Plasmon-Modulated Excitation-Dependent Fluorescence and Antimicrobial Activities of Silver and Gold Nanoparticles Prepared with *Eugenia uniflora* L. extracts

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Abstract

Green synthesis using plant extract is a sustainable method to obtain silver and gold nanoparticles (Ag and AuNPs) and was employed in this work. The *Eugenia uniflora* L. fruits and leaves extracts were used in nanoparticles synthesis. The photoreduction process with a xenon lamp and pH control improved optical properties and nanoparticles stability. The UV-vis, TEM, FTIR, and Zeta potential of the prepared solutions were obtained. The fluorescence spectra of Ag and AuNPs were investigated at different excitation wavelengths, which showed two kinds of fluorescence peaks. The shorter wavelength peaks red-shift with the increasing excitation wavelength, which results from the electron interband transitions, and the longer fixed wavelength peaks due to the local field enhancement. Finally, the antimicrobial tests were performed with Gram-negative and Gram-positive bacteria and *Candida albicans*. The best results were obtained with EuAgNPs prepared with fruits extract, photoreduction, and pH 7.0 (with a mean of $95.12\% \pm 10.29\%$ of inhibition).

1. Introduction

Green methods to synthesize nanoparticles are inexpensive and ecofriendly[1–6]. In the green synthesis using plant extracts, phytochemicals agents can reduce silver at the same time, inhibit the ions agglomerations to produce nanoparticles[7–11]. The use of light can facilitate reduction. This process is denominated photoreduction. The formation of nanoparticles (NPs) can be triggered by photoirradiation reactions[12, 13]. The main advantages of photochemical synthesis are the high spatial resolution, excellent versatility, and local control of reducing agents[14]. The photochemical synthesis enables NPs synthesis in various systems, for example, plants extracts[15].

Eugenia uniflora Linnaeus belongs to the Myrtaceae family, with 142 genera and 5.500 species[16, 17]. Native to South America, the *E. uniflora* L., known as Brazilian cherry, is widely distributed in Brazil, Argentina, Uruguay, and Paraguay. The leaves and fruits of *E. uniflora* L. have many therapeutic properties: antimicrobial, antifungal, antiviral, antitumor, antidiarrheal, anti-helminth, insecticide, antihypertensive and anti-rheumatic effects[18–23]. The composition of *E. uniflora* L. extracts includes phenolic compounds such as flavonoids, terpenes, tannins, anthraquinones, anthocyanins, flavonoids, carotenoids, and essential oils [16, 17, 23–26]. Tannins, present in *E. uniflora* L. have activity against a broad spectrum of viruses: enteroviruses, caliciviruses, rotavirus, influenza virus A, rhabdo-virus, paramyxoviruses, human immunodeficiency virus, herpes simplex virus, adenoviruses [27, 28] [29] and could be used hypothetically to treat the outbreak of COVID-19.

Although there are several publications reporting silver and gold nanoparticles prepared with plants extracts, little attention has been done to the fluorescence properties of these systems[30]. The interaction of light with metal nanoparticles in diameters much smaller than the light wavelength leads to the localized surface plasmon resonance (LSPR)[31]. LSPR produces the local fields greatly enhanced around the nanoparticles. Metal-enhanced fluorescence (MEF) can occur by two mechanisms: local electric field effect and intrinsic radiative decay rate effect[32]. In the first mechanism, the metal surface

or particles produce an LSPR effect which can strengthen the local electric field. The other mechanism is that the nanoparticles can strengthen the intrinsic radiative decay rate of the fluorophore. MEF signal has been used for DNA and protein sensing[33]. Another intriguing effect is plasmon-modulated excitation-dependent fluorescence (EDF). The emission peak of EDFs red-shifts as the excitation wavelength increases, which is usually explained as the red-edge effect in the multilevels[34] .

In this paper, we have employed a photoreduction method to improve the optical properties of silver (*EuAgNPs*) and gold (*EuAuNPs*) nanoparticles prepared using the fruit and leaves extracts of *Eugenia uniflora* L.. The nanoparticles were characterized, and their antimicrobial properties were investigated. The fluorescence spectra of silver and gold nanoparticles under different excitation have also been studied.

