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Optimization of the Biogenic Synthesis of Silver Nanoparticles and their Application in the Degradation of Dyes

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Abstract

Using plant extracts for the green synthesis of silver nanoparticles (AgNPs) is being recognized as a promising and environmentally sustainable approach. Plant extracts in producing AgNPs obviate cell culture maintenance and allow scalability in a non-aseptic setting. This work aims to identify the categories of phytochemicals and to conduct the biosynthesis and characterization of AgNPs utilizing extracts from the leaves and stems of Clinacanthus nutans. The present work involved the synthesis of AgNPs using aqueous extracts derived from the leaves (L) and stems (S) of C. nutans. This synthesis approach was chosen due to its non-toxic nature, cost-effectiveness, and environmentally favorable characteristics. The Transmission Electron Microscopy (TEM) examination revealed the presence of silver nanoparticles, denoted as AgNP-L and AgNP-S, which exhibited size ranges of 20 to 200 nm and 25 to 250 nm, respectively. The average sizes of AgNP-L and AgNP-S were determined to be 99.38 nm and 68.47 nm, respectively. The AgNP has shown remarkable degrading efficiency towards commercial dyes, namely Nile Blue (NB), with a removal rate of 94% within 110 minutes, and Reactive Yellow 160 (RY160), with a removal rate of 82% within 110 minutes. The extract derived from the Clinacanthus nutans exhibited considerably elevated concentrations of biologically active chemicals (Flavonoid and Phenolic). The utilization of biogenic AgNPs presents a significant prospect for the remediation of water resources polluted with industrial dye, owing to their notable reusability, photocatalytic effectiveness, and compatibility with environmentally friendly synthesis methods.

Keywords: *Biogenic synthesis, silver nanoparticles, dye degradation, Clinacanthus nutans, Nile Blue, Reactive Yellow.*

Introduction

In recent years, nanoparticles have become increasingly significant due to their wide-ranging utilization in research and technology. According to the established definition, these entities are minuscule particles with dimensions less than 100 nm in at least one axis [1]. The possession of distinctive biological, chemical, and physical characteristics by nanoparticles is attributed to their tiny dimensions, morphology, and spatial arrangement, which distinguish them from individual atoms and molecules. Metal nanoparticles have been widely investigated owing to their wide range of applications in several fields, such as catalysis, biosensing, medicine, and drug delivery. Silver nanoparticles (AgNPs) have garnered significant interest in the scientific community due to their distinct physicochemical characteristics [2].

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There have been reports indicating that colloidal silver particles exhibit notable antibacterial properties against both bacteria and viruses. In addition, these materials have notable catalytic capabilities that facilitate the degradation of harmful synthetic dyes, including methylene blue, congo red, methyl orange, and methyl red [3]. Furthermore, it has been demonstrated that they can be very effective detectors for identifying heavy metals such as mercury and copper ions. AgNPs can be synthesized using several techniques, including physical, chemical, and biological approaches. The utilization of reducing and stabilizing agents in physical and chemical synthesis procedures presents an environmental hazard since these agents have been identified as both hazardous and non-biodegradable [4].

The utilization of biological synthesis, also known as 'Green Synthesis', for the creation of AgNPs is regarded as an innovative method due to its several benefits, including its environmentally friendly characteristics, convenient production process, ability to be scaled up for large-scale synthesis, and absence of the need for dangerous chemical agents [5]. The biological technique primarily involves the utilization of plant extracts or microorganisms in the synthesis of AgNPs. However, when considering commercial practicality, the plant-mediated green synthesis of AgNPs is seen as a more cost-effective and safer option compared to microbe-assisted synthesis. This is because managing and sustaining microbial cultures may sometimes be laborious.

