

## ***Evaluation of the antimicrobial effect of silver nanoparticles obtained by the green synthesis route against bacteria of medical importance***

Metal nanoparticles are useful in the most varied fields of application, in catalysis processes, in optical systems, biological systems, in the pharmaceutical and electronics industry, among others. This is explained by the differential characteristics arising from its large surface area and the characteristics inherent to the properties related to the metallic nature of each particle, such as antimicrobial, antibacterial, antiviral and anticancer effects, observed for silver nanoparticles (AgNPs), for example. AgNPs can be obtained through green synthesis routes, an environmentally friendly alternative, cheap, fast and without the generation of toxic reagents, compared to the traditional physicochemical methodology. In this sense, the objectives of the present work were the synthesis of AgNPs using aqueous foliar extracts of *Panicum maximum* and the evaluation of their effect against bacteria with relevance to public health. The presence of AgNPs was confirmed by the color change of the reaction medium and by the spectroscopic profile obtained. Applying the disk diffusion technique in petri dishes containing Mueller Hinton culture medium, formation of inhibition halos was observed against *E. coli* (ATCC 1122), *S. aureus* (ATCC 29213), *S. epidermidis* (ATCC 12228) and *P. aeruginosa* (ATCC 25619). This is the first record of the use of aqueous foliar extracts of *P. maximum*, originating in the Midwest of Minas Gerais, used in the synthesis of AgNPs whose antimicrobial effects are marked by the uniqueness and whose results are quite promising.

**Keywords:** *Panicum maximum*; AgNPs; Bacteria; Inhibition.

## ***Avaliação do efeito antimicrobiano de nanopartículas de prata obtidas por rotas de síntese verde frente a bactérias de importância médica***

Nanopartículas metálicas são úteis nos mais variados campos de aplicação, em processos de catálise, em sistemas ópticos, sistemas biológicos, na indústria farmacêutica e eletroeletrônica, dentre outros. Isso se explica pelas características diferenciais advindas de sua grande área superficial e as características inerentes as propriedades relacionadas à natureza metálica de cada partícula, como efeitos antimicrobianos, antibacterianos, antivirais e anticâncer, observados para nanopartículas de prata (AgNPs), por exemplo. As AgNPs podem ser obtidas por meio de rotas de síntese verde, alternativa ambientalmente correta, barata, rápida e sem geração de reagentes tóxicos, comparativamente a metodologia físico-química tradicional. Neste sentido, os objetivos do presente trabalho foram a síntese de AgNPs utilizando-se de extratos aquosos foliares de *Panicum maximum* e a avaliação de seu efeito frente a bactérias com relevância em saúde pública. A presença das AgNPs confirmou-se pela mudança de coloração do meio reacional e pelo perfil espectroscópico obtido. Aplicando-se a técnica de disco-difusão em placas de petri contendo meio de cultura Mueller Hinton observou-se formação de halos de inibição frente a *E. coli* (ATCC 1122), *S. aureus* (ATCC 29213), *S. epidermidis* (ATCC 12228) e *P. aeruginosa* (ATCC 25619). Trata-se do primeiro registro de uso de extratos foliares aquosos de *P. maximum*, originários do Centro Oeste de Minas, utilizados na síntese de AgNPs cujos efeitos antimicrobianos são marcados pelo indetimento e cujos resultados obtidos são bastante promissores.

**Palavras-chave:** *Panicum maximum*; AgNPs; Bactérias; Inibição.

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## INTRODUCTION

Metallic nanoparticles presents an immense surface area as a peculiar characteristic, implying a series of relevant properties, including physical, biological, optical and magnetic (MELO, 2020). This characteristic stimulates intense research in various fields of application, such as in catalysis processes (NASROLLAHZADEH, 2019), optical systems (CHEN, 2018), electronics area (TAN et al., 2019), biomaterials production (HOSTERT, 2018). In addition to presenting, in some cases, antibacterial, antimicrobial and anticancer effects (PATIL, 2016).

