Int. J. Nanosci. Nanotechnol., Vol. 15, No. 3, Sept. 2019, pp. 179-188

Green Synthesis of Silver Nanoparticles Using Amazon Fruits

Alan Kelbis Oliveira Lima[1], Arthur Abinader Vasconcelos[2], José Jeosafá Vieira de Sousa Júnior[3], Silvia Katrine Silva Escher[3], Gerson Nakazato[4] and Paulo Sérgio Taube Júnior[2,*]

[1]Department of Genetics and Morphology, University of Brasilia, Brasilia, Brazil.
[2]Institute of Biodiversity and Forests, Federal University of Western Pará, Pará, Brazil.
[3]Microbiology Laboratory, Federal University of Western Pará, Pará, Brazil.
[4]Laboratory of Basic and Applied Bacteriology, State University of Londrina, Londrina, Brazil.

(*) Corresponding author: pstjunior@yahoo.com.br
(Received: 18 August 2017 and Accepted: 13 May 2019)

Abstract

In this study, we report the green synthesis of silver nanoparticles (AgNPs) from extracts of native fruits from Amazonia, Brazil. AgNPs were characterized by UV/Vis and medium infrared (MIR) spectroscopy. Their antimicrobial activities were evaluated against the growth of bacteria and leavers, as well as the evaluation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The colloidal solutions had a maximum absorption peak around 440 nm, as was well reported in the literature for colloidal silver. The MIR spectra identified the functional groups of carboxylic acids and several phenolic compounds as possible factors responsible for the stabilization and coating of AgNPs. All synthesized AgNPs showed antimicrobial activity against Candida albicans, Escherichia coli and Staphylococcus aureus. In this sense, the obtained results showed that the native plants of Amazonia have potential to be used for the green synthesis of AgNPs and that more studies related to their applications should be performed.

Keywords: *Antimicrobial activity, Minimal inhibitory concentration, Minimum bactericidal concentration, AgNPs.*

1. INRODUCTION

Nanotechnology is an interdisciplinary field focused on the manufacture of materials with a scale of 1-100 nm [1-3]. Within this size range, the properties (chemical, physical and biological) of atoms and molecules change and take on new applications based on their size, distribution and morphology [4].

Metal nanoparticles (NPs) have attracted interest of several researches of pharmaceutical and medical areas, especially due their antimicrobial activities [5-6]. In addition, because their catalytic, optical and electrical properties, they are quite studied in the areas of biotechnology, chemistry and physics [7]. Silver nanoparticles (AgNPs) are among the most studied NPs once they present several biological applications, such as: antimicrobial and antioxidant compound [8], antifungal agents [9-10], anti-inflammatory [10-11] and anti-cancer [12-13].

AgNPs are usually prepared by chemical or physicochemical methods, which can generate residues harmful to the environment or present high energy cost [1, 14]. However, new synthetic processes have been used for the preparation of AgNPs, mainly using low-cost and non-polluting biological materials [15]. These green syntheses are usually fast and sustainable [16-18]. These biological compounds have a great amount of molecules capable of reducing and stabilizing AgNPs [15, 18-19]. Among the most used biological compounds in the synthesis of AgNPs, plant extracts are the most reported since

they contain high amounts of secondary metabolites (*e.g*. phenolic compounds) capable of reducing metal ions and forming NPs [2, 20]. However, it is important to note that the toxicity of AgNPs, even obtained by green routes of synthesis, is still not well evidenced [1, 21].

The Brazilian Amazon presents about 220 edible fruit species, which corresponds to 4% of Brazilian native fruits species [22]. Amazonian exotic fruit market has been growing in recent years, which reflect world demand for natural products with diverse nutritional and functional properties. The Brazilian population has increased the consumption of typical fruits, mainly exotic fruits of the Amazon region. This increase in consumption is mainly due to the presence of nutrients capable of preventing some diseases, mainly degenerative diseases [23].

In this sense, the consumption of pulps and derivatives of tropical fruits such as Açaí (*Euterpe oleraceae*), Cupuaçu (*Theobroma grandiflorum*), Murici (*Byrsonima crassifolia*) and Taperebá (*Spondias mombin*) has increased mainly due to their richness in vitamins, phytosterols and phenolic compounds [24]. However, most of the studies already carried out in relation to the Amazonian fruits were focused only on the nutritional analyzes or chemical composition [25].

In this context, considering the potential benefits of Amazonian plants for nanotechnology, this study aims at the production, characterization and biological activity of silver nanoparticles (AgNPs) synthesized from Açaí (*Euterpe oleraceae*), Cupuaçu (*Theobroma grandiflorum*), Murici (*Byrsonima crassifolia*) and Taperebá (*Spondias mombin*).

2. MATERIAL AND METHODS

2.1. Preparation of Extracts

Commercial samples of fruit pulp native to the Amazonian region (Açai, Cupuaçu, Murici and Taperebá) were purchased, in September 2014, in popular markets of Santarém, Brazil (2°41’99.99’’S and 54°72’94.23W). The extracts were prepared in Falcon® tubes using 5 g of pulp of each fruit and ultrapure water (Milli-Q®) to make up to 15 mL and after the extract were filtered through a porosity filter between 8-12 μm.