The green synthesis approach primarily involves the utilization of capping and reducing chemicals found in plant extracts to produce nanoparticles. Plant extracts may be obtained from many vegetative components, including stems, leaves, fruits, roots, and flowers. The above components serve as abundant reservoirs of reducing agents, including membrane proteins, phenols, flavonoids, and other secondary metabolites [6]. Furthermore, it should be noted that plant extracts possess capping agents, including extracellular tannic acids, peptides, and enzymes. The careful selection of plant material is crucial to acquire the desired mix of plant biomolecules. Numerous research studies have effectively conducted the synthesis of AgNPs by utilizing extracts derived from freshly harvested plant material such as Ocimum sanctum (commonly known as Tulsi), olive leaf, Amaranthus gangeticus (also referred to as Chinese red spinach), Datura stramonium, coffee, Azadirachta indica (neem), Matricaria recutita (commonly known as Babunah), Aloe vera, and other similar plant species. In addition, the utilization of fruit and vegetable wastes has been employed to synthesize AgNPs to address the significant environmental impact caused by these wastes [7].

C. nutans is well recognized as 'Belalai gajah' in Malaysia, Phaya yo or Phaya plongtong in Thailand, and Dandang gendis in Indonesia. Numerous bioactive substances derived from extracts of C. nutans have been identified and subjected to scientific investigation. Lupeol, isoorientin, orientin, isovitexin, schaftoside, vitexin, and β-sitosterol were extracted from the stem and leaf of C. nutans [8]. Additional beneficial compounds extracted from this plant include glucosides containing sulfur and derivatives of chlorophyll. In addition, the methanol extracts of the leaves included saponin, phenolics, flavonoids, diterpenes, and phytosterols. This research aimed to perform the biogenic synthesis of AgNPs using extracts from the leaves and stems of C. nutans and to optimize the factors involved in this synthesis process.

Related Works

The present review contains a comprehensive collection of research that centers around the sustainable production of AgNPs employing diverse plant extracts, as well as their efficacy in the photocatalytic degradation of colors.

Aslam et al. (2021) propose making silver nanoparticles from Sanvitalia procumbens aqueous extract [9]. This study uses a plant extract to reduce silver ions to make AgNPs. The implementation phase includes nanoparticle characterization, production method optimization, and photocatalytic degradation of azo dyes Orange G and Direct Blue-15.

AgNPs are characterized, synthesis parameters are refined, and the nanoparticles photocatalyst azo dye degradation. This approach uses eco-friendly plant-mediated synthesis and can be used in wastewater treatment. Potential drawbacks include plant extract composition inconsistencies and the need for adjustment to achieve desired results.

Plant extracts induce AgNP biogenic synthesis, according to Naseem et al. (2020). The authors also investigate using nanoparticles as catalysts to break down hazardous dyes [10]. AgNPs are synthesized by reducing silver ions with plant extracts. Quantifying nanoparticles and testing their catalytic degradation of hazardous dyes is part of the implementation. The values include silver nanoparticles, which have been shown to degrade dyes. The benefits of biogenic synthesis include eco-friendly catalytic applications. Potential drawbacks include plant extract diversity and careful regulation of reaction conditions.

Hashemi et al. (2022) propose a green AgNP synthesis method. Sambucus ebulus phenolic extract is used in this method. Additionally, the authors assess the potential applications of AgNP in various fields [11]. The method uses plant extract to produce AgNPs and improve synthesis greenly. The implementation phase involves the characterization of the nanoparticles, photocatalytic degradation of methyl orange, and in vitro antibacterial and anticancer testing. Well-described AgNPs, carefully adjusted synthesis conditions, and useful insights into their many uses are presented. The eco-friendly synthesis process and wide range of applications are benefits. Plant extracts' compositional diversity and need for careful adjustment may be drawbacks.

Kadam et al. (2020) used cauliflower waste to produce AgNPs environmentally. These NPs were then tested for photocatalysis of methylene blue dye and biosensing of Hg2+ ions. This study used plant waste as a precursor for silver nanoparticle synthesis to improve the process. The implementation phase involves NP characterization, photocatalytic testing, and Hg2+ biosensing [12]. Well-defined silver nanoparticles, refined production conditions, and dye degradation and biosensing applications are output. This approach's advantages are its environmentally sustainable synthesis and many environmental remediation applications. Variety in plant waste and the need to optimize it for certain purposes may be drawbacks.

