



CHEMICAL SCIENCES

Novel biosynthesis of silver nanoparticles catalytically active using extract of non- native species (*Eragrostis plana* Nees) of Brazilian Pampa

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Abstract: Novel silver nanoparticles (AgNPs) were synthesized using biomass from *Eragrostis plana* Nees, commonly known as Tough lovegrass, an invasive grass species in the Brazilian Pampa. AgNPs were synthesized in a one-step synthesis using the extract as a reducing agent for Ag⁺ ions and stabilizing agent. The results showed that the colloidal solution of AgNPs had a surface plasmon resonance (SPR) band at 410 nm. Through vibrational analysis, they showed that the OH, NH, CONH₂, COC, COOH, and CO present in the extract were associated with reducing Ag⁺ ions. The synthesized nanoparticles were crystalline and, through morphological analysis, showed that the AgNPs produced had different shapes, with an average diameter of 23 ± 8 nm. The biosynthesized AgNPs exhibited high catalytic activity for reducing Toluidine blue (TB). The total reduction of TB with AgNPs in the presence of NaBH₄ at 25 °C occurred after only 5 min of reaction.

Key words: AgNPs, botanical extract, nanocatalysis, toluidine blue, tough lovegrass.

INTRODUCTION

Synthetic dyes such Toluidine blue an azo dye mainly used in industries for several applications and well known as carcinogenic and toxic to aquatic life (Benkhaya & El- Harfi 2018, Filho et al. 2017). Therefore, the development of efficient methods to remove these contaminants is therefore crucial.

The reduction process in the presence of metal nanoparticles and sodium borohydride (NaBH₄) has been demonstrated as a rapid and effective method for removing organic dyes from water (Ali et al. 2024, Begum et al. 2019). Among the metal nanoparticles employed for this end, AgNPs have gained particular interest due to their good stability and high catalytic activity (Ahmed et. al 2023, Hossain et al. 2022).

Traditionally, AgNPs are manufactured with success by chemical and physical routes of synthesis (Aboyewa et al. 2021, Nguyen et al. 2023). Using these approaches of synthesis, the AgNPs are commonly produced using solvents and synthetic chemical products or use high quantities of energy that may have specific toxicity capable of generating hazardous residues during the synthesis process (Harish et al. 2022, Ramrakhiani & Ghosh 2018). Intending to reduce the use of dangerous toxic substances during the production of metallic nanoparticles (MNPs) that can damage both humans and the environment, some researchers have now preferred ecologically friendly synthesis (Bhardwaj et al. 2020, Khan et al. 2022, Koçer & Özçimen 2025, Pattanayak et al. 2022).

Plant-based nanoparticle synthesis (biosynthesis) is gaining attention among the existing methods to produce MNPs (Zulfiqar et al. 2024, Abada et al. 2024, Vijayaram et al. 2024). Brazil has continental dimensions with a rich endemic biodiversity of plants with potential applications in MNPs manufacture. Still, many of these plants have not yet been exploited for this purpose, as is the case of *Eragrostis plana* Nees.

Eragrostis plana Nees belongs to the Poaceae family is an invasive, no commercial value, non-toxic, renewable, and fast-growing grass commonly found in pastures in the south of Brazil (Cimirro et al. 2020). Previous phytochemical studies showed that the aqueous extract of *Eragrostis plana* Nees leaves contains phenolic compounds (ellagic acid, caffeic acid, chlorogenic acid, gallic acid), flavonoids (rutin, quercetin), and tannins (epicatechin and catechin), which are typically related to trigger the process of biosynthesis of MNPs (Fiorenza et al. 2016). To our best knowledge, there is no report about using the extract of *Eragrostis plana* Nees leaves for the green synthesis of MNPs (e.g., AgNPs). Grass-mediated biogenic synthesis of metal nanoparticles is still poorly explored. The biosynthesis of AgNPs was achieved through an innovative single-step process, highlighting its simplicity and eco-friendliness.

