticles are being used in drug delivery, drug discovery, and new drug therapies to conquer many dreadful diseases. Nanoparticles can use the body's natural transport system and uptake mechanisms to transport the drugs [6].

Inflammation is the complex immune response to infection or injury that results in the removal of offending factors and restoration of the underlying structure and function of the tissue [7]. The symptom of Inflammation causes pain, heat, redness, swelling, and loss of function. Even though started as a protective mechanism, loss of the ability to control this complex process can lead to various inflammatory conditions. The majority of the pharmacological treatment of inflammation consists of anti-inflammatory drugs, including both steroidal and non-steroidal options. There are still numerous side effects associated with conventional drugs, which has led to a need to identify alternative substances that can effectively resolve inflammation in a way that is homeostatic, modulatory, effective, and well tolerated by the body [8]. Phytomedicine is one such alternative rationale for treating inflammatory disorders. Purified natural plant compounds have aided in the synthesis of future-generation anti-inflammatory drugs with higher therapeutic value and lower toxicity [9].

There are different primary sources for nanoparticle synthesis by biological methods, including microorganisms (yeast, fungi, bacteria, viruses, and actinomycetes) and plants [10]. Nanoparticles synthesized using plant extracts are more advantageous than those synthesized using microorganisms because they do not require complex and specialized processes such as bacterial isolation, culture maintenance, and multiple purifications. Consequently, it has become a major focus, leading to the development of green methods utilizing various parts of the plant [11]. Plant extracts contain several compounds that play an important role in the mechanisms of metal ions uptake, reduction of precursor salt as well as capping agents, some of which have antimicrobial properties.

During the dyeing process, over 15% of the world's total dye production is wasted and discharged in textile effluents. The release of colored waste waters into the ecosystem is a significant source of non-aesthetic pollution, eutrophication, and perturbations in aquatic life. As international environmental standards are becoming more rigorous (ISO 14001, October 1996), technological systems for removing organic pollutants such as dyes have recently been developed. Among the various dye removal methods, new oxidation methods, or advanced oxidation technology (AOT), heterogeneous photocatalysis appears as an emerging destructive technology that leads to the total mineralization of most organic pollutants [12]. Biosynthesized silver nanoparticles have been effectively used as dye degradation and detoxification catalysts [13]. Non-biodegradable dyes are used in the textile, paper, and leather industries, which are the most hazardous and may cause environmental problems. To reduce these issues, dye detoxification techniques such as redox medications, UV-light degradation, and carbon sorption flocculation have been used. As a result of their excellent biocompatibility and catalytic behavior, green synthesized nanoparticles are more economical and valuable [14,15].

The application of *Pogostemon speciosus* in nanotechnology has not received much attention, according to a thorough review of the literature. The present study aims for the first time to evaluate the anti-inflammatory, antibacterial and photocatalytic activities of the synthesized nanoparticles.

MATERIALS AND METHODS

Inhibition of Protein denaturation assay

By using the inhibition of the Protein denaturation method, PSLASNPs (*Pogostemon speciosus* leaf aqueous extract silver nanoparticles) samples have been examined for anti-inflammatory activity [16,17]. A sample with 15.65, 31.25, 62.5, 125, 250, 500, and 1000 µg/ml concentration was studied**.** Each sample concentration was mixed with 2 mL of egg albumin and 2.8 mL of PBS, and the mixture was incubated at 37 °C for 15 minutes. A reaction mixture of 0.5 mL (pH 6.3 EUTECH-OAKION) was prepared by mixing 0.45 mL of bovine serum albumin (5% aqueous solution) with 0.05 mL of distilled water. Various concentrations of the sample were added to the reaction mixture then incubated at 37 ºC for 20 min and heated at 57 ºC for 5 minutes. Finally, 2.5 ml of phosphate buffer saline was added after cooling the sample. Percentage inhibition was measured through an

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ELISA reader at 600 nm. Inhibition percentage calculation was done using a particular formula.