In recent decades, with the development of nanotechnology, the use of silver nanoparticles (AgNPs) have stood out as a promising alternative in the prevention of microbial diseases and cancer (FATTAH et al., 2018; KAILASA, 2019). They are among the most popular metallic nanoparticles due to their chemical and physical properties and versatility of applications (SRIKAR et al., 2016). However, their toxicity and low biocompatibility, due to the synthesis methodology, has limited its potential for use (SOUZA et al., 2019). Usually, silver nanoparticles are prepared by physical and chemical methods, often expensive, energy-intensive and use chemical reagents with varying degrees of toxicity. Such chemical reagents promote the generation of waste potentially hazardous to health and the environment (RAO, 2017). Thus, the development of biosynthesis methodologies aiming to obtain silver nanoparticles based on silver ions and natural plant extracts, rich in reducing and stabilizing agents, changes this perspective.

Green synthesis is a biological process in which biomolecules present in natural plant extracts reduce metal ions, forming nanoparticles. The green synthesis methodology allows obtaining nanoparticles with greater biocompatibility, absence of the use of toxic chemicals, faster than conventional processes, low cost and sustainable from an environmental point of view (AWWAD, 2020; KUMAR, 2017; RAFIQUE, 2017). Brazil, for being recognized worldwide as one of the largest holders of plant biodiversity on the planet, gathering values greater than 40,000 plant species already cataloged, has great potential for development and global autonomy in this particular, besides the fact that it is among the main participants of the Global Strategy for Plant Conservation - GSPC (FIORAVANTI, 2016; FILARD, 2018). Some studies have indicated that secondary metabolites found in essential oils of some plants represent the main agents that act in the reduction of metal ions and formation of nanoparticles from the use of plant material (MARSLIN, 2018; OWONUBI, 2019; OVAIS, 2018). In addition, these phytochemicals are also responsible for encapsulation preventing subsequent aggregation of AgNPs (AHMAD, 2019).

Based on the above, the present work aimed to use leaf extracts of *Panicum maximum* (Poales: Poaceae) to obtain AgNPs through green synthesis. *P. maximum* is a grass native to Africa, forage, perennial and easily accessible, where in Brazil it is widely cultivated for livestock activity (QUARRY, 2015; YAMORI et al., 2013). It has a cespitosis growth, forming clumps, decumbent leaves without pilosity, and lightly purplish stems (GOMIDE, 2016). The innovative character of this report deserves special attention, given that there are no records in the literature on the evaluation of the green synthesis and antimicrobial potential of AgNPs using leaf extracts of the exotic species *P. maximum*, from the Cerrado of southeastern Brazil. It has already

been proven antimicrobial effect of AgNPs, obtained through green synthesis routes, medically important bacteria (KUMAR, 2017). We sought to characterize the AgNPs obtained and evaluate their antimicrobial effect against *Enterococcus faecalis* (ATCC 14506), *Escherichia coli* (ATCC 1122), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus mutans* (ATCC 10449) and *Pseudomonas aeruginosa* (ATCC 25619)

## MATERIALS AND METHODS

### Plant species

To obtain plant extracts, aerial parts (leaves) of *P. maximum* known as 'Mombaça grass', were collected on the campus of the UEMG Unit Divinópolis, MG.

### Preparation of plant extracts

Leaves of *P. maximum specimens* were washed with tap water, followed by distilled water and a cleaning cycle with ethanol, then removed with ultrapure water. The leaves were air-dried and then fragmented into small pieces for later weighing. Portions of 10 g of the obtained material were boiled for 3 minutes in 50 mL of ultrapure water, using beakers and watch glasses, placed on a magnetic stirrer with heating. After resting for one minute, the material was filtered on Whatman N. 1 filter paper and the filtrate was used in the subsequent green synthesis step of AgNPs. All experimental steps were performed at the Microbiology Laboratory of UEMG-Divinópolis MG Unit, between October and November 2020 and January 2021.