The present study proposes the biosynthesis of AgNPs using an aqueous extract of *Eragrostis plana* Nees leaves. The AgNPs biosynthesized were characterized by ultraviolet-visible spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FT-IR), x-ray diffraction analysis (XRD), Transmission electron microscope (TEM) and energy-dispersive x-ray spectroscopy (EDS) techniques. Furthermore, their catalytic efficacy was evaluated for reducing Toluidine blue (TB) dye in aqueous solutions in the presence of NaBH_4 at 25 °C.

MATERIALS AND METHODS

Material

Silver nitrate (AgNO_3 ; 99.8%), Toluidine blue (TB) and sodium borohydride (NaBH_4) were acquired from Sigma Aldrich, Brazil. All the other chemicals used were of analytical grade. The double distilled water was used as a medium for all experiments.

Plant material

The *Eragrostis plana* Nees (Fig.1) plant sample were collected on the farm of the Federal University of Pampa in Bagé, Brazil. The biomass was authenticated at the Brazilian Agricultural Research Corporation (EMBRAPA, Brazil) of Herbarium, and voucher number UILH/001/1263 was assigned to it. The leaves were manually separated from the other parts of the plant, washed with running tap water to remove dirt particulates adhering to it, and dry in an oven at 60 °C for 48 h. After drying, it was grinded.

Preparation of the extract

To prepare aqueous extract, 2 grams of dry powdered leaves were boiled in 100 mL of distilled water for 10 min. The mixture was filtered and the resulting extract was immediately used to synthesize AgNPs.

Biosynthesis of AgNPs

In a typical experiment, 10 mL of prepared plant extract was mixed with 50 mL of AgNO_3 (1mM) for 240 min, when it was observed clearly a change in the coloration of the mixture, indicating the formation of AgNPs.

Characterization of AgNPs

The absorption spectrum of AgNPs, UV-Vis was recorded using a spectrophotometer (Agilent, Cary 50 Bio, USA). The functional groups responsible for nanoparticle biosynthesis were identified using Spectrum-Two FT-IR spectrophotometer



Figure 1. Image of the *Eragrostis plana* Nees plant.

(PerkinElmer, USA), combined with the ATR accessory. The FT-IR spectrum was taken in the wavenumber region of 400–4000 cm^{-1} with a resolution of 4 cm^{-1} , accumulating 32 scans. To evaluate the phase formation of nanoparticles, X-ray diffraction (XRD) analysis of AgNPs, was performed using a Rigaku X-Ray diffractometer (Model ULTIMA IV) supplied with a $\text{CuK}\alpha$ radiation source ($\lambda = 1.5418 \text{ \AA}$) and Bragg-Brentano geometry operating at a voltage of 40 kV, current of 30 mA, to obtain information about. The 2θ angles were registered in the range of 20° - 80° at room temperature. The crystallite size of nanoparticles was calculated using Scherer' formula (Fardood et al. 2018). The morphology and size were analyzed by using a TEM (JEOL JEM 1400 microscope), operating at an acceleration voltage of 100 kV. The silver was examined using SEM coupled with an EDS microprobe, performed on a JEOL JSM 6610LV

instrument. The samples were metallized with gold sputtering, using equipment from Dentun Vacuum, where the samples were exposed for 120 s to a current of 50 mA.

Catalytic degradation of Toluidine blue

The catalytic activity of biosynthesized AgNPs, was tested on reduction of Toluidine blue (0.09 mM) dye in the presence of NaBH_4 at 25°C . The reduction of dyes is carried out by adding 0.5 mL of NaBH_4 (0.1M), 2.5 mL of dye and 100 μL of AgNPs at pH of 7 (maintained using the natural pH of the plant extract). The control experiment was also carried out without AgNPs. The rate reduction of dye was monitored in a UV-Vis spectrophotometer at defined intervals of time and wavelength range between 300-750 nm. The residual dyes were calculated at fixed intervals by determining the absorbance peaks with wavelength (λ_{max} value) at 620 nm for TB. A decline in the color intensity of the dye solutions was taken as evidence of the successful reduction processes. The kinetics reduction of dye was calculated using the pseudo-first-order model expressed in eq. (1) (Biswas et al. 2017).

$$\ln \left(\frac{A}{A_0} \right) = -k * t \quad \text{eq. (1)}$$

Where A_0 was the initial absorbance of dye at zero time, A_t is the apparent absorbance at the time "t" at $\lambda_{\text{max}} = 620 \text{ nm}$ for TB dye, while, k is the pseudo-first-order rate constant, and t is time in minutes.