2.2. Synthesis of AgNPs

Silver nanoparticles (AgNPs) were synthesized using silver nitrate ($AgNO_3$) (Sigma-Aldrich®, St. Louis, USA). To each 1.00 mL of the diluted extract under stirring was added slowly 50 mL of a 3 mmol L^{-1} solution of $AgNO_3$. After the colloidal suspensions containing the AgNPs were separated in polyethylene containers (previously sterilized) and protected from light, being kept on refrigeration between 4°C and 9°C for further analysis. After resting for 5 hours, the samples were separated for characterization and biological testing.

The suspensions were named with acronyms of the initials of their popular names for better interpretation, being Aç-AgNPs, Cp-AgNPs, Mu-AgNPs and Ta-AgNPs for Açaí, Cupuaçu, Murici and Taperebá silver nanoparticles, respectively.

2.3. UV/Vis Spectroscopy

To investigate the formation and stability of AgNPs, a UV/Vis absorption spectrophotometer Nova Instruments NI1600 model (Piracicaba, São Paulo, Brazil) was used. After the reaction time, a 2.0 mL aliquot of each sample was separated and analyzed in UV/Vis with wavelength scanning ranging from 190 at 800 nm.

2.4. Medium Infrared Spectroscopy (MIR)

The infrared measurements were performed on spectrometer Bruker Vertex 70 model (Milan, Italy) in the medium infrared ranging from 4000 cm^{-1} to 400 cm^{-1}, with the use of OPUS 6.5 software (Bruker®, Milan, Italy), with attenuated

total reflectance accessory. 1.0 mL aliquot of each sample of AgNPs was separated for characterization using KBr (Sigma-Aldrich®, St. Louis, USA) pellets which were vacuum-dried, dried and used for analysis.

2.5. Antimicrobial Activity

The antimicrobial activity of AgNPs against bacteria and fungi of clinical interest was tested. The microorganisms used in the antimicrobial assays were *Candida albicans* (AC 01), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 12228) (CEFAR®, São Paulo, Brazil) from the Microbiology Laboratory of the Federal University of West of Pará - UFOPA.

The technique used for sowing was well diffusion [26] in which the Petri dishes used for the test were sterilized and prepared with Saboraund agar medium (Synth, Diadema, São Paulo, Brazil) for growth of *Candida albicans* and Muller-Hinton agar medium (MHA, Sigma-Aldrich®, St. Louis, USA) for growth of *Escherichia coli* and *Staphylococcus aureus*. To achieve the antimicrobial activity test an aliquot of 100 µL of each microorganism suspension was adjusted to 0.5 of the MacFarland scale, corresponding to 1.5 x 10^8 CFU mL^{-1}, were seed in Petri dishes (15 mm x 90 mm) containing 0.9% NaCl sterilized solution (Sigma-Aldrich®, St. Louis, USA). Cylindrical wells with 6 mm of diameter were prepared and after dispensed 100 µL of each AgNP suspension, in triplicate. The negative control was carried out with 100 µL of 3 mmol L^{-1} $AgNO_3$ solution.

The plates were incubated at 35°C for 24 and 48 hours, and the antibacterial activity was detected by the presence of inhibition halos around the wells and expressed in millimetres. It is worth noting that in this test the guarana NPs were used, but since they did not show action against bacterial growth, this suspension was not used for the characterization techniques, considering only the AgNPs synthesized with the extracts of the other fruits.

2.6. Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) is a quantitative method used to determine the minimum antimicrobial concentration required to inhibit the growth of microorganisms. Minimum bacterial concentration (MBC) is reported as the lowest concentration of antimicrobial that causes total absence of microorganisms in the inoculum.

The MIC was determined in 96-well polystyrene plates using broth dilution technique according to the norms of the Clinical Laboratory Standard Institute (CLSI) [27]. MIC were evaluated only for extracts which showed antimicrobial effect in the well diffusion technique. A non-diluted sample of each extract was added in the first well and a serial dilution of extract was established to obtain the following AgNPs concentration: 250, 125, 62, 31, 16, 8, 4 and 2 µg mL^{-1}. All analyses were realized in triplicate.

Posteriorly, for the determination of MIC and MBC, 100 µL of Mueller Hinton broth (MH, Sigma-Aldrich®, St. Louis, USA) and 100 µL of Saboraund broth (SD, Synth, Diadema, São Paulo, Brazil) were added to the wells with 10 µL of bacteria and 10 µL of fungus, respectively. 15 µL of resazurin solution (0.01%) (Sigma-Aldrich®, St. Louis, USA) was used as chemical colorant. Negative control was performed replacing extracts samples by Mueller Hinton Broth (MHB) and the positive control was performed with ceftriaxone (Agila, Barra da Tijuca, Rio de Janeiro, Brazil) (100 µg mL^{-1}) for bacteria and nystatin (Multilab, São Jerônimo, Rio Grande do Sul, Brazil) (250 µg mL^{-1}) for fungus. Suspension of microorganisms was adjusted to MacFarland scale (corresponding to 1.5 x 10^8 CFU mL^{-1}). All plates were incubated ate 37 ºC for 24 hours.