2. Materials And Methods

Silver nitrate (AgNO_3) and chloroauric acid (HAuCl_4) were purchased from Sigma-Aldrich. *E. uniflora* L. fruits and leaves were collected from spontaneous germination trees in São Paulo, SP, Brazil. All solutions were prepared with double-distilled water. The pH was adjusted by the addition of sodium hydroxide (NaOH).

2.1. Preparation of nanoparticles

E. uniflora L. leaves and fruits (pitanga) of different colors and sizes were washed abundantly with double distilled water and finely chopped. Extracts were prepared with 1.03 ± 0.03 g of leaves or fruits in 40 mL of double-distilled water. Solutions were boiled for 5 minutes.

Leaves extracts or fruits extracts still hot were mixed with 1 mmol of AgNO_3 to prepare silver nanoparticles solution (*EuAgNPs*). The color change was observed due to AgNO_3 interaction with extracts and reduction to elemental silver. The pH of the solution after reaction ($\sim 5.3 \pm 0.3$).

To prepare gold nanoparticles solution (*EuAuNPs*), HAuCl_4 (1mmol) was added to the extracts still hot. The formation of nanoparticles was initiated immediately and was completed after 10 min. After the reaction, the suspensions become acidic ($\text{pH} \sim 2.7 \pm 0.3$).

Nanoparticles properties were improved by the photoreduction process in which silver and gold nanoparticles solutions (10 mL) were illuminated with a 300 Watt Cermax xenon lamp for 1 min. The pH of the solution after the reaction was adjusted to ~ 7.0 . Some pictures of the synthesis process are shown in Figure 1.

2.2. Characterization of nanoparticles

Shimatzu MultiSpec 1501 spectrophotometer was used in the spectrophotometry analyzes in the UV-vis region. For this measurement, 50 mL of NPs were diluted in 500 mL of doubly distilled water, and the measurements were carried out in a 10 mm optical path quartz cuvette in the range 200 and 800 nm.

The Fourier transform infrared spectroscopy (FTIR) was obtained with Shimatzu IRPrestige. In this case, 200 ml of the NPs and their extracts were deposited on microscope slides and dried in an oven at 60 degrees. The process was repeated three times. The material deposited on the slides was scraped off to make KBr pellets.

The stability of the colloidal suspensions was analyzed by Zeta potential measurements using the Zetasizer Nano ZS Malvern apparatus. The analyses were carried out in an electrophoresis cell, where a potential difference was applied to the electrodes. Three measurements were made for each sample.

Transmission electron microscopy images were obtained by the JEM 2100 JEOL microscope.

Fluorescence measurement was recorded on a Fluorolog III Horiba fluorimeter. The fluorescence spectra were accomplished on a 1 × 1 cm quartz cell at various excitation wavelengths: 280, 300, 325, 340, and 410 nm

2.3. Determination of Antibacterial Efficacy plant extracts and NPs

The *EuAg*NPs and *EuAu*NPs, (table 1) were evaluated against Gram-negative bacteria, namely: *Escherichia coli* American Type Culture Collection (ATCC) 25922; *Escherichia coli* O44:H18 EAEC042[35] (clinical isolate); *Salmonella Thiphymurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, Gram-positive bacteria *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, and against the yeast *Candida albicans* ATCC 90112. The clinical strains present the following resistance profile: *E. coli* O44:H18 EAEC042 - chloramphenicol, tetracycline, and streptomycin, MRSA - amikacin, clindamycin, gentamicin, oxacillin, penicillin, and sulfonamide, and *E. faecalis* - amikacin, clindamycin, gentamicin, oxacillin, penicillin, and erythromycin.

Table 1. Characteristics of samples used in antimicrobial tests.