RESULTS AND DISCUSSION

Biosynthesis of AgNPs

During the synthesis of AgNPs, the color of the reaction mixture containing *Eragrostis plana* Nees leaves extract and aqueous silver nitrate solution changed from yellow to brown (Fig. 2). This change in the color reaction mixture confirms the reduction of Ag^+ ions to Ag^0 the

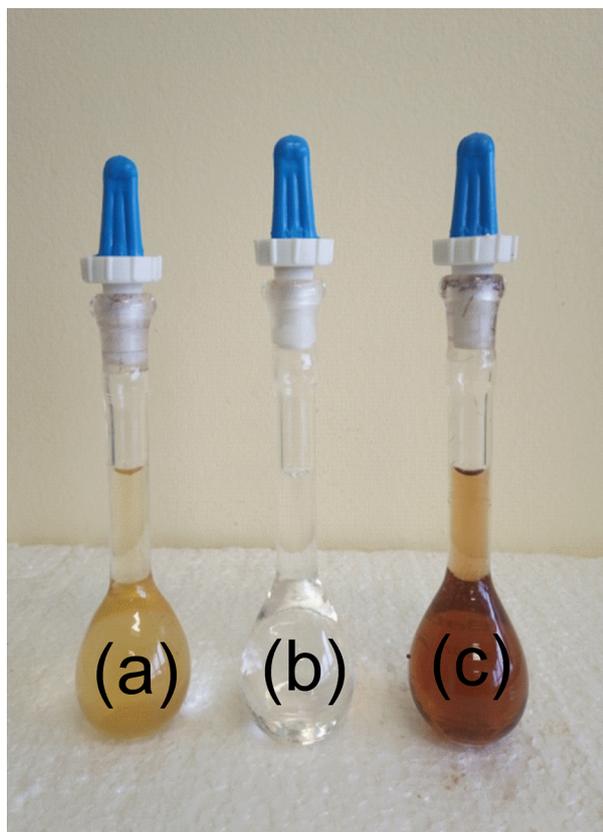


Figure 2. a) *Eragrostis plana* Nees leaves extract, b) 1mM AgNO₃ solution and c) biosynthesized AgNPs.

by the extract (John et al. 2019, Singh et al. 2018). The water-soluble phytochemicals such as phenols, flavonoids, and tannins present in aqueous *Eragrostis plana* Nees leaves extract acted as reductants of Ag⁺ ions, promoting the formation of the nanoparticles (Velgosova et al. 2024).

UV-vis spectra analysis

The UV-Vis spectroscopy analysis was employed for to confirm the formation of AgNPs, these results are presented in the Fig. 3. The Fig. 3 shows that colloidal nanoparticle suspension exhibited a typical absorption band with wavelength (λ_{\max} value) centered at 410 nm, corresponding to SPR of AgNPs, proving the occurrence of AgNPs formation (Flieger et al. 2021). The SPR peak remained practically unchanged after

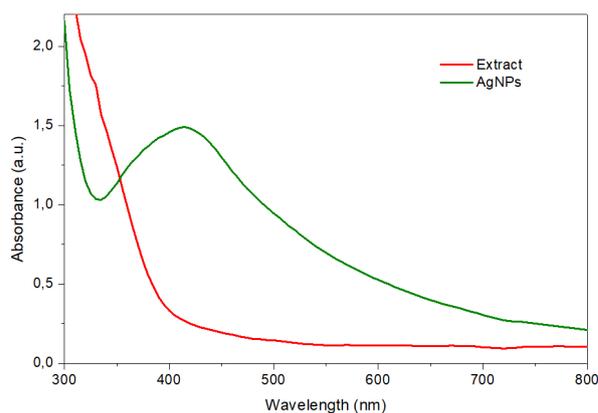


Figure 3. UV-Vis spectrum of the AgNPs

four months, indicating no notable variation in optical properties and probably structural features of the AgNPs.