Singh et al. (2019) proposed biogenic copper oxide NPs from plant extract. Researchers test nanoparticles' photocatalytic dye degradation efficacy [13]. Copper oxide NPs are made by reducing copper ions with a plant extract. Nanoparticles are characterized, and their photocatalytic dye breakdown efficacy is tested during implementation. The values describe copper oxide NPs and explain their dye degradation potential. Benefits include an environmentally sustainable synthesis approach and wastewater treatment implementation. Unpredictable plant extract content and dye type optimization may be drawbacks.

Mat Yusuf et al. (2020) optimized the biogenic manufacturing process for AgNPs using aqueous extracts from Clinacanthus nutans leaves and stems, which contain high flavonoids [14]. The method uses plant extracts to synthesize AgNPs and refine the process. Implementation involves nanoparticle characterization and finding the best synthesis conditions. The results show well-defined AgNPs, refined synthesis parameters, and insights into synthesizing flavonoid-rich plant extracts. A sustainable, plant-mediated synthesis method has many benefits and applications. Plant extracts may have drawbacks like their heterogeneity and the need for careful adjustment to achieve desired results.

In conclusion, optimizing the biogenic production of silver nanoparticles through the utilization of plant extracts and their subsequent application in the degradation of dyes represents a highly promising and ecologically sound methodology that holds significant promise across a range of applications. Researchers are now engaged in extensive investigations into various plant sources, optimization methodologies, and applications, with a primary emphasis on augmenting the efficiency and scalability of these procedures.

Biogenic Synthesis of Silver Nanoparticles and their Application in the Degradation of Dyes

Collection of C.nutans

The dried C. Nutans Leaves (CN-L) and Stems (CN-S) were acquired from Botani Sdn. Bhd, located in Manjung, Perak. Clinacanthus nutans was authenticated using voucher specimen No. 11465, which was subsequently placed in the Herbarium Unit of the School of Biological Sciences at Universiti Sains Malaysia. The dried leaves and stems were ground into a fine powder using an Ultra Centrifugal Mill ZM200 grinder manufactured by Retsch in Haan, Germany. Subsequently, the powder was hermetically enclosed within a glass container and stored at ambient temperature until further utilization.

Preparation of Plant Extract

The extraction methodology was derived from a prior investigation, incorporating some adjustments. In this experiment, 45 grams of finely powdered CNL was subjected to marinating in 550 milliliters of distilled water (dH_2O). The aqueous extract was subsequently filtrated and lyophilized using the EYELA FDU 1200 freeze-drying system. The specimen was appropriately labeled, accurately measured, and stored in a desiccator for future utilization. The determination of the percentage yield of the aqueous extract has been carried out using equation (1). The protocols mentioned above have been repeated for the powdered CNS samples.

$$\%\ yield = \left\{\frac{[m_2 - m_1]}{m_0}\right\} \times 100\% \quad (1)$$

where m_2 denotes the mass of the container and dried extract, m_1 denotes the mass of the container and m_0 is the mass of the original dried sample. A qualitative examination of phytochemicals was conducted, specifically targeting tannins, saponins, alkaloids, proteins, carbohydrates, and flavonoids. The results of each test were categorized as either negative (-) or positive (+) responses.