$$Percentage\,stabilization(\%) = \frac{(control - sample}{control} \times 100$$

HRBC membrane stabilization method

For membrane stabilization assay, PSLASNPs sample at 15.65, 31.25, 62.5, 125, 250, 500, and 1000g/ml concentrations were incubated separately with HRBC (human red blood cells) solution [18]. The blood of healthy volunteers (2 ml) was mixed with an equal volume of sterilized Alsevers solution (2 % dextrose, 0.8 % sodium citrate, 0.5% citric acid, and 0.42 % Sodium chloride in distilled water) and centrifuged at 3000 rpm. A solution of Isosaline was used for washing the packed cells. The sample was prepared in various concentrations in normal saline, while Aspirin was used as the standard control. The assay mixtures were incubated at 37 ºC for 30 minutes and centrifuged at 3000 rpm for 20 minutes, and the hemoglobin content of the supernatant solution was determined spectrophotometrically at 560 nm. The following formula was used to calculate the various plant extract HRBC membrane stabilization or protection percentages:

$$Percentage\,stabilization(\%) = \frac{(control - sample}{control} \times 100$$

Antibacterial activity

Antibacterial activity of PSLASNPs sample was analyzed on five bacterial organisms by well diffusion method. Among five bacterial pathogens, three gram-positive bacteria (*Staphylococcus aureus, Staphylococcus gallinarium,* and *Bacillus subtilis*) and two gram-negative bacteria (*Pseudomonas stuberia* and *Escherichia coli*) were used for this study. Three different concentrations like 10 µg/ml, 5 µg/ml, 2.5 µg/ml, and Standard (Amoxicillin) were used. The test microorganisms were seeded into the respective medium by spread plate method 10 µl (10 cells/ml) with 24 hrs cultures of bacteria growth in nutrient broth. After solidification, the filter paper wells (5 mm in diameter) impregnated with the samples were placed on test organism-seeded plates. The antibacterial assay plates were incubated at 37 °C for 24 hrs. The diameters of the inhibition zones were measured in mm.

Photocatalytic dye degradation

The photocatalytic activity of PSLASNPs on the degradation of dyes was demonstrated by using organic dyes like Crystal Violet (CV), Coomassie Blue (CB), and Congo Red (CR) was tested on nanoparticles under the effect of sunlight [19]. To ensure the equilibrium of the solution, 1 mg of each dye was dissolved in 100 mL of double distilled water and stirred for 30 minutes with a magnetic stirrer. Each dye solution received 10 mg of Ag-Cs nanoparticles sample and was exposed to sunlight. At various time intervals, 3 ml suspensions were centrifuged at 10,000 rpm for 10 minutes. The absorbance of the sample was measured using an Ultraviolet-visible spectrophotometer (Hitachi Double Beam Model No. UH5300).

RESULTS AND DISCUSSION

In vitro anti-inflammatory activity of PSLASNPs sample

Protein denaturation assay

Inflammation is a significant global health issue that is triggered by irritants and infections [20]. Despite this, inflammation is often a part of the body's healing process, but it is extremely painful and uncomfortable. Furthermore, inflammation has been linked to malignant cell persistence and invasion via a variety of cellular and molecular mechanisms [21]. Many anti-inflammatory synthetic drugs are available to treat the symptoms of inflammation. These anti-inflammatory chemical drugs interfere with the body's natural repair and healing ability. Thus, natural products can be a better substitute for chemical drugs in order to achieve true and ultimate healing of the body, which is a true property of anti-inflammatory drugs. Fibrinolytic assays, platelet aggregation, uncoupling of oxidative phosphorylation, erythrocyte membrane stabilization, lysosomal membrane stabilization, anti-proteinase assays, and protein denaturation inhibition have all been used to screen drugs for *in vitro* anti-inflammatory activity [22]. The anti-inflammatory activity of the PSLASNPs sample was analyzed through inhibition of albumin denaturation assay by checking the ability of the silver nanoparticles to inhibit protein denaturation. The PSLASNPs were very effective in inhibiting heat-induced albumin denaturation at various concentrations (Table 1 and Fig. 1). Previous studies have evidenced that denaturation of proteins especially blood proteins as albumin is a major cause of rheumatoid arthritis and inflammation [23,24]. Protein denaturation is the process by which proteins lose their secondary and

Table1. Anti-inflammatory activity of PSLASNPs sample

S.No.	Sample Concentration µg/ml	Protein denaturation	
		PSLASNPs	Aspirin
1	1000	71.92±1.37b	83.75±1.17a
2	500	63.80±1.11b	73.67±2.13a
3	250	52.45±1.35b	63.12±2.28a
4	125	42.06±1.55b	50.28±2.36a
5	62.5	33.10±1.57b	42.12±1.57a
6	31.25	21.28±1.56b	32.13±1.64a
7	15.62	09.92±1.59b	21.07±1.58a