Name	Method
<i>EuAg</i> NP1	Eu leaves extract + Xe (1 min) + pH 7.0
<i>EuAg</i> NP2	Eu fruit extract + pH 7.0
<i>EuAg</i> NP3	Eu fruit extract + Xe (1 min)+ pH 7.0
<i>EuAu</i> NP	Eu fruit extract + Xe (1 min)+ pH 7.0

The antimicrobial activity of NPs was evaluated in 96-well microtiter plates, according to the CLSI guidelines. Aliquots of 50µL of Mueller-Hinton (MH) broth (bacteria) or Sabouraud (Sab) broth (*C. albicans*), with bacterial or fungal inoculum adjusted to approximately 10⁶CFU/mL was incubated with 50µL of NPs solutions diluted 10 times in MH or Sab broth, resulting in a final volume of 100 µl with 10⁴ CFU/well and the final dilution of solutions of 20 times. The plates were incubated at 37°C for 20 hours, after which the microbial growth was measured based on optical density (OD) at 595 nm in an enzyme-

linked immunosorbent assay (ELISA) reader (Multiskan®EX - Thermo Fisher Scientific, EUA). The results were expressed as inhibition percentage of OD compared with the control (microorganisms in the absence of treatment) and expressed as means ± standard deviation of triplicate assays. The following formula was used to calculate the rate of microbial:

$$\% \text{Cell inhibition} = \frac{\text{Control at } OD_{595nm} - \text{Test at } OD_{595nm}}{\text{Control at } OD_{595nm}} \times 100$$

2.4. Statistical analysis

Statistics were performed using GraphPad Prism 4.00 software. Differences were considered significant when $P < 0.05$, by the Student's t test.

3. Results

3.1 Synthesis and characterizations

Figure 2a shows the UV-Vis spectra of the obtained extracts. In general, flavonoids show two UV absorption bands, one between 240-280 nm and the other one around 300-370 nm, which are attributed to conjugations in the B-ring and A-ring, respectively [36]. Leaves extract presents absorption bands around 247 nm, 338 nm, probably due to luteolin[37-39], and absorption at 462 nm due to the presence of carotenoids[40]. Fruits extract presents bands around 248, 303, 371 nm, probably due to quercetin, absorption at 462 nm, due to the carotenoids, and around 520 nm due to anthocyanins [16].

Figure 2b shows the UV-Vis spectrum of AgNPs synthesized using aqueous extracts. The *Eu*AgNPs produced with leaves extracts without pH adjustment presented a broad absorbance band with a maximum of around 490 nm, similarly obtained by Dugganaboyana et al. using fruits extract[30]. Surface plasmon resonance (SPR) peak at 420 nm was observed for *Eu*AgNPs-leaves solution illuminated with Xe lamp for 1 min (pH ~7.0). These nanoparticles have sizes of about 17 nm (TEM image). For *Eu*AgNPs prepared with pitanga extract, it was observed a peak around 444 nm that shifted to ~422 nm, illuminating the solution with 1 min of Xe lamp and setting pH set to 7.0, which indicated probably reduction in particle size.

The results obtained for the synthesis of *Eu*AuNPs are shown in Figure 2c. The nanoparticles prepared with leaves and fruits extracts present SPR bands around 540 and 534 nm, respectively.

Zeta potential measurements were performed to determine the stability of the suspensions. For *Eu*AgNPs, the more stable particles were obtained with fruit extract irradiated with Xe lamp and pH 7.0 (-21.7mV) (Figure 3). In this case, the polydispersity index (PI) was 0.369. *Eu*AuNPs prepared with pitanga present the followed values:-13.7mV, PI 0.675.

Figure 4a presents the Fourier transform infrared spectroscopy (FTIR) obtained for *E. Uniflora L.* leaves extract, *EuAgNPs* and *EuAuNPs* prepared with leaves extract, carried out to identify the possible biomolecules responsible for reduction, capping, and efficient stabilization of the Ag and Au nanoparticles. Bands around 3400 cm^{-1} , probably related to -NH and bonded -OH groups of carboxylic acids[15, 41] are observed. Bands in the region $2980 - 2800\text{ cm}^{-1}$, indicate the presence of C-H₂ asymmetric and symmetric stretching. Bands in the region 1600 and 1700 cm^{-1} , could be related to C=C stretching vibration of aromatic rings [29] [23] [21] and to the vibration of N-H of amines, C=O of amides, and carboxylic groups [26]; in addition, the band around 1635 cm^{-1} could be related to flavonoids and amino acids, $\nu(\text{C}=\text{O})$, $\nu(\text{C}=\text{C})$, $\delta_{\text{as}}(\text{N-H})$ [24]. The peak observed around 1380 cm^{-1} , indicates that the C-H vibration of the different compounds of extracts interacts significantly with *EuAgNPs* but not with *EuAuNPs*.