Overall Phenolic Concentration (OPC)

The Folin–Ciocalteu technique was derived from a prior investigation and subsequently modified. In summary, the extracted sample was made at a concentration of 1 mg ml-1 using deionized water (dH2O). Subsequently, 25 µl of the sample was combined with 155 µl of a diluted Folin–Ciocalteu's phenol solution (10% v/v) in a 95-well plate. The resulting mixture was allowed to incubate at room temperature for 10 minutes. Subsequently, 125 µl of sodium carbonate (7% weight/volume) was introduced into the solution, followed by an incubation period of 20 minutes in a light-restricted environment. The absorbance measurement was conducted at a wavelength of 770 nm using the FLUOstar Omega microplate reader. OPC was quantified by determining the concentration in micrograms of Gallic Acid Equivalent (GAE) per milligram of extract, utilizing the GAE standard curve.

Overall Flavonoid Concentration (OFC)

OFC was determined using the aluminum chloride technique, with several changes included. In summary, the preparation of the extract involved the dissolution of 1 milligram of CNL or CNS in 1 milliliter of dH_2O. Subsequently, 20 µl of each extract was combined with 120 µl of deionized water and 7 µl of sodium nitrate (5% weight/volume). The solution was subjected to incubation for 10 minutes at ambient temperature. Subsequently, a volume of 20 µl of an $AlCl_3$ solution with a concentration of 15% w/v was introduced into the combination, which was then allowed to remain at ambient temperature for 5 minutes. Following this, 55 µl of a NaOH solution with a concentration of 1M, along with 30 µl of dH_2O, were included in the mixture. The solution was subjected to incubation in a light-restricted environment for 25 minutes. The absorbance measurement was conducted at a wavelength of 515 nm using a microplate reader. The OFC was quantified in

micrograms of Quercetin Equivalents (QE) per milligram of extract, with quercetin as the reference standard curve.

Biogenic Synthesis of Silver Nanoparticles

The sequential methodology for the manufacture of biogenic silver nanoparticles (AgNPs) is depicted in Fig. 1. The process of synthesizing AgNPs started by combining 90 ml of a 1.5mM solution of silver nitrate ($AgNO_3$) with 10 ml of CNL extract, which had a concentration of 1 mg/ml in deionized water. The mixture was subjected to continuous stirring for 24 hours. The occurrence of AgNPs was evidenced by the observed alteration in color, transitioning from a yellow hue to a dark brown shade. Following 24 hours, the combination underwent centrifugation at a speed of 6500 revolutions per minute for 20 minutes. Subsequently, the mixture was subjected to three rounds of washing using DI water. The pellets were dried in an oven set at 45°C for further characterization. An identical methodology was employed for the CNS extract.

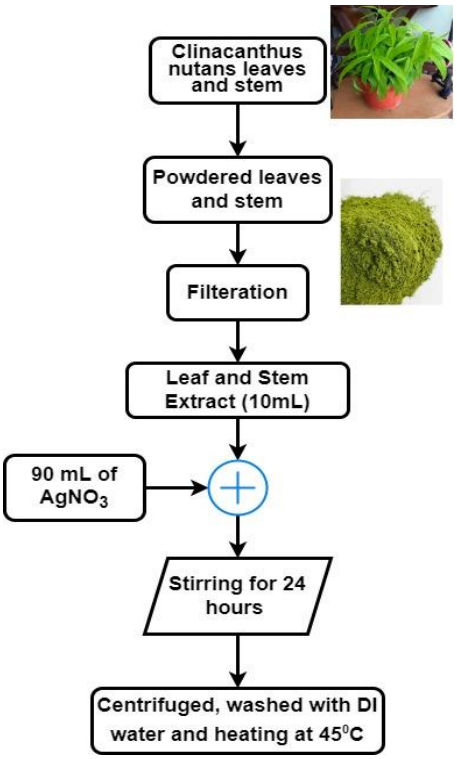


Figure 1: Biogenic synthesis of silver nanoparticles

The study investigated many parameters that impact the production of AgNPs, including the concentration of extract and $AgNO_3$, as well as the incubation duration and temperature. Table 1 displays the parameter conditions that were employed in the optimization investigation.