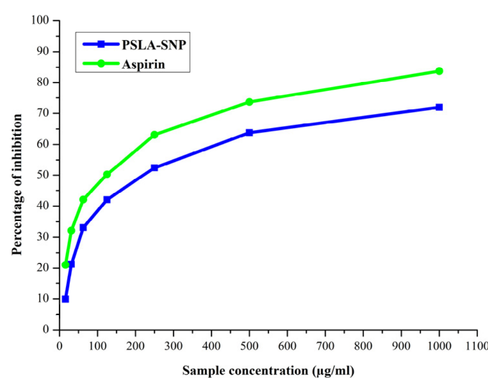

Fig. 1. Anti-inflammatory activity of PSLASNPs sample

tertiary structure as a result of external stress caused by physical or chemical agents such as heat shock. Protein denaturation is well known to cause the loss of biological function(s) [25]. The percentage of inhibition was calculated at the concentrations of 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/ml of the extract. The percentage of inhibition varied from 09.92±1.59, 21.28±1.56, 33.10±1.57, 42.06±1.55, 52.45±1.35, 63.80±1.11 and 71.92±1.37 form lowest concentration (15.62µg/ml) to highest concentration (1000 µg/ml) of PSLASNPs sample respectively. The 50% inhibition concentration (IC_{50}) was found at 237.46±2.46 µg/ml, whereas the IC_{50} value of control Aspirin was 124.26±2.17µg/ml (Table 3). As part of the investigation of the mechanism of the anti-inflammation activity, the ability of the synthesized PSLASNPs to inhibit protein denaturation was investigated. The result shows that PSLASNPs were effective in inhibiting heat-induced albumin denaturation. Maximum inhibition of 71.92±1.37 % was observed at 1000 µg/ml indicating the capability of the bio-generated nano silver to prevent protein denaturation involved in the inflammatory process. As a result, the anti-inflammatory properties of the nanoparticles in this study could be attributed to the individual or synergistic action of various phytochemical compounds like Naphthalene, Hexadecanoic acid, Phytol coated on the nanoparticles.

HRBC membrane stability activity

Several nonsteroidal anti-inflammatory drugs inhibit heat-induced erythrocyte lysis, most likely by stabilizing the cell membrane. This is the fundamental HRBC membrane stability test [26]. Some of the herbal preparation was capable of stabilizing the RBC membrane and this may be evidence of their potential to extract anti-inflammatory activity [27]. The results of the anti-inflammatory activity of PSLASNPs were evaluated through membrane stabilization assay and presented in Table 2. Different concentrations of the extract prevented heat-induced hemolysis. The samples may be attached to the erythrocyte membranes and alter the surface charges of the cells. This may arrest physical relations with aggregating agents or encourage dispersal by repulsion of similar charges which are involved in the red blood cell hemolysis. The sample was incubated individually with HRBC solution at concentrations of 15.62, 31.25, 62.5, 125,250,500, and 1000µg/ml, and hemolysis percentage was compared with standard Aspirin.

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Table2. Anti-inflammatory activity of PSLASNPs sample

S.No.	Sample Concentration µg/ml	HRBC membrane stabilization	
		PSLASNPs	Aspirin
1	1000	64.39±1.61c	80.76±2.48a
2	500	55.11±2.31c	71.91±2.54a
3	250	42.42±1.44c	62.72±0.96a
4	125	30.22±2.62c	51.11±1.50a
5	62.5	23.18±1.55c	42.80±1.95a
6	31.25	12.80±1.86c	32.70±1.00a
7	15.62	06.44±1.20c	21.68±1.76a

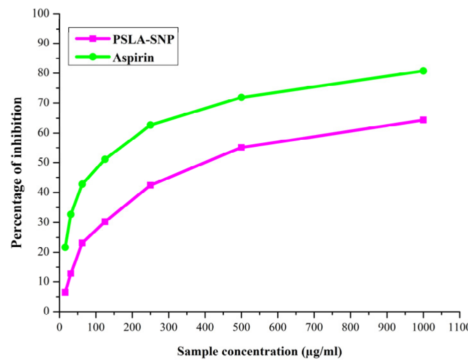

Fig. 2. Anti-inflammatory activity of PSLASNPs sample

Table 3. Anti-inflammatory IC_{50} value of PSLASNPs sample

S.No.	Sample	IC_{50} value (µg/ml	
		Protein denaturation	HRBC membrane stabilization
1	**PSLASNPs**	237.46±2.46	413.87±1.65
2	**Aspirin**	124.26±2.17	119.84±1.93