### Green synthesis of AgNPs

Initially, a volume of 45 mL of silver nitrate aqueous solution (Dinamica®) at 1 mM was prepared, to which 5 mL of the aqueous leaf extract obtained in the previous step were added by titration. From this experimental stage, special attention was paid to the change in the coloration of the plant extract, turning from light yellow to brown, after the addition of silver nitrate solution, indicative of the presence of silver nanoparticles in solution. Subsequently, the reaction mixture was centrifuged at 6000 rpm (Thermo®), the supernatant was discarded and the pellet was resuspended in ultrapure water, being kept in aluminum foil-lined falcon tubes in a refrigerator. The evaluation of the presence of AgNPs was confirmed via spectroscopy in the ultraviolet visible region (UVVis), from the analysis of the absorption curves obtained in scanning spectra using a spectrophotometer with selected wavelengths for the range from 300 to 600 nm.

### Microorganisms

The microorganisms used in this study come from the American Type Culture Collection (ATCC), provided by the Oswaldo Cruz Foundation's Collection of Reference Microorganisms in Sanitary Surveillance (FIOCRUZ, Rio de Janeiro, Brazil). A total of six bacterial strains, two Gram-negative species (*Escherichia coli*

ATCC 1122 and *Pseudomonas aeruginosa* ATCC 25619), and four Gram-positive species (*Enterococcus faecalis* ATCC 14506, *Staphylococcus aureus* ATCC 29213, *Streptococcus mutans* ATCC 10449 and *Staphylococcus epidermidis* ATCC 12228) were used in the biological assays. The isolates were reactivated under refrigeration at 4°C in Nutrient Agar (Himedia®, India) for use in the experimental stages, obtained from stock kept in an ultrafreezer at -80°C.

### **Evaluation of the antimicrobial effect of silver nanoparticles obtained by green synthesis**

Initially, bacterial strains were reactivated in Nutrient Agar (Himedia®, India). and then incubated in a bacteriological oven at 37°C for 24h in order to evaluate the presence of pure cultures on the plates. To obtain bacterial inoculum, isolated colonies grown on solid media were inoculated into Erlenmeyers containing Mueller Hinton Agar (Himedia®, India), subsequently incubated in a Shaker orbital shaker at 37°C for 24h.

After that period, bacterial aliquots of the different samples were adjusted, with the aid of a spectrophotometer at 625nm, to obtain suspensions containing approximately  $1.5 \times 10^6$  CFU/mL. After that, in order to evaluate the antimicrobial effect of the synthesized AgNPs, the disk-diffusion technique was applied using petri dishes containing Mueller Hinton Agar medium, previously inoculated with bacterial strains by the spread-plate technique. Volumes of 10 µL of aqueous solutions containing the silver nanoparticles were dispensed into the disks deposited on the surface of the plates with the solid medium. After incubation in a bacteriological oven at 37°C for 24h, the plates were evaluated for inhibition of microbial growth around the discs by measuring the diameter (mm) of the inhibition halos formed. Assays were performed in triplicate and control plates containing only aliquots of plant extracts added to discs were also evaluated.

### **Data analysis**

For data analysis, the mean and standard deviation of the diameter measurements of the inhibition halos formed for the control and treatment plates were calculated. Three replications were performed for all tests carried out; the controls being represented by plates with disks containing samples of plant extracts only.

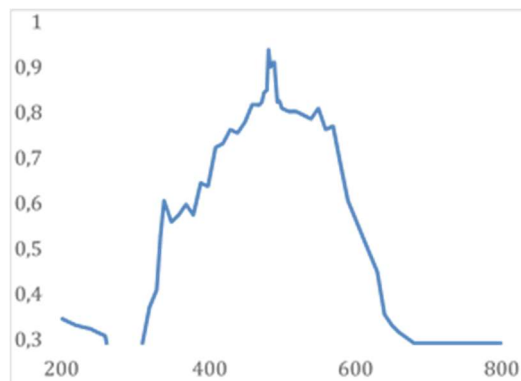
## **RESULTS AND DISCUSSION**

### **Synthesis of AgNPs**

From the volume titration of the aqueous leaf extract of *P. maximum* in silver nitrate solution, a dark brown solution was obtained, indicating the presence of silver nanoparticles (Fig. 1). This fact is reported in the literature by several other authors, such as in the work of Raj et al. (2018). The presence of silver nanoparticles in solution was also confirmed by spectroscopy in the ultraviolet visible region (UV-Vis), due to the plasmon resonance effect, as shown in Graph 1, with higher absorption peaks in the range of 450-500nm.