2.7. Statistical Analysis

All antimicrobial activity, MIC and MBC analysis were performed in triplicate and the results were represented as the mean ± standard deviation. The differences among the analyses were assessed with the analysis of variance model (ANOVA) followed by Tukey's test at a 95% confidence level ($p < 0.05$) using the Minitab® 14 software (Minitab, State College, PA, USA). Tukey's test was performed at 95% confidence level ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Synthesis and Formation of AgNPs

Molecular absorption spectrometry in the UV/UVvis region is an important tool to identify the formation of AgNPs. AgNPs exhibit yellow-brown staining in the suppression, which refers to the molecular absorption bands around 400-450 being in accordance with the response to plasmon surface resonance (SPR), and confirming the formation of AgNP [2, 28-30]. A single SPR band is characteristic of the spherical nanoparticles in solution, while two or more bands correspond to the anisotropic particles [15, 31].

In some studies, absorption bands similar to those found in this study were found. As for example for AgNPs synthesized using *Azadirachta indica* extract with absorption bands between 446-448 nm [29], *Lawsonia inermis* leaves extract with 430 nm [32] and extracts of leaves of *Catharanthus roseus* with a value of 425 nm [33].

3.2. Medium Infrared Spectroscopy (MIR)

Through infrared spectrometry it was possible to identify the main functional groups present in the suspension that may be able to stabilize the AgNPs. Figure 1 shows the medium infrared spectrum of suspension containing AgNPs.

The major bands detected were similar for Aç-AgNPs (Figure 1A) and Cp-AgNPs (Figure 1B). The MIR spectra of these extracts showed intense bands around 3350-3000 cm^{-1} and weaker bands around 1600-1700 cm^{-1}, which may refer respectively, to stretches of the hydroxyl O-H bonds and/or phenolic compounds and stretching of C=O bonds of carbonyl or carboxylic groups [30, 34]. These results corroborate with the literature since açaí, taperebá, muruci and cupuaçu present in their composition flavonoid contents and several phenolic compounds [23, 35], besides the cupuaçu have in the chemical composition of the seed about nine flavonoids antioxidants [36].

MIR spectra for the Mu-AgNPs (Figure 1C) and Ta-AgNPs (Figure 1D) were relatively similar, with broad and smoothly elongated bands around 600-700 cm^{-1}, a strong band around 1700 cm^{-1}, a weak band around 2100-2300 cm^{-1} and an elongated band around 3300-3550 cm^{-1}. These bands correspond, respectively, to the C-H deformation, C=C and C=O stretches, C≡C, C≡N, C=O stretches and the O-H stretch. Both murici and tapereba are fruits rich in fatty acids, being that a murici species has already been reported with different concentrations of proteins, starch, carbohydrates and minerals [37]. Significantly different levels of phenol compounds of taperebá in comparison to other Brazilian tropical fruits, such as gallic acid, quercetin and myricetin, and the main functional groups of these compounds are represented in the peaks observed in the murici and taperebá spectra [23].

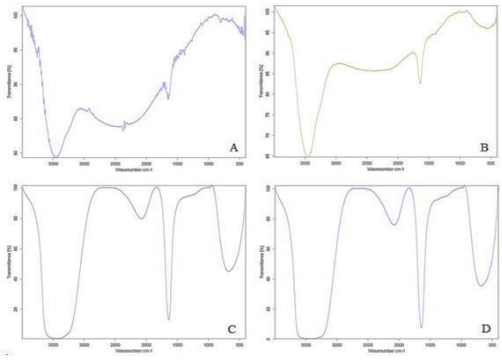

Figure 1. *Medium infrared spectra (MIR) of AgNPs: (A) Aç-AgNPs, (B) Cp-AgNPs, (C) Mu-AgNPs, (D) Ta-AgNPs.*

3.3. Antimicrobian Activity

Due to the emergence of microorganisms resistant to various antibiotic synthetics, new antimicrobial agents have been developed for the prevention of infections caused by pathogenic microorganisms. In this context, the AgNPs appear as possible antimicrobial agents and in this study their action was tested against Gram-positive attributed to Mu-AgNPs (10.7 ± 0.58 mm). In relation to the control samples, this microorganism was the most susceptible to $AgNO_3$ action with an overall mean of 15.3 ± 0.58 mm, but no significant difference was observed between AgNPs results against yeast ($p < 0.05$).

Other results were similar to those of this study working with *Candida albicans*, such as that which synthesized AgNPs from the leaf extract of a coastal plant and obtained an average inhibition of 12 mm [38], the AgNPs synthesis using banana peel extract, which had halos Inhibition of about 11 mm [39] and the synthesis of AgNPs using a culture supernatant of *Enterococcus* sp. which obtained inhibition halos around 26 mm [40].