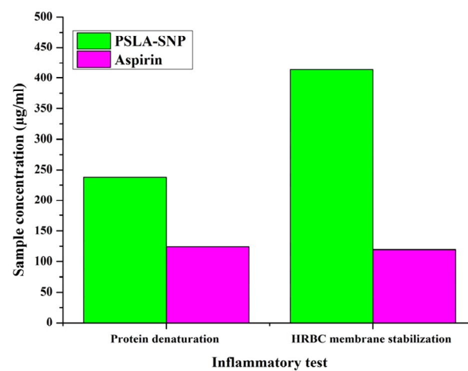

Fig. 3. Anti-inflammatory IC_{50} value of PSLASNPs sample

The highest percentage of inhibition was observed at 1000 µg/ml concentration. Among these the sample exhibited maximum significant anti-inflammatory activity with 64.39±1.61 percent, followed by 55.11±2.31, 42.42±1.44, 30.22±2.62, 23.18±1.55,12.80±1.86, and 06.44±1.20 percentage respectively (Fig. 2). This result was almost similar to *Solanum trilobatum* mediated silver nanoparticles [28]. The lowest IC_{50} value of the sample was found (413.87±1.65 µg/ml) compared to the control aspirin 119.84±1.93 µg/ml (Table 3 and Fig. 3). Oyedapo *et al* [29], reported that some

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Table 4. Antibacterial activity of PSLASNPs sample

S. No.	Organisms	Zone of inhibition (mm)			
		10 μg/ml	05 μg/ml	2.5 μg/ml	Standard (Amoxicillin)
1	*Staphylococcus aureus*	5.3±1.56	3.2±1.32	2.7±2.61	1.3±1.12
2	*Bacillussubtilis*	8.2±1.75	5.1±1.85	4.2±2.06	1.6±1.74
3	*Staphylococcus gallinarium*	4.5±0.65	3.6±2.40	2.8±1.28	5.1±2.35
4	*Pseudomonas stuberia*	6.2±2.52	5.7±0.21	3.3±2.19	4.2±0.75
5	*Escherichia coli*	6.4±1.76	5.8±2.11	2.4±1.82	1.5±1.83

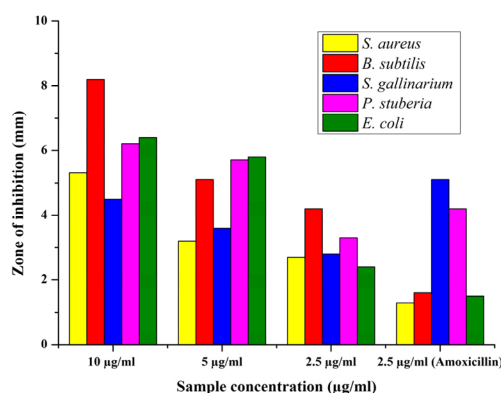

Fig. 4. Antibacterial activity of PSLASNPs sample

of the secondary metabolites exerted an extensive stabilizing effect on a lysosomal membrane and also possess the ability to bind caution leads to erythrocyte and other biological macromolecules stabilization.

Antibacterial activity

The PSLASNPs were more active against the Gram-negative bacteria as compared to the Gram-positive bacteria. This is likely due to the different membrane structures and compositions in the cell wall of the microorganisms [30]. Gram-positive bacteria have a thick layer of peptidoglycan in their cell wall, whereas, in Gram-negative bacteria, the cell wall contains a thin peptidoglycan layer [31]. The antibacterial assay of the PSLASNPs sample is summarized in Table 4 with standard amoxicillin. Fig. 4 and Fig. 5 exhibit the size of the zone of inhibition and antibacterial activity formed around the different concentrations (10, 05, 2.5 μg/ml, and Standard) of PSLASNPs. The PSLASNPs sample showed the best antibacterial activity at the concentration of 10 μg/ml against *Bacillus subtilis* (8.2 mm), followed by *Pseudomonas stuberia* (6.2 mm) and *Escherichia coli* (6.4 mm), *Staphylococcus aureus* (5.3 mm), *Staphylococcus gallinarium* (4.5 mm) respectively at the same concentrations (Plate 1). These results are in agreement with other findings in the literature where Gram-negative bacteria were more susceptible to AgNPs than Gram-positive bacteria [32,30]. In this study, the PSLASNPs also exhibited better antibacterial activity than the antifungal activity, which is consistent with previous findings [33,34]. The high antibacterial activity of AgNPs is due to their large surface area, which allows the nanoparticles to make better contact with the cell wall of microorganisms [35]. The capacity of AgNPs to bind to phosphorus- and sulfur-containing cell components, such as DNA and certain proteins, and cause their degradation is one of their additional antibacterial mechanisms. AgNPs also release silver ions into the bacteria cell wall, which can lead to bacterial death [36].