The *EuAgNPs* prepared with fruits extract with or without photoreduction FTIR spectra can be observed in Figure 4b. Both present the same functional groups in extract responsible for the bioreduction of Ag^+ and capping/stabilization of silver nanoparticles, but differences in ratios between bands around 1730 and 1600 cm^{-1} are observed. The increase in the absorption peaks at 1724 cm^{-1} is observed for Nps submitted to photoreduction. The peak at 1724 cm^{-1} corresponds to C=O stretch (carbonyl). The existence of these bands in the case of plant-mediated AgNPs, clearly demonstrating the involvement of phenolic compounds.

The fluorescence spectra of leaves extracts, *EuAgNPs*, and *EuAuNPs* were investigated under different excitation wavelengths (Figure 5). *Eu* leaves extract (Figure 5a), excited at 340 nm presents two emission peaks, around 450 nm and $\sim 515\text{ nm}$. For *EuAgNPs*, a shorter emission wavelength was red-shifted (from $320 - 570\text{ nm}$), along with the increase in excitation wavelength (Figure 5b). For *EuAuNPs*, as observed in Figure 5c, is observed intense fixed bands around 450 and 515 nm (excitation 325 and 340 nm) and a shorter emission wavelength that red-shifts, along with the increase in excitation wavelength. Figure 5d shows the dependence between excitation wavelength and narrow high energy peak fluorescence wavelength for *EuAgNPs* and *EuAuNPs*.

A theoretical model described by Ding et al. [34] explained plasmon-modulated excitation-dependent fluorescence strongly coupled to gold nanoparticles. By their theory, the fluorophores (without NPs, in their case activated hexadecyltrimethyl ammonium bromide-CTAB*) present long lifetime multilevel states due to the surface states from the functional group. The fluorescence peak λ appears at the wavelength,

$$\bar{\lambda} = \frac{2\lambda_{\text{exc}}\lambda_{\text{low}}}{\lambda_{\text{exc}} + \lambda_{\text{low}}} \quad (1)[34]$$

Where λ_{exc} is the excitation wavelength and λ_{low} is the wavelength of photons emitted from the lowest state relative to the HOMO of fluorophores. To obtain this equation, the authors have assumed the same

radiative/nonradiative rate and the same spectral width for all intermediate states. This equation demonstrates that the fluorescence peak wavelength increases with the excitation wavelength[34].

The red-shifted emission peaks for EuAg and EuAuNPs observed in Figure 5 b and c were plotted in function for the excitation wavelengths 280, 300, 325, and 340 nm and presented in Figure 5d. The fit curve (red line) plotted using equation (1) indicated that the results agree well with this equation. From the obtained parameter “A” it is possible to calculate the frequency of photons emitted from the lowest state with frequency ω_{low} relative to the HOMO of excitation-dependent fluorophores.

3.2 Antimicrobial tests

The antimicrobial activity of EuAgNPs, and EuAuNPs was tested using the broth microdilution assay, which provides quantitative data on inhibition efficacy, and results are presented in Figure 6 for tested NPs described in Table 1. The results demonstrated that most of the microbial species, including *C. albicans*, showed high inhibition percentual (> 90%) when exposed to EuAgNPs prepared with fruit extract and photoreduction (Figure 6) except for *E. faecalis* (54%). In contrast, EuAgNPs prepared with leaves extract and photoreduction showed more inhibition of *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *E. faecalis* than EuAgNPs prepared with fruit extract and photoreduction.