**Fig. 1:** Illustration of the dark brown colored solution indicative of the presence of AgNPs.



**Fig. 2:** Profile in absorption gives radiation at region UV-VIS for The solution of AgNPs.

### Evaluation of the antimicrobial effect of AgNPs

Through the disk-diffusion technique, different diameters of inhibition halos were obtained for the evaluated bacteria (Table 1). For *E. coli* (ATCC 1122) an average of 13.6 mm was obtained, for *E. faecalis* (ATCC 14506) the average obtained was 4.0 mm, for *S. aureus* (ATCC 29213) an average of 13.6 mm, while *S. epidermidis* (ATCC 12228) showed growth inhibition with an average diameter of 13.6 mm. For *S. mutans* (ATCC 10449), the average found was 12.3 mm and for *P. aeruginosa* (ATCC 25619) an average inhibition halo of 13.6 mm.

**Table 1:** Diameters (mm) of the inhibition halos obtained against the species reference bacterial tested.

Microorganisms	Diameter of the inhibition halos (mm)			Average	Standard deviation
<i>AND. coli</i> (ATCC 1122)	13.0	15.0	13.0	13.6	1.15
<i>E. Faecalis</i> (ATCC 14506)	3.0	6.0	3.0	4.0	1.73
<i>S. aureus</i> (ATCC 29213)	13.0	15.0	13.0	13.6	1.15
<i>S. epidermidis</i> (ATCC 12228)	13.0	13.0	15.0	13.6	1.15
<i>S. mutans</i> (ATCC 10449)	12.0	12.0	13.0	12.3	0.57
<i>P. aeruginosa</i> (ATCC 25619)	15.0	13.0	13.0	13.6	1.15

For the control plates, the formation of inhibition halos was not observed in any of the analyzed cases. Among the components used in the green synthesis of metallic nanoparticles, with application in studies of the biological potential and reduction of ions by extracts from various parts of different plant species such as leaves, flowers, tissues and organs have been reported in the literature (KHAN, 2018; YADI et al., 2018). Secondary compounds, such as amino acids, flavonoids, alkaloids, phenolics, terpenoids, peptides, polysaccharides and functional groups (alcohols, aldehydes, amines) present in plants can act as chemical agents to reduce metallic salts, resulting in the production of nanoparticles, example of silver nanoparticles (SILVA, 2017; MASHWANI, 2016; CHUNG, 2016).

It is important to note that the concentration of these constituents in plants depends on a number of factors, such as location, time of year, climate, soil, humidity and sun exposure, to which plants are subjected (YANG et al., 2018). In this study, leaf extracts of the plant *P. maximum* were used, through which the reduction of silver ions in solution was effected, leading to the formation of nanoparticles. It is important to note that there are few reports in the literature on the use of *P. maximum* for the green synthesis of silver nanoparticles, this being the first study related to plant samples from the Midwest Region of Minas Gerais.

Nanoparticles have optical properties that vary according to the wavelength of visible light. Because

are nanostructured particles, when in contact with metallic silver, these optical properties change (BONATO, 2014). Due to the excitation of the SPR vibrations of the AgNPs during the biosynthesis process, a color change occurs in the solution (REN, 2016).

The leaf extract of the plant *P. maximum*, used in the study, has alkaloids, tannins, saponins and flavanoids as its main phytochemical constituents in different percentages (KANIFE, 2012), which can act in the process of silver reduction, formation of nanoparticles and complexation of same. AgNPs have the ability to attach to the cell membrane surface and penetrate into the bacterial cell, causing disturbances and complications in the functioning of cellular respiration (SHARMA, 2009).