Photocatalytic activity of PSLASNPs sample
Crystal violet dye degradation

The degradation of crystal violet dye was carried out in the presence of PSLASNPs various times in the visible region. The absorption spectrum showed decreased peaks for crystal violet at 05, 10, 15, 20, 25, and 30,35 min time intervals. Initially, the absorption peaks at 560 nm (Fig. 6 and Fig.

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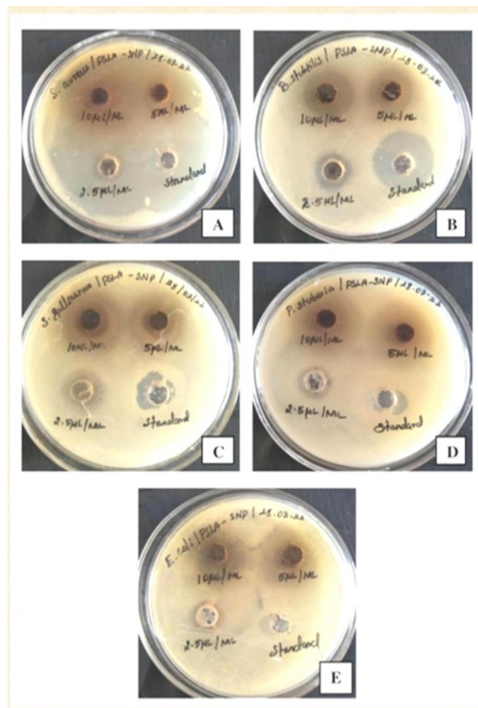

Fig. 5. Antibacterial activity of PSLASNPs sample

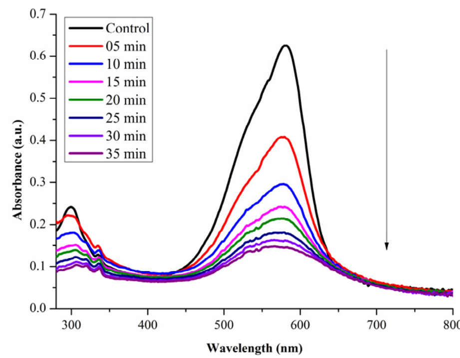

Fig. 6. Spectrum analysis of crystal Violet dye degradation of *Pogostemon speciosus* SNPs

Fig. 7. Crystal Violet dye degradation of *Pogostemon speciosus* SNPs at various time intervals

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7) for crystal violet dye decrease gradually with the increase of the exposure time and that indicates the photocatalytic degradation reaction of crystal violet. The completion of the photocatalytic degradation of the dyes is known from the gradual decrease of the absorbance value of dye approaching the baseline. Initial degradation of dye is indicated by a change in the color of the solution. The deep blue color of the dye changed into light blue after 25 min of incubation with silver nanoparticles while exposed to solar light. Finally, the degradation process was completed at 35 min and was identified by the change of reaction mixture color to colorless (Fig. 7). Using this catalytic degradation process, colored industrial effluent can be treated and made into a pollution-free environment. The photocatalytic efficiency of AgNPs reported in this study was significantly higher than many other findings reported in the literature [37,38,39]. As a result, the PSLASNPs synthesized in the study are the best choice for photocatalytic degradation of crystal violet, coomassie blue, and congo red.

Coomassie blue dye degradation

The photocatalytic activity of the PSLASNPs was determined using the detoxification of CB under sunlight for a particular time. Initially, the catalytic degradation of the dyes in the presence of SNPs was observed visually by the change in color. Because of their large surface area, silver nanoparticles can act as efficient photocatalysts in the electron transfer process [40]. When the valence electrons of AgNPs were exposed to UV/ solar irradiation, they gained energy and emitted from the valence shell. Following emission, these highly energetic electrons are used to generate hydroxyl radicals, which are responsible for dye decomposition [41,42,43,44]. The catalytic activity of biosynthesized PSLASNPs was analyzed for degradation of Coomassie blue under solar irradiation. Coomassie blue degradation was initially identified by visible color changes immediately after adding PSLASNPs. The blue color of the dye changed into light blue within 25 min of incubation with PSLASNPs under sunlight. The absorbance of Coomassie blue was observed at 600nm, which was identified by the change of reaction mixture color to colorless within 30 min (Fig. 8 and Fig. 9).