In general, in this study, antifungal activity was more prominent than antibacterial activity, but silver action on bacterial growth is well recognized and this can be attributed to the fact that silver tends to adsorb on the bacterial surface [41], but not commonly in fungal cells. Table 1 shows that AgNPs were less efficient in inhibiting the growth of *Escherichia coli* bacteria, although this is a Gram-negative bacterium, which presenting cell wall composed of a less thick layer of peptidoglycan compared to *Staphylococcus aureus* bacteria where the cell wall is more rigid and less exposed to the action of substances with bactericidal effects [28].

Mu-AgNPS showed also lower activity, presenting inhibition halos around 10.7 ± 0.58 mm for the two bacterial strains. Ta-AgNPs showed equal halos for both bacteria (12.0 ± 0 mm). Among the two bacteria, Cp-AgNPs were mildly better at and Gram-negative bacteria and fungus specie. The antimicrobial activities of the AgNPs synthesized are shown in Table 1.

Table 1 shows that the Cp-AgNPs and Ta-AgNPs had the highest averages of inhibition halos against *Candida albicans* growth, being around 15.3 ± 0.58 mm and 16.3 ± 0.58 mm, respectively (Figure 2). The lowest effect on yeast growth was inhibiting *Staphloccocus aureus* growth (13.7 ± 0.58 mm) relative to *Escherichia coli* (12.3 ± 0.58 mm) (Figure 3), but between mean of halos of these bacteria was also not observed significant difference ($p < 0.05$) (Table 1). Several studies have already been published showing the antibacterial activity of AgNPs against strains of *Escherichia coli* and *Staphyloccocus aureus*: 15 mm and 20 mm [10], 7 mm and 19 mm [42], 14 mm and 19 mm [43], 10 and 16 mm [15], 9 mm for the two pathogens [26], 9.7 mm and 9.2 mm [44], 12.8 mm and 11 mm [45] and 12 and 10 mm [46] respectively.

The antimicrobial activity of AgNPs is influenced by several factors, including pH, temperature, salt and solvent concentration used in the synthesis, size and shape of AgNPs, type of pathogen used in the test and time of exposure to NPs [47]. Therefore, the possible mechanisms of action may be related to the surface and size of the AgNPs [41, 48], the release of Ag^+ ions and electrostatic attraction between them and the negatively charged membrane of microorganisms [49-50], silver interaction with microbial compounds containing sulfur and phosphorus leading to protein inactivation and damage in DNA [15, 51-52], as well as the reactive oxygen species (ROS) generated by the AgNPs [52-53].

Kim et al. (2012) observed that inhibition of fungal growth increased with increasing of AgNPs concentrations. This may be related to the high density in which the solution was able to saturate and associate with fungal hyphae and deactivate the plant pathogenic fungi. In summary, the antifungal activity of AgNPs probably

occurs through the destruction of membrane integrity [54].

Table 1. *Antimicrobial activity (mm) of AgNPs against Candida albicans (Ca), Escherichia coli (Ec) and Staphylococcus aureus (Sa) after 24 h.*

Candida albicans		*Escherichia coli*		*Staphylococcus aureus*	
Aç-AgNPs	11.7 ± 0.58[a*]	Aç-AgNPs	11.0 ± 0.01[a*]	Aç-AgNPs	11.3 ± 0.58[a*]
Cp-AgNPs	15.3 ± 0.58[a*]	Cp-AgNPs	12.3 ± 0.58[a*]	Cp-AgNPs	13.7 ± 0.58[a*]
Mu-AgNPs	10.7 ± 0.58[a*]	Mu-AgNPs	10.0 ± 0.01[a*]	Mu-AgNPs	10.0 ± 0.01[a*]
Ta-AgNPs	16.3 ± 0.58[a*]	Ta-AgNPs	12.0 ±0.01[a*]	Ta-AgNPs	12.0 ± 0.01[a*]
Control	15.3 ± 0.58[a*]	Control	12.0 ± 0.01[a*]	Control	12.0 ± 0.01[a*]

Control containing only the $AgNO_3$ solution.
*Mean of the diameters of inhibition zones (mm). Average of three replicates. Means followed by the same letter in the same column, do not differ from each other, by the Tukey test at 5% probability.

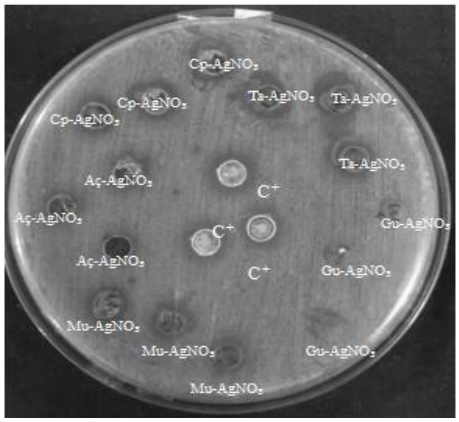

Figure 2*. Candida albicans well diffusion assay (AC 01) showing zones of inhibition in the presence of AgNPs synthesized with Amazonian fruit extracts.*