The bacteria *E. coli* (ATCC 1122), *E. faecalis* (ATCC 14506), *S. aureus* (ATCC 29213), *S. epidermidis* (ATCC 12228), *S. mutans* (ATCC 10449) and *P. aeruginosa* (ATCC 25619) are potential infectious agents, with great relevance and repercussion in public health. The antimicrobial effect obtained for the synthesized nanoparticles indicated similar inhibition halo diameters for *E. coli* (ATCC 1122) isolates (Figure 3), *S. aureus* (ATCC 29213) (Figure 4), *S. epidermidis* (ATCC 12228) (Figure 5), and *P. aeruginosa* (ATCC 25619) (Figure 6). The average value of the diameter of the inhibition halos corresponded to 13.6 mm, proving the efficiency of AgNPs in terms of inhibiting the growth of those microorganisms.



Fig. 3: Control plates and treatment against *E. coli* (ATCC 1122).



Fig. 4: Control and treatment plates against *S. aureus* (ATCC 29213)



Fig. 5: Control and treatment plates for *S. epidermidis* (ATCC 12228).



Fig. 6: Control plates and treatment against *P. aeruginosa* (ATCC 25619).

For the *S. mutans* isolate (ATCC 10449) (Figure 7), silver nanoparticles also inhibited microbial growth. However, compared to the bacteria mentioned above, the value related to the diameter of the inhibition halos was smaller, 12.3 mm.



**Fig. 7:** Control and treatment plates for *S. mutans* (ATCC 10449).



**Fig. 8:** Control plates and treatment against *E. faecalis* (ATCC 14506).

For *E. faecalis* (ATCC 14506) (Figure 8) the mean value of the inhibition halo was significantly lower, ie, 4.0mm.

It is important to highlight that no inhibition halo formation was observed for any of the control plates, the antimicrobial action was achieved due to the nanoparticles present in the disks, and not because of the inhibitory action of eventual metabolites present in the aqueous extracts themselves, as evidenced in the plates control.

The green synthesis process of AgNPs using *P. maximum* leaf extracts, was satisfactory, indicating for the formation of nanoparticles in solution, with proven antimicrobial effect. The results of the present work are in agreement with the findings of Ingle, with regard to the antibacterial effects of nanoparticles obtained by the green route (INGLE, 2008). Based on the studies developed, it was not possible to establish a relationship between the bacterial cell wall composition and the growth effects observed for the microbial species tested in this work.

Obtaining AgNPs from plant extracts is utmost importance, relevant from a therapeutic point of view, given the absence of toxic constituents in the final formulation (KHAMENEH, 2016; MANANDHAR, 2019). Numerous sectors of society can benefit from the development of new products generated by green synthesis, due to the environmental, economic and social benefits, incentive and expansion of interactions between industry, academia and government institutions, with the ultimate purpose of improving quality of life of society as a whole (CASANOVA, 2010). The relevance of this work stands out for the promising results obtained, from the microbiological point of view, for the originality of the application of the plant sample used for the purpose for which it was intended.

## CONCLUSIONS

From the results obtained in this work, it can be concluded that *P. maximum* leaf extracts are useful for application in processes of green synthesis of AgNPs. Moreover, the nanoparticles obtained were effective in inhibiting microbial growth, especially against *E. coli* (ATCC 1122), *S. aureus* (ATCC 29213), *S. epidermidis* (ATCC 12228) and *P. aeruginosa* (ATCC 25619). It is necessary to carry out additional studies with the purpose of elucidating the mechanism of action of silver nanoparticles on microbial cells, as well as expanding the list of bacterial species evaluated, evaluating possible additive or synergistic effects when associated with other compounds, as well as studying possible effectiveness when using other parts of the

plant.

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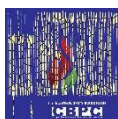
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