Congo red dye degradation

At different periods, the solar irradiated degradation of CR dye by PSLASNP nanoparticles was studied. The typical absorption peak of CR solution was observed to be 500 nm (Fig. 10and Fig. 11). In industries, the most commonly used dye is CR, a secondary diazo anionic dye. The carcinogenic metabolite benzidine of CR has been linked to bladder cancer in humans. It has also been discovered that CR effluents are highly colored and have a high chemical oxygen demand, as well as a high amount of dissolved solids [45]. Within 60 minutes of incubation, the peak intensity of CR had decreased. The adsorption of silver nanoparticles on CR solution was initially low and gradually increased with increasing time. The color change was the first indicator of dye degradation. Initially, the color of the dye shows red color changed into light red after 25 min of incubation with silver nanoparticles while exposed to solar light. Finally, after 35 mins the degradation process was completed and was identified by the change of reaction mixture color to colorless (Fig. 11). The probable mechanism of degradation could be attributed to the SPR effect where the excited surface electrons might interact with the dissolved oxygen molecules and ultimately produce hydroxyl radicals while allowing Ag^+ ions to interact with the anionic dye

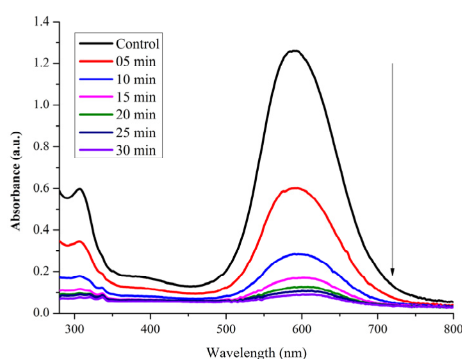

Fig. 8. Spectrum analysis of Coomassie blue dye degradation of *Pogostemon speciosus* SNPs

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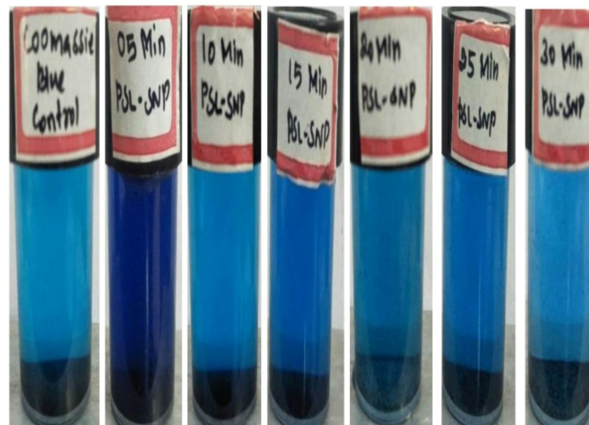

Fig. 9: Coomassie blue dye degradation of *Pogostemon speciosus* SNPs at various time intervals

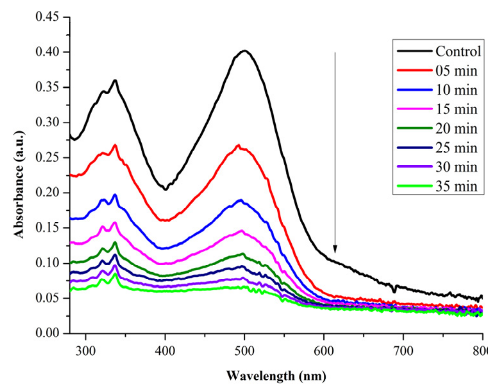

Fig. 10. Spectrum analysis of Congo red dye degradation of *Pogostemon speciosus* SNPs

Fig.11. Congo red dye degradation of *Pogostemon speciosus* SNPs at various time intervals

[46]. Hence, it is evident that the PSLASNPs sample is a highly potential photocatalytic agent for dye degradation in the presence of sunlight.

CONCLUSION

The leaf extract of the *pogostemon speciosus* plant was used successfully to synthesize silver nanoparticles that were economical, safe, non-toxic, and environmentally friendly. The characterization shows that the PSLASNPs sample was crystalline, coated with organics, polydispersed, and of various sizes. The *in-vitro* anti-inflammatory, antibacterial

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and photocatalytic activities from aqueous leaf extract were assessed, and the results indicated their effectiveness in the environment. Thus, PSLASNPs could have potential applications as anti-inflammatory, antibacterial, and photocatalytic activities in industrial traits. Greenly synthesized AgNPs can be used in biomedical applications, as a new drug combination.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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