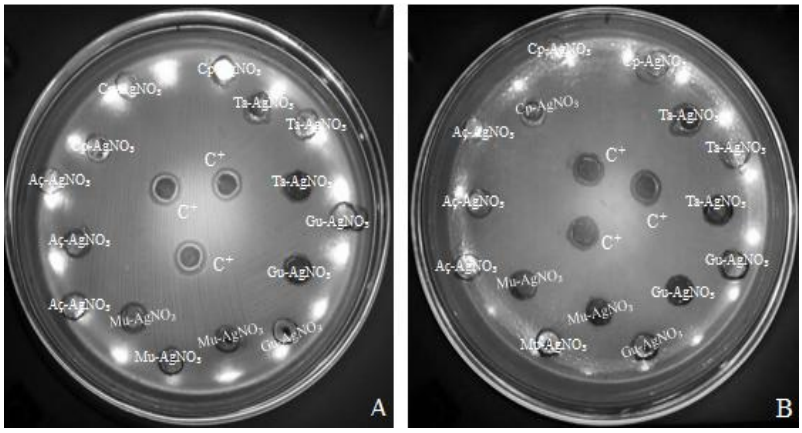

Figure 3*. Antibacterial activity of AgNPs against strains of Escherichia coli - ATCC 25922 (3A) and Staphylococcus aureus - ATCC 12228 (3B).*

3.4. Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC)

MIC and MBC values provide quantitative data on the antimicrobial efficacy of the AgNPs tested and are determined as the lowest concentration in which no pathogen growth is observed and the lowest concentration of the antimicrobial agent in which the presence of microorganism is not observed, respectively. The values of these tests are shown in Table 2.

Among the different concentrations tested, the Ag-AgNPs showed a lower MIC for *Candida albicans* and *Staphyloccocus aureus* being up to 4 times lower when compared to the concentrations of the antibiotics used as controls. Even so, when analyzing the statistic ($p<0.05$), no significantly different values were found among the microorganisms. In a recent study, an MIC concentration of 0.5 μg mL^{-1} was found for yeast *Candida albicans* [3]. The MIC versus *Staphylococcus aureus* results of this study were higher than those reported in the literature [38, 55], but also in a lower concentration compared to other findings [56].

The lowest inhibitory concentration for *Escherichia coli* was also of Ag-AgNPs with MIC value similar to that reported in the AgNPs extraction from the methanolic extract of an Indian medicinal plant (12.5-25 μg mL^{-1}) [42], but were higher than those found in other studies [38, 56-57].

Data from MBC showed that the values obtained for *Candida albicans* did not differ significantly from the others ($p<0.05$), whereas MBC results for *Escherichia coli* and *Staphyloccocus aureus* were statistically different ($p<0.05$). Concerning MBC against *Staphyloccocus aureus*, samples Aç-AgNPs and Mu-AgNPs had a greater effect than reported in the literature using *Zingiber officinale* which was 40 μg mL^{-1}, while the lowest MBC value for *Escherichia coli* was 62 μg mL^{-1} for Aç-AgNPs.

Table 2. *MIC and MBC values of AgNPs, silver nitrate ($AgNO_3$) and control (standard antibiotics) against human pathogens.*

AgNPs	MIC (μg mL^{-1})*			MBC (μg mL^{-1})*		
	C. albicans	*E. coli*	*S. aureus*	*C. albicans*	*E. coli*	*S. aureus*
Aç-AgNPs	8.0^{a}	16.0^{a}	8.0^{a}	31.0^{a}	$62.0^{a,b}$	$16.0^{a,c}$
Cp-AgNPs	31.0^{a}	31.0^{a}	16.0^{a}	62.0^{a}	$125.0^{a,b}$	$62.0^{a,c}$
Mu-AgNPs	31.0^{a}	31.0^{a}	16.0^{a}	62.0^{a}	$125.0^{a,b}$	$31.0^{a,c}$
Ta-AgNPs	31.0^{a}	31.0^{a}	31.0^{a}	62.0^{a}	$125.0^{a,b}$	$62.0^{a,c}$
$AgNO_3$	31.0^{a}	31.0^{a}	31.0^{a}	62.0^{a}	$125.0^{a,b}$	$62.0^{a,c}$
Control	125.0^{a}	125.0^{a}	125.0^{a}	125.0^{a}	$125.0^{a,b}$	$125.0^{a,c}$

Positive control was performed with ceftriaxone (100 μg/ml) for bacteria and nystatin (250 μg/ml) for fungus.
*Average of three replicates. Values followed by the same letter in the same column, do not differ from each other, by the Tukey test at 5% probability.

4. CONCLUSION

In this work AgNPs were synthesized using extract of native fruit from, by green route. The formation of AgNPs was evaluated using UV/Vis spectrophotometry, where a maximum absorption band around 440 nm was observed in all suspensions, which is characteristic of the presence of colloidal silver. Using MIR, it was possible to identify that the main

functional groups present in the extracts and that may have been responsible for the stabilization of AgNPs were those of carboxylic acids and phenolic compounds. The antimicrobial activity was tested against gram-negative, gram-positive and fungus and the results showed that there was considerable inhibition of microbial growth by the qualitative test of diffusion in wells and that by the quantitative tests of MIC and MBC. Thus, it was demonstrated that the Amazonian plants could be promising in the production of AgNPs, which could act as antimicrobial agents. In addition, Amazonian native species should be furthers studied in order to discover new biomedical applications, mainly for use in nanotechnology.