FT-IR analysis

The FT-IR analysis was carried out to identify the nature of biomolecules responsible for the reduction and capping of silver nanoparticles. The FT-IR spectrum of AgNPs is presented in Fig. 4. In Fig 4 is possible to observe a strong band at 3288 cm⁻¹ in the spectrum was attributed to alcohol and phenol groups (-OH) (Cimirro et al. 2020). The minor band at 2965 and 2915 cm⁻¹ region indicates alkane compounds (-CH) (Xiao et al. 2017). The band at 1633 cm⁻¹ relates to C-N and C-C stretching is a characteristic of proteins (Varadavenkatesan et al. 2019). The band at 1454 cm⁻¹ was assigned for N-H stretch vibrations present in the amide bonds of the proteins. The band 1375 cm⁻¹ can be due to the asymmetrical and symmetrical stretching of carboxylate ions (-COO-) (Raghupathy et al. 2024). The band at 1037 cm⁻¹ corresponds to the ethers (-COC), ester (-COO), and/or carboxylic (-COOH) groups (Younus 2018). The FT-IR results is confirmation that different functional groups are involved in the bioreduction mechanism of AgNPs. Furthermore, these results suggest that AgNPs

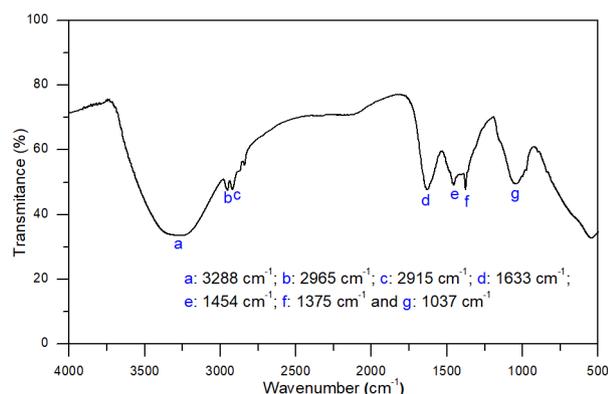


Figure 4. FT-IR spectrum of the AgNPs.

might be covered with a layer of phytochemicals derived from *Eragrostis plana* Ness.

XRD analysis

The XRD analysis was employed to explore the crystalline nature of biosynthesized nanoparticles (Fig. 5). XRD showed four distinct Bragg peaks at $2\theta = 38^\circ, 46^\circ, 65^\circ$ and 78° values, which correspond to the (111), (200), (220) and (311) planes, respectively. All these 2θ values obtained are in concordance with the lattice constant (JCPDS card no. 4-0783) for the standard spectrum of pure silver metal. The characteristic diffraction peaks observed in XRD confirm the crystalline nature of the biosynthesized of AgNPs (Das et al. 2024). The crystallite size of AgNPs calculated using Scherrer's formula was found to 11 nm.

TEM and SEM/EDS analyses

In the TEM analysis was conducted to investigate the morphology and size of AgNPs (Fig.6). The micrographic image in Fig. 6a displays that particules are well dispersed and spherical, elliptical, and hexagonal in forms. TEM-based particle size distribution histogram (Fig. 6b) shows that the diameters of AgNPs vary from 10 to 40 nm, with an average particle size of 23 ± 8 nm.