The results indicated high inhibition percentual of Gram-positive, Gram-negative bacteria and yeast treated with EuAgNps (33.00% to 100%, with a mean of $89.95\% \pm 19.78\%$), in comparison with EuAuNPs treatment ($P < 0.05$). The antimicrobial effect of EuAuNPs ranged from 0% - 71.27%, presenting a mean of $32.39\% \pm 18.00$. EuAg Fruit inhibited 53.71% to 100.0% (with a mean of $95.12\% \pm 10.29\%$) of microorganisms, whereas EuAu Fruit inhibited 19.85% to 71.27% (with a mean of $32.39\% \pm 18.00\%$). EuAg Fruit without Xe and Ag Fruit exposed to Xe did not possess a significant ($P > 0.05$) difference.

All treatments tested exhibited high inhibition percentual of *B. subtilis*, especially EuAgNPs (98.83% - 99.47%), whereas, with EuAuNPs, it reached 66.23% to 75.88%. On the other hand, Au Fruit 1 Xe pH 7.0 was not effective against *K. pneumoniae* ATCC 700603.

4. Discussion

Chemically, phenolic substances present in plant extracts are defined as those that have in their structure one (or more) aromatic rings with one or more hydroxyl substitutes [42]. These substances have variable structures and are therefore multifunctional. Phenolic metabolites include phenolic acids, flavonoids, simple phenolics, phenylpropanoids, coumarins, tannins, and tocopherols[16].

In general, phenolic substances are potent antioxidants, acting by several mechanisms such as electron donation and interruption of the chain of oxidation reactions [42]. In addition to their antioxidant capacity, phenolic substances, mainly phenolic acids, flavonoids, and tannins, have other health-beneficial properties such as anticancer, antimicrobial, antiallergic, hepatoprotective, antithrombotic, antiviral,

vasodilator, antimutagenic and anti-inflammatory activity since many of these biological functions have been correlated with their antioxidant capacity.

The absorption spectrum for the aqueous extracts of *E. uniflora* L. (Figure 2a) shows absorbance peaks in the ultraviolet region at wavelengths compatible with the presence of phenolic metabolites[43].

Absorption bands around 247 nm, 338 nm (flavonoids probably luteolin), and a peak around 462 nm probably due to carotenoids were observed for leaves extracts and bands around 248, 303, 371 nm, that can be attributed to flavonoids as quercetin[44], were observed for fruits extracts.

The use of plant extracts for nanoparticles synthesis is a straightforward green method and economically viable[45]. There are several works in the literature evidencing good results of synthesis and antimicrobial activities of nanoparticles prepared with *plants* extracts[46-49]. Here, *Eu*NPs with leaves and fruits extracts are associated with the photoreduction method and pH control.

E. uniflora L. phytochemicals as saponin, tannins, phenolic compounds, and flavonoids [23-25, 50, 51], interact with AgNO_3 or HAuCl_4 that are reduced to elemental silver or gold, respectively. Biosynthesis probably involves quercetin [52] and anthocyanins in fruits extract and luteolin[53] in leaves extract.

The results showed that Xenon lamp irradiation and pH adjustment improve optical properties of *E. uniflora* L. nanoparticles. The stabilization of NPs can be divided into three different categories, including steric, electrostatic, and unification of steric and electrostatic stabilization[54]. Although electrostatic stabilization is easier to maintain in colloidal media, due to strong forces of interactions between oppositely charged ions, it is impossible to separate agglomerated particles. So immediately after mix plants extracts and silver nitrate or HAuCl_4 , the SPR bands are wide, indicating the presence of agglomerates. Electrostatic stabilization is regulated by pH adjustment, and the SPR bands become narrowed, indicating monodispersed nanoparticles. Steric stabilization is a thermodynamic stabilization method; therefore, particles can be redispersed. This stabilization can be achieved using ionic surfactants but also the photoreduction method. Photoreduction offers steric repulsion within nanoparticles, thus preventing the agglomeration and giving rise to a mutual stabilization system[55].