Table 1: Parameters for optimization

Parameter	Variable
Concentration of the extract	5,10,15 & 20% v/v
$AgNO_3$ concentration	1.5, 3.5, 5.5 mM
Temperature	25℃, 45℃, and 65℃
Time	20 minutes, 30 minutes, 2,4,8,12,18,20,22, and 24 Hours

Photocatalytic Degradation of Dyes

In a standard experimental procedure, a solution consisting of 25 mL of NB dye with a concentration of 1.2×10^{-5} mol/L and 4.6×10-5 mol/L of RY160 dye (both at a concentration of 40 ppm) in DI water was subjected to stirring. This solution was combined with 10 mg of biogenic AgNPs to achieve adsorption-desorption equilibrium. The entire process was

conducted in darkness and allowed to proceed for one hour. It should be noted that the alteration in dye concentrations was employed to ascertain the extent of dye adsorption in the absence of light. Subsequently, the resultant solution was exposed to direct sunshine. The photocatalytic experiment was conducted with a solar flux of 635 W/m^2. The measurement of solar flux was conducted using a Pyranometer. At regular intervals of 20 minutes during the reaction, a 2 mL sample was obtained and subjected to centrifugation at a speed of 6500 revolutions per minute for 10 minutes. The residual liquid was subjected to analysis using a UV-visible spectrophotometer. The maximum wavelengths (β_{max}) observed for the NB and RY160 compounds were 630 nm and 425 nm, respectively. The assessment of dye absorbance accomplished the quantification of the residual dye concentration subsequent to the photocatalytic experiment. It should be noted that the absorbance of a solution is directly proportional to its concentration. In addition, control tests were conducted to assess the effects of the catalyst, both in the presence and absence of it. These experiments were afterward examined using a UV-visible spectrophotometer. The below equation was employed to ascertain the efficiency (γ) of photocatalytic degradation of AgNPs:

$$\gamma = [(\mu_0 - \mu_t)/\mu_0] \times 100 \quad (2)$$

The variables μ_0 and μ_t represent the absorbance of a dye at the initial time $t = 0$ and at a subsequent time $t = t$, respectively. To evaluate precision, three separate tests were conducted to analyze the degradation of NB and RY160. These experiments were carried out simultaneously in three distinct reaction containers under comparable experimental circumstances. Both dyes were given a degradation time of 2 hours. The calculations for the Limit of Detection (LOD) and Limit of Quantification (LOQ) have been calculated as follows:

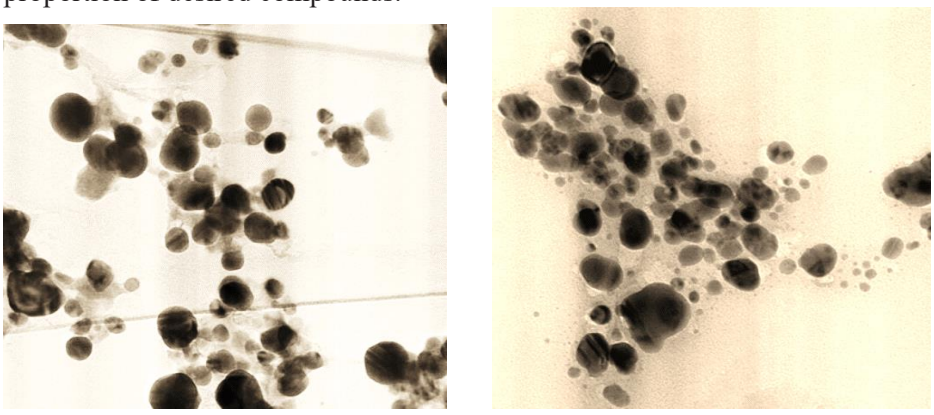
$$LOD = M_{bl} + 3SD_{bl} \quad (3a)$$

$$LOQ = M_{bl} + 10SD_{bl} \quad (3b)$$

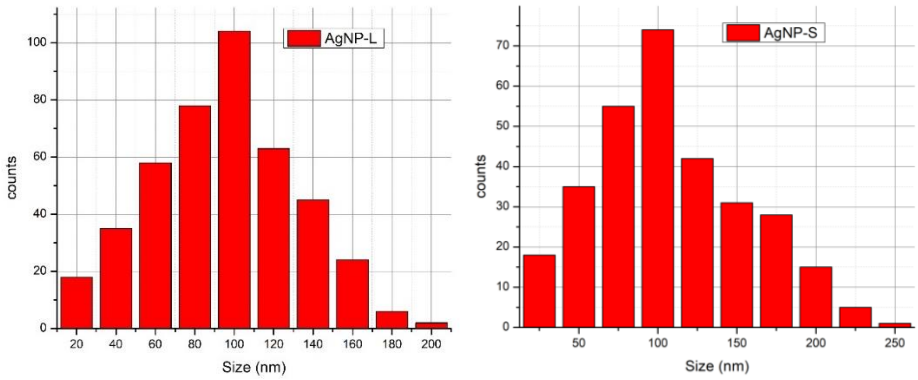
Where M_{bl} and SD_{bl} denotes the mean and standard deviation of the blank. The LOD values for NB and RY160 were determined to be 5 and 10 mg/L, respectively. The LOQ values for NB and RY160 were 8 and 17 mg/L, respectively.

Results and Discussion

The leaf and stem extracts of C. nutans were obtained using the maceration technique at ambient temperature. The leaf extract demonstrates a percentage yield of 26.42%, whereas the stem extract exhibits a percentage yield of 14.34%. The maceration method was chosen due to the presence of thermolabile chemicals in plants, making it a viable technique for large-scale applications. Thermolabile substances undergo degradation or decomposition with prolonged exposure to heat. For this investigation, DI water was selected as the extraction solvent due to its widespread availability and ability to generate a high proportion of desired compounds.



(a) (b)



(c) (d)

Figure 2: TEM analysis of (a) AgNP-L, (b) AgNP-S, and size range of (c) AgNP-L and (d) AgNP-S

Fig. 2 depicts the TEM analysis of (a) AgNP-L, (b) AgNP-S, and the size range of (c) AgNP-L and (d) AgNP-S. TEM analysis revealed the presence of silver nanoparticles, denoted as AgNP-L and AgNP-S, which exhibited size ranges of 20 to 200 nm and 25 to 250 nm, respectively. The average sizes of AgNP-L and AgNP-S were determined to be 99.38 nm and 68.47 nm, respectively. The data presented exhibits a diverse array of particle sizes, wherein most nanoparticles are observed to fall between the range of 60 to 100 nm. This particular size range together represents a substantial proportion of the overall distribution. The maximum number of AgNP-L, totaling 104, is detected at a size of 100 nm, indicating a prominent peak in the size distribution at this particular dimension. Nanoparticles of smaller dimensions, around 40 nm, and those of higher dimensions, ranging from 120 to 140 nm, significantly contribute to the overall dispersion. Nevertheless, as the dimensions surpass 140 nm, there is a notable decline in the quantity of NPs, accompanied by a limited presence of particles ranging from 160 to 200 nm. This observation indicates a gradual reduction in the overall size distribution. The observed distribution exhibits a distinct peak of 100 nm, characterized by the greatest frequency count of 74 AgNP-S. This finding suggests a notable concentration of NPs within this particular size range.