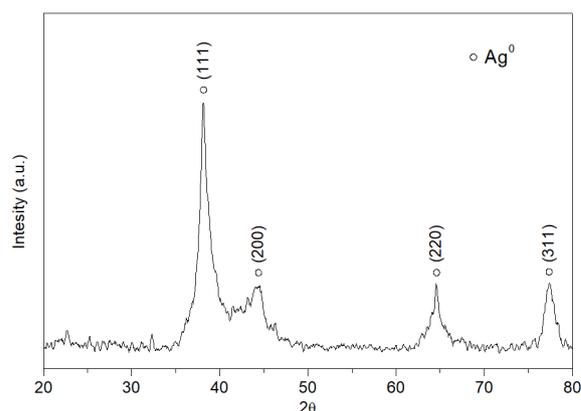


Figure 5. XRD pattern of the AgNPs

SEM/EDS analysis was performed to investigate the elemental composition of AgNPs, as shown in Fig. 6c. The EDS of AgNPs presented in Fig. 6c displayed a typical peak of Ag at 3 keV, associated with the reduction of Ag^+ to Ag^0 (Bharathi et al. 2023). The other signals, C and O atoms, may be related to X-ray emissions produced by the phytochemicals in *Eragrostis plana* Nees. Based on EDS analysis, the percentage relative composition of Ag metal found was 65% in occurrence with other chemical elements such as C (2%) and O (18%).

Catalytic degradation of Toluidine blue

The catalytic action of the AgNPs was investigated to reduce Toluidine blue (TB) from aqueous media in the presence of NaBH_4 at 25°C and pH7. The absorption spectra of TB ($\lambda_{\text{max}} 620$ nm) reductions at different reaction times are shown in Fig. 7. As can be seen in Fig.7a, the reduction of TB by AgNPs was completed within and 5 min of reaction. For comparison, a similar test was performed without the use of a nanocatalyst. Fig.7b shows the UV-visible absorption spectra profiles, referring to the reduction of TB only with NaBH_4 . Based on the results obtained, it is obvious that NaBH_4 cannot reduce dyes without the presence of AgNPs. Studies involving AgNPs, NaBH_4 and dyes demonstrate evidence of a