ACKNOWLEDGEMENT

Brazilian Federal entities CNPQ (National Council for Scientific and Technological Development) and CAPES (Coordination of Improvement of Higher Education Personnel.

REFERENCES

1. Ahmed, S., Ahmad, M., Swami, B. L., Ikram, S., (2016). "A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise", *Journal of advanced research*, 7(1): 17-28.
2. Shams, S., Pourseyedi, Sh., Hashemipour Rafsanjani, H., (2014). "Green synthesis of silver nanoparticles: eco-friendly and antibacterial", *International Journal of nanoscience and nanotechnology*, 10(2): 127-132.
3. Xue, B., He, D., Gao, S., Wang, D., Yokoyama, K., Wang, L., (2016). "Biosynthesis of silver nanoparticles by the fungus *Arthroderma fulvum* and its antifungal activity against genera of *Candida, Aspergillus* and *Fusarium", International journal of nanomedicine.*, 11: 1899-1906.
4. Kaviya, S., santhanalakshmi, J., viswanathan, B., (2011). "Green synthesis of silver nanoparticles using *Polyalthia longifolia* leaf extract along with D-sorbitol: study of antibacterial activity", *Journal of nanotechnology*, Article ID 152970.
5. Saxena, A., Tripathi, R. M., Zafar, F., Singh, P., (2012). "Green synthesis of silver nanoparticles using aqueous solution of *Ficus benghalensis* leaf extract and characterization of their antibacterial activity", *Material Letters.*, 67(1): 91-94.
6. Khalil, K. A., Fouad, H., Elsarnagawy, T., Almajhdi, F. N., (2013). "Preparation and characterization of electrospun PLGA/silver composite nanofibers for biomedical applications", *International journal of electrochemical science*, 8(3): 3483-3493.
7. Jha, R. K., Jha, P. K., Chaudhury, K., Rana, S. V., Guha, S. K., (2014). "An emerging interface between life science and nanotechnology: present status and prospects of reproductive healthcare aided by nano-biotechnology", *Nano reviews*, 5(1): 22762.
8. Niraimathi, K. L., Sudha, V., Lavanya, R., Brindha, P., (2013). "Biosynthesis of silver nanoparticles using *Alternanthera sessilis* (Linn.) extract and their antimicrobial, antioxidant activities", *Colloids and surfaces B: biointerfaces*, 102, 288-291.
9. El-Chaghaby, Ghadir A., Ahmad, Abeer F., (2011). "Biosynthesis of silver nanoparticles using *Pistacia lentiscus* leaves extract and investigation of their antimicrobial effect", *Oriental journal of chemistry*, 27(3): 929-936.
10. Veerasamy, R., Xin, T. Z., Gunasagaran, S., Xiang, T. F. W., Yang, E. F. C., Jeyakumar, N., Dhanaraj, S. A., (2011). "Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities", *Journal of saudi chemical society*, 15(2): 113-120.
11. Wong, K. K., Cheung, S. O., Huang, L., Niu, J., Tao, C., Ho, C. M., Tam, P. K., (2009). "Further evidence of the anti-inflammatory effects of silver nanoparticles", *ChemMedChem: Chemistry enabling drug discovery*, 4(7): 1129-1135.
12. Baruwati, B., Polshettiwar, V., Varma, R. S., (2009). "Glutathione promoted expeditious green synthesis of silver nanoparticles in water using microwaves", *Green chemistry*, 11(7): 926-930.
13. Popescu, M., Velea, A., Lőrinczi, A., (2010). "Biogenic production of nanoparticles", *Digest journal of nanomaterials & biostructures (DJNB)*, 5(4): 1035-1040.
14. Sowbarnika, R., Anhuradha, S., Preetha, B., (2018). "Enhanced antimicrobial effect of yeast mediated silver nanoparticles synthesized from baker's yeast", *International Journal of nanoscience and nanotechnology*, 14(1): 33-42.
15. Kurian, M., Varghese, B., Athira, T. S., Krishna, S., (2016). "Novel and efficient synthesis of silver nanoparticles using *Curcuma longa* and *Zingiber officinale* rhizome extracts", *International Journal of nanoscience and nanotechnology*, 12(3): 175-181.