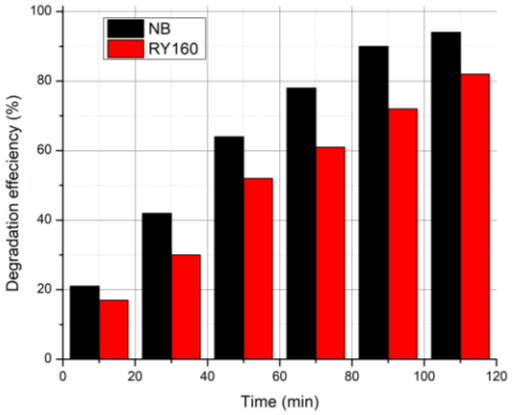


Figure 3: Photocatalytic degradation efficiency (%) of AgNP for the removal of NB and RY dyes

Fig. 3 presents data on the efficacy (%) of AgNP (Silver Nanoparticles) in the process of photocatalytic degradation, specifically in the removal of NB (Nile Blue) and RY160 (Reactive Yellow 160) dyes. The data is organized based on different time intervals measured in minutes. The data indicates that with time, there is a notable enhancement in the degrading efficiency of AgNP for both dyes. After a duration of 10 minutes, NB's degradation efficiency is 21%, but RY160 exhibits a little lower degradation efficiency of

17%. Nevertheless, when the duration is extended to 110 minutes, the degrading efficiency of NB exhibits a significant increase to 94%, but for RY160, it experiences a rise to 82%. The findings suggest that the AgNP photocatalyst has enhanced efficacy as the exposure period increases, leading to significant dye degradation. Notably, NB exhibits superior efficiency throughout the whole duration of the experiment.

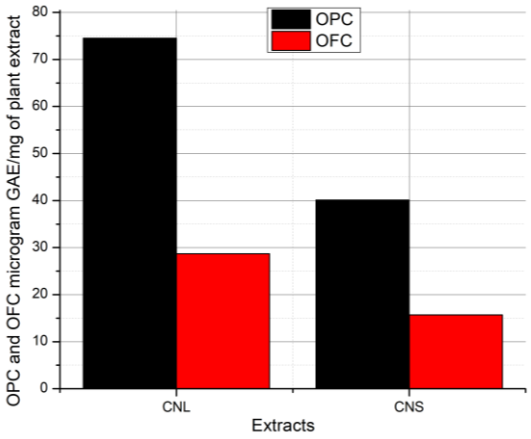


Figure 4: OPC and OFC of CNL and CNS extracts

Fig. 4 presents a comprehensive comparison of the OPC and OFC in extracts obtained from the leaves and stems of Clinacanthus nutans, namely CNL and CNS. The data indicates that the CNL extract demonstrates a much higher OPC of 74.5 units, in contrast to the OPC of 40.1 units for the CNS extract. Similarly, the CNL extract has a notably higher OFC (optical flow correlation) value of 28.7 units, in contrast to the OFC value of 15.7 units observed in the CNS extract. The findings highlight a significant disparity in the phenolic and flavonoid content between the extracts obtained from the leaves and stems. Notably, the extract derived from the CNL exhibited considerably elevated concentrations of these biologically active chemicals.

Conclusion

This paper aims to categorize phytochemicals and perform the biosynthesis and characterization of silver nanoparticles (AgNPs) using extracts derived from the leaves and stems of Clinacanthus nutans. The current study focused on synthesizing AgNPs utilizing aqueous extracts obtained from the leaves (L) and stems (S) of C. nutans. This synthesis technique was selected based on its non-toxic properties, cost-effectiveness, and ecologically advantageous attributes. The utilization of TEM in the analysis unveiled the existence of AgNPs, specifically AgNP-L and AgNP-S, which displayed size distributions ranging from 20 to 200 nm and 25 to 250 nm, respectively. The mean sizes of AgNP-L and AgNP-S were measured to be 99.38 nm and 68.47 nm, correspondingly. The AgNP has demonstrated a notable capacity for degradation of commercial dyes, namely NB with a removal efficiency of 94% within 110 minutes and RY160 with a removal efficiency of 82% within the same time frame. The sample obtained from the C. nutans plant had much higher levels of bioactive compounds, specifically flavonoids and phenolics. The application of biogenic AgNPs has great promise for purifying water contaminated with industrial dyes. This is due to their remarkable capacity to be reused, their high efficiency in photocatalysis, and their compatibility with ecologically sustainable synthesis techniques.

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