The Zeta potential results, presented in Figure 3, indicated that the photoreduction process can improve the stability of *Eu*NPs. The high zeta potential values mean that *Eu*NPs are highly stable due to the presence of a high surface charge, which prevents agglomeration.

The increase in the absorption peaks at 3878 cm^{-1} is observed for *EuAg*Nps prepared with fruits extract and photoreduction (Figure 4), indicating that C-H vibration of the different compounds of extracts interacts significantly with *EuAg*NPs. The peak around 1727 cm^{-1} , due to C=O stretch clearly in *EuAg*NPs prepared with fruits extract, indicates the involvement of carbonyl functional group in silver reduction and stabilization, probably attributed to the presence of quercetin in the fruits extract.

The fluorescence spectra of leaves extracts, *EuAg*NPs, and *EuAu*NPs were investigated under different excitation wavelengths (Figure 5). The changes in fluorescence behaviors showed in Figure 5 are induced

by several fluorescence mechanisms. Figure 5a show emission observed from extract fluorophores due basically to flavonoids molecules at around 450 and 515 nm (excitation 340 nm) [39, 56]. In Figure 5b is observed that *EuAgNPs* have two emission regions, a narrow emission peak around 320–380 nm, and a broad emission band around 380-500 nm. We speculated that the narrow emission is attributed to electron-hole recombination or quantum size effect, while the broad emission is attributed to surface fluorophores (flavonoids) [57].

Figure 5c presents the fluorescence spectra of *EuAuNPs* with different excitation wavelengths from 280 to 410 nm. The narrow emissions observed for *EuAuNPs* are attributed to recombination of sp electrons with holes in the d band[58], and the broad fixed are attributed to LSP- enhanced radiative emission. In the presence of *EuAuNPs*, the surface plasmon resonance leads to the local field enhancement. *EuAuNPs* emission intensity around 450 nm enhanced 4 times in comparison to the *Eu* extract emission. Figure 5d clearly shows that the position of high-energy peak red-shifts as the excitation wavelength increases for both *EuAg* and *EuAuNPs*, and the theoretical model proposed by Ding et al. [34] can describe excitation-dependent fluorescence spectra of extract fluorophores with metallic nanoparticles.

Recently, metal-enhanced fluorescence has led to new developments and applications by improving the fluorescence intensity of various materials. When the fluorophores or emitters are placed in proximity but to an optimum distance from silver or gold nanoparticles, they are benefitted from the additional plasmon-enhanced optical fields. The observed enhanced fluorescence intensity is due to the local field enhancement associated with the excitation of LSPRs in the metal nanostructures. The plasmonic nanoparticle here serves as a transmitting optical antenna to transfer the near field to the far-field at the fluorescence wavelength[59].

According to Sobeh et al., the essential oil extracted from *E. uniflora L.* presents antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus licheniformis*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida parapsilosis* and *Candida albicans*[60]. In the present paper, we obtained the antimicrobial activities of silver and gold nanoparticles synthesized with *E. uniflora L.* extracts.

The obtained results (Figure 6) indicated that low concentrations of synthesized *EuAgNPs* (50µL of NPs solutions diluted 10 times) showed inhibitory activity against Gram-positive and Gram-negative bacteria. Higher inhibition was obtained against spherical bacteria as *S. Thiphymurium*, *B. subtilis*, *S. aureus*. *E. faecalis*, and *Candida albicans*. *EuAgNPs* prepared with fruit extract are more effective than those prepared with leaf extract. All tested bacteria treated with *EuAgNPs* prepared with fruit extract show inhibition > 90%.

The solutions prepared with controlled pH and Xe illumination presented excellent results for all tested microorganisms except, Gram-negative bacteria *E. coli*, *P. aeruginosa* *K. pneumoniae*, and *C. albicans*, whose have a thicker peptidoglycan layer in their cell wall. Probably the best results could be obtained with these nanoparticles in lower concentrations.

EuAuNPs presented low antimicrobial inhibition, but *B. subtilis* treated with AuNPs presented inhibition >70%.