16. Ajitha, B., Reddy, Y. A. K., Reddy, P. S., (2015). "Green synthesis and characterization of silver nanoparticles using *Lantana camara* leaf extract", *Materials science and engineering: C*, 49: 373-381.
17. Guo, M., Li, W., Yang, F., & Liu, H., (2015). "Controllable biosynthesis of gold nanoparticles from a *Eucommia ulmoides* bark aqueous extract", *Spectrochimica acta part A: molecular and biomolecular spectroscopy*, 142: 73-79.
18. Mondal, S., Roy, N., Laskar, R. A., Sk, I., Basu, S., Mandal, D., Begum, N. A., (2011). "Biogenic synthesis of Ag, Au and bimetallic Au/Ag alloy nanoparticles using aqueous extract of mahogany (*Swietenia mahogani* JACQ.) leaves", *Colloids and surfaces B: biointerfaces*, 82(2): 497-504.
19. Thuesombat, P., Hannongbua, S., Akasit, S., Chadchawan, S., (2014). "Effect of silver nanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth", *Ecotoxicology and environmental safety*, 104: 302-309.
20. Aromal, S. A., Philip, D., (2012). "Green synthesis of gold nanoparticles using Trigonella foenum-graecum and its size-dependent catalytic activity", *Spectrochimica acta Part A: molecular and biomolecular spectroscopy*, 97: 1-5.
21. Lok, C. N., Zou, T., Zhang, J. J., Lin, I. W. S., Che, C. M., (2014). "Controlled-release systems for metal-based nanomedicine: encapsulated/self-assembled nanoparticles of anticancer gold (III)/platinum (II) complexes and antimicrobial Silver Nanoparticles", *Advanced materials*, 26(31): 5550-5557.
22. Neves, L. C., Campos, A. J. D., Benedette, R. M., Tosin, J. M., Chagas, E. A., (2012). "Characterization of the antioxidant capacity of natives fruits from the Brazilian amazon region", *Revista brasileira de fruticultura*, 34(4): 1165-1173.
23. Bataglion, G. A., da Silva, F. M., Eberlin, M. N., Koolen, H. H., (2015). "Determination of the phenolic composition from Brazilian tropical fruits by UHPLC–MS/MS", *Food chemistry*, 180: 280-287.
24. Cetó, X., Capdevila, J., Mínguez, S., del Valle, M., (2014). "Voltammetric BioElectronic Tongue for the analysis of phenolic compounds in rosé cava wines", *Food research international*, 55: 455-461.
25. Cerón, I. X., Higuita, J. C., Cardona, C. A., (2015). "Analysis of a biorefinery based on *Theobroma grandiflorum* (copoazu) fruit", *Biomass conversion and biorefinery*, 5(2): 183-194.
26. Groove, D. C., Randal, W. A., (1955). "Assay methods antibiotic activity: a laboratory manual", *New York, Medical Enciclopedia*.
27. Wayne, P. A., (2011). "Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing".
28. Ankanna, S. T. N. V. K. V. P., Prasad, T. N. V. K. V., Elumalai, E. K., Savithramma, N., (2010). "Production of biogenic silver nanoparticles using *Boswellia ovalifoliolata* stem bark", *Digest Journal of nanomaterials and biostructures*, 5(2): 369-372.
29. Ahmed, S., Saifullah, Ahmad, M., Swami, B. L., & Ikram, S., (2016). "Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract", *Journal of radiation research and applied sciences*, 9(1): 1-7.
30. Khatami, M., Soltani Nejad, M., Pourseyedi, S., (2015). "Biogenic synthesis of silver nanoparticles using mustard and its characterization", *International journal of nanoscience and nanotechnology*, 11(4): 281-288.
31. Cruz, D., Falé, P. L., Mourato, A., Vaz, P. D., Serralheiro, M. L., Lino, A. R. L., (2010). "Preparation and physicochemical characterization of Ag nanoparticles biosynthesized by *Lippia citriodora* (*Lemon verbena*)", *Colloids and surfaces B: biointerfaces*, 81(1): 67-73.
32. Ajitha, B., Reddy, Y. A. K., Reddy, P. S., Suneetha, Y., Jeon, H. J., Ahn, C. W., (2016). "Instant biosynthesis of silver nanoparticles using *Lawsonia inermis* leaf extract: Innate catalytic, antimicrobial and antioxidant activities", *Journal of molecular liquids*, 21: 474-481.
33. Ahmad, N., Shree, K., Srivastava, M., & Dutta, R., (2014). "Novel rapid biological approach for synthesis of silver nanoparticles and its characterization", *International journal of pharmacology*, 1(1): 28-31.
34. Velmurugan, P., Anbalagan, K., Manosathyadevan, M., Lee, K. J., Cho, M., Lee, S. M., Oh, B. T., (2014). "Green synthesis of silver and gold nanoparticles using *Zingiber officinale* root extract and antibacterial activity of silver nanoparticles against food pathogens", *Bioprocess and biosystems engineering*, 37(10): 1935-1943.
35. de Lima Yamaguchi, K. K., Pereira, L. F. R., Lamarao, C. V., Lima, E. S., da Veiga-Junior, V. F., (2015). "Amazon acai: Chemistry and biological activities: A review", *Food chemistry*, 179: 137-151.
36. Yang, H., Protiva, P., Cui, B., Ma, C., Baggett, S., Hequet, V., Kennelly, E. J., (2003). "New bioactive polyphenols from *Theobroma grandiflorum* ("Cupuaçu")", *Journal of natural products*, 66(11): 1501-1504.
37. Domingues de Souza, L. C., de Sá, M. E., de Moraes, B., Maria, S., de Carvalho, C., Antonio, M., Abrantes, F. L., (2012). "Composição química e nutrientes em sementes das espécies florestais Pente de Macaco, Flor de Paca, Itaúba, Jatobá e Murici Manso", *Bioscience journal*, 478-483.
38. Nabikhan, A., Kandasamy, K., Raj, A., Alikunhi, N. M., (2010). "Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum* L", *Colloids and surfaces B: biointerfaces*, 79(2): 488-493.