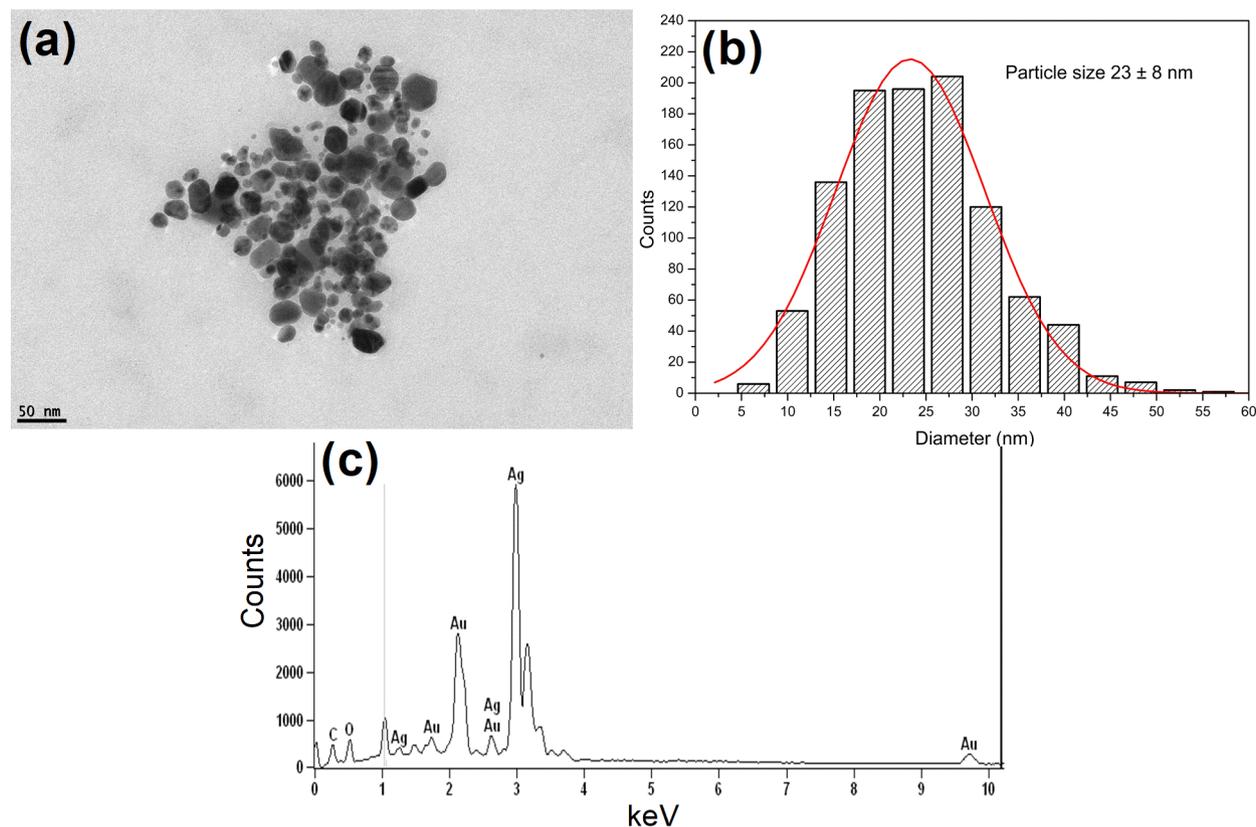


Figure 6. TEM image of the (a) AgNPs, (b) particle size distribution histogram and (c) EDS spectrum of the AgNPs

coupled adsorption/reduction mechanism (Filho et al. 2023). AgNPs act as promoters of the reduction reactions by accelerating electron transfer. NaBH_4 , on the other hand, is a strong reducing agent that provides the essential BH_4^- ions for the reaction. The combination of these two components creates a synergistic system where the AgNPs facilitate the adsorption of both the dye and the reducing agent (Asmare et al. 2022). Research on the catalytic degradation of toluidine blue (TB) using silver nanoparticles (AgNPs) is still limited. One relevant study by Aranaz et al. (2019) explored the reduction kinetics of TB with colloidal AgNPs synthesized using low molecular weight chitosans. The ability of four sets of silver nanoparticles to reduce TB was used as a test. In their work, a known volume (5-20 μL) of AgNPs and 200 μL of

0.3 M NaBH_4 (three times more concentrated and in a larger volume than in our study) were used. Under these experimental conditions, complete TB reduction for the F3L-AgNps sample is achieved after more than 10 minutes of reaction, which is twice the duration of our experiment. This comparison underscores the excellent catalytic activity exhibited by biosynthesized AgNPs with extract of *Eragrostis plana* Nees. The kinetics reduction of TB was monitored and calculated using the pseudo-first-order model (Eq.1). The results of the linear regression plot of the logarithm of absorbance ($\ln A_t/A_0$) of TB versus reaction time (t) are presented in Fig. 7c, and reveal an excellent linear correlation between the plot of $\ln A_t/A_0$ versus t . The rate constant (k) value determined for TB ($k=0.0151\pm 0.00635 \text{ min}^{-1}$) only with NaBH_4 was