The results showed that *EuAgNPs* exhibited superior antimicrobial activity when compared to *EuAuNPs*, against tested strains. These nanoparticles could be impregnated in the equipment of individual protection to improve reducing the bacterial growth of the device and promoting the reduction of hospital-acquired infections. Another possible application is suppressing both viral (as COVID-19) and bacterial respiratory infections by inhalation delivery.

Metal nanoparticles can penetrate the bacterial cell and interact with sulfur and phosphorous bases from DNA molecules, decreasing the capacity for cell replication. Another mechanism of the inhibitory action is the induction of oxidative stress due to the generation of the reactive oxygen species (ROS), including free radicals. They are capable of damaging the cell membrane, making it porous, denaturing proteins, and inhibit cellular respiratory enzymes, leading to cell death (Maillard and Hartemann 2013, Franci et al. 2015, Baptista et al. 2018). *EuNPs* may be employing one or more of these mechanisms to inhibit microbial growth.

The facile synthesis and excellent properties, such as good stability and low polydispersity, guarantee several essential applications in biology and medicine to the synthesized nanoparticles.

5. Conclusions

The *EuAgNPs* were successfully prepared using the aqueous extract of leaves and fruits as a reducing agent source. The photo-induced synthesis has provided a clean and convenient way to improve the optical properties of nanoparticles. The outcome of the present study showed that *E. uniflora* L. extracts are suitable for reducing agents to synthesize silver nanoparticles with Zeta potential around -20 mV and polydispersity ~0.3. To the best of our knowledge, first-time plant-mediate metallic nanoparticles have been used to study MEF under aqueous conditions. With the addition of suitable *EuAuNPs*, the fluorescence intensity of the flavonoids was found to be increased ~4-fold. The comparisons of antibacterial activity of the *EuNPs* further confirm the antibiotic efficiency of the green-synthesized *EuNPs* for the development of novel antibacterial agents for treatment against Gram-negative and Gram-positive pathogens.

Declarations

Data Availability

The authors declare that the data supporting the findings of this study are available in the article.

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Contributions

LCC and MRF designed the study and carried out the data analysis and interpretation of the results. MRF and DSC performed antimicrobial studies. All authors drafted the manuscript authors to read and approved the final manuscript.

Ethics declarations

Not applicable

Competing Interests

The authors declare that there are no conflicts of interest.

Ethics Approval

For this type of study, the ethical approval was not required, because this study does not involve cell or animal manipulation.

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Figures

Figure 1

a) *E. uniflora* L. (pitangueira), b) Leaves extract preparation, c) Photoreduction.

Figure 2

UV-Vis absorbance spectra of a) *E. uniflora* L. leaves and fruits extracts; b) synthesized EuAgNPs prepared with leaf extract, pitanga extract, and Xenon irradiation for 1 min and pH ~ 7.0 of leaves extract (figure inside TEM) and pitanga extract. c) UV-Vis absorbance spectra of *E. uniflora* fruits and leaves extracts, EuAuNPs+ Xe (1 min)+ pH 7.0.

Figure 3

Zeta potential analysis of synthesized EuAgNPs using *E. uniflora* fruit extract, Xe illumination and pH 7.0.

Figure 4

a) FTIR spectra of *E. uniflora* L. leaves extract, and EuAgNPs and EuAuNPs prepared with leaves extract.
 (b) FTIR spectra of EuAgNPs (fruits extract) prepared without and with photoreduction process.

Figure 5

Fluorescence spectra were obtained for excitations from 280 to 410 nm for a) *E. uniflora* L. leaves extracts; b) synthesized EuAgNPs prepared with leaves extract, Xenon irradiation for 1 min and pH ~ 7.0, c) synthesized EuAuNPs prepared with leaves extract Xenon irradiation for 1 min and pH ~ 7.0, d) Relationship of excitation and emission wavelength of extract fluorophores in EuAg and EuAuNPs.

Figure 6

Antimicrobial activity of EuAgNP and EuAuNP against *Escherichia coli* ATCC 25922, *Escherichia coli* O44:H18 EAEC042, *Salmonella Typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, and *Candida albicans* ATCC.