39. Ibrahim, H. M., (2015). “Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms”, *Journal of radiation research and applied sciences*, 8(3): 265-275.
40. Rajeshkumar, S., Malarkodi, C., Vanaja, M., Annadurai, G., (2016). “Anticancer and enhanced antimicrobial activity of biosynthesizd silver nanoparticles against clinical pathogens”, *Journal of molecular structure*, 1116: 165-173.
41. McQuillan, J. S., Groenaga Infante, H., Stokes, E., Shaw, A. M., (2012). “Silver nanoparticle enhanced silver ion stress response in *Escherichia coli* K12”, *Nanotoxicology*, 6(8): 857-866.
42. Bindhu, M. R., Umadevi, M., (2015). “Antibacterial and catalytic activities of green synthesized silver nanoparticles”, *Spectrochimica acta part A: molecular and biomolecular spectroscopy*, 135: 373-378.
43. Ahmed, S., Ikram, S., (2015). “Silver nanoparticles: one pot green synthesis using *Terminalia arjuna* extract for biological application”, *Journal of nanomedicine and nanotechnology*, 6(4): 309-314.
44. Anandalakshmi, K., Venugobal, J., Ramasamy, V., (2016). “Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity”, *Applied nanoscience*, 6(3): 399-408.
45. Rao, N. H., Lakshmidevi, N., Pammi, S. V. N., Kollu, P., Ganapaty, S., & Lakshmi, P., (2016). “Green synthesis of silver nanoparticles using methanolic root extracts of *Diospyros paniculata* and their antimicrobial activities”, *Materials science and engineering: C*, 62: 553-557.
46. Hadi Soltanabad, M., Bagherieh-Najjar, M. B., Kohan Baghkheirati, E., & Mianabadi, M., (2018). “Ag-Conjugated nanoparticle biosynthesis mediated by Rosemary leaf extracts correlates with plant antioxidant activity and protein contente”, *International journal of nanoscience and nanotechnology*, 14(4): 319-325.
47. Durán, N., Durán, M., de Jesus, M. B., Seabra, A. B., Fávaro, W. J., Nakazato, G., (2016). “Silver nanoparticles: a new view on mechanistic aspects on antimicrobial activity”, *Nanomedicine: nanotechnology, biology and medicine*, 12(3): 789-799.
48. M Vanaja, M., Annadurai, G., (2013). “Coleus aromaticus leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity”, *Applied nanoscience*, 3(3): 217-223.
49. Liu, J., Sonshine, D. A., Shervani, S., Hurt, R. H., (2010). “Controlled release of biologically active silver from nanosilver surfaces”, *ACS nano*, 4(11): 6903-6913.
50. Gopinath, V., MubarakAli, D., Priyadarshini, S., Priyadharsshini, N. M., Thajuddin, N., Velusamy, P., (2012). “Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach”, *Colloids and surfaces B: biointerfaces*, 96: 69-74.
51. Nel, A. E., Mädler, L., Velegol, D., Xia, T., Hoek, E. M., Somasundaran, P., Thompson, M., (2009). “Understanding biophysicochemical interactions at the nano–bio interface”, *Nature materials*, 8(7): 543-557.
52. Marambio-Jones, C., Hoek, E. M., (2010). “A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment”, *Journal of nanoparticle research*, 12(5): 1531-1551.
53. Hussain, S. M., Javorina, A. K., Schrand, A. M., Duhart, H. M., Ali, S. F., Schlager, J. J., (2006). “The interaction of manganese nanoparticles with PC-12 cells induces dopamine depletion”, *Toxicological sciences*, 92(2): 456-463.
54. Kim, S. W., Jung, J. H., Lamsal, K., Kim, Y. S., Min, J. S., Lee, Y. S., (2012). “Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi”, *Mycobiology*, 40(1): 53-58.
55. Sharma, V. K., Yngard, R. A., Lin, Y., (2009). “Silver nanoparticles: green synthesis and their antimicrobial activities”, *Advances in colloid and interface science*, 145(1-2): 83-96.
56. Verma, D. K., Hasan, S. H., Banik, R. M., (2016). “Photo-catalyzed and phyto-mediated rapid green synthesis of silver nanoparticles using herbal extract of Salvinia molesta and its antimicrobial efficacy”, *Journal of photochemistry and photobiology B: biology*, 155: 51-59.
57. Dipankar, C., Murugan, S., (2012). “The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts”, *Colloids and surfaces B: biointerfaces*, 98: 112-119.