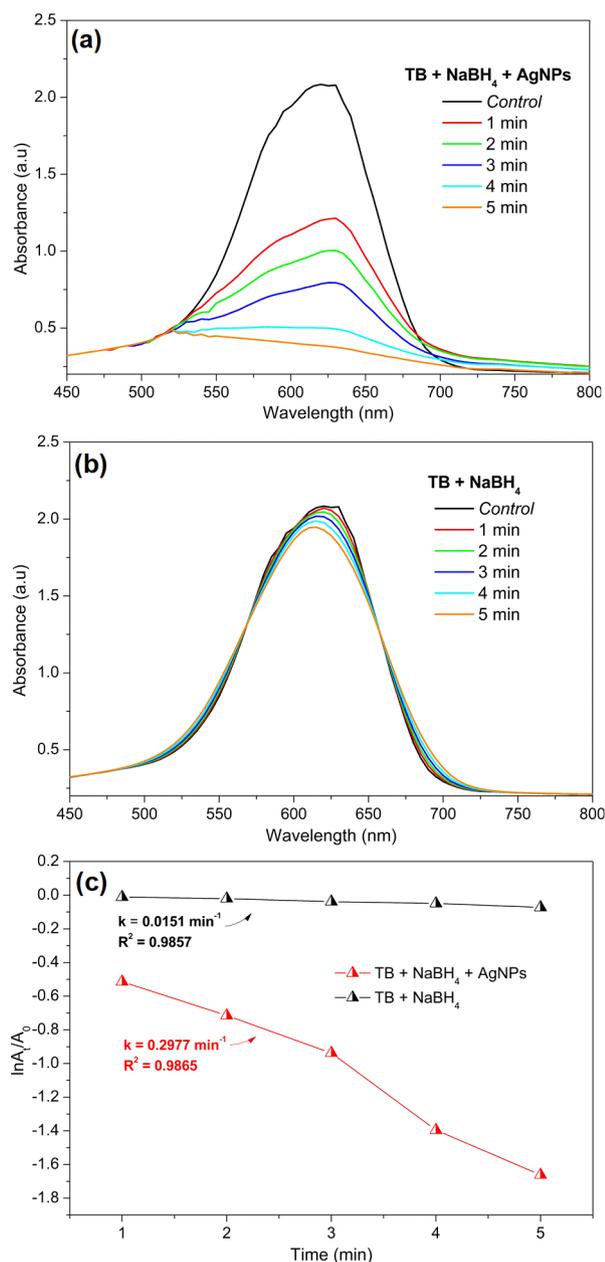


Figure 7. UV-Vis spectra of catalytic reduction of (a) TB in the presence of AgNPs, (b) TB in the absence of AgNPs (c) kinetic plots ($\ln A_t/A_0$) versus time.

significantly smaller when compared with the k value obtained for TB ($k = 0.2977 \pm 0.0330 \text{ min}^{-1}$) in the presence of AgNPs. These data demonstrate that TB reduction reactions only with NaBH_4 was slower when compared in the presence of green nanocatalyst.

CONCLUSIONS

For the first time, this study highlights the potential of *Eragrostis plana* Nees, an invasive plant species, as a renewable and environmentally friendly source for producing silver nanoparticles (AgNPs). The successful synthesis of AgNPs was confirmed through various analytical techniques, including UV-Vis spectroscopy, FT-IR, XRD, TEM, and SEM-EDS, ensuring the method's reliability and consistency. FT-IR analysis identified water-soluble phytochemicals such as phenols, flavonols, and tannic acid in the aqueous extract of *Eragrostis plana* Nees, which facilitated the reduction of silver ions to metallic silver. Characterization techniques, including UV-Vis, XRD and TEM, revealed that the biosynthesized nanoparticles featured a surface plasmon resonance (SPR) band at 410 nm, a crystalline structure, and diverse morphological forms. AgNPs efficiently catalyzed the reduction of the azo dye Toluidine Blue (TB) in the presence of NaBH_4 , completing the process within 5 minutes. The reaction followed a pseudo-first-order kinetic model with a rate constant (k) of 0.2977. In future studies, the extract of *Eragrostis plana* Nees can be an alternative to synthesizing other metallic nanoparticles (MNPs) with catalytic potential for azo dyes.

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VALESKA M. SCURO: research, funding acquisition and formal analysis; AUGUSTO C. DOTTA FILHO: funding acquisition and discussion; DANIEL RAPACHI: funding acquisition and validation; ANA C. MAZZOCATO: methodology and conceptualization; MARCOS A. GELESKY: methodology, discussion and conceptualization; FLÁVIO A. PAVAN: project administration, supervision, writing – original draft, writing – review and editing.

