

Bactericidal potential of two species typical of Brazilian cerrado: *Stryphnodendron obovatum* and *Lafoensia pacari*

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ABSTRACT: This study aimed to evaluate the bactericidal potential of plant extracts from medicinal plants of the Brazilian cerrado against microorganisms associated with gastrointestinal infections, through the disc-diffusion method. Two typical cerrado plants were selected: *Stryphnodendron obovatum* and *Lafoensia pacari*. The leaves were crushed and reduced to the powder state, and for the extraction of substances 100% methanol was used as the solvent followed by the proportion of 1 g and 2 g of vegetable powder 10 ml of solvent [extracts 1:10 and 2:10 (p/v)] and the microorganisms submitted to the test were *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella sp.* The results showed that *L. pacari* presented a better percentage of bactericidal activity by the disc-diffusion method in all microorganisms tested with inhibition halos between 15 and 20 mm in both concentrations; *S. obovatum* showed only antimicrobial activity in *S. aureus* cultures at both concentrations with inhibition halos between 12 and 16 mm. Both species present great possibilities of being useful in the fight against multiresistant bacteria, allowing the investigation and the isolation guided by bioassays as well as the future identification of new bioactive compounds. It's concluded that it is necessary to fill gaps related to the scientific knowledge of bioactive compounds produced by species of the Brazilian Cerrado biome.

Key words: Brazilian cerrado, bactericidal, extracts, medicinal plants, herbal medicines

RESUMO: Potencial bactericida de duas espécies típicas do cerrado brasileiro: *Stryphnodendron obovatum* e *Lafoensia pacari*. Este trabalho teve por objetivo avaliar o potencial bactericida de extratos vegetais de plantas medicinais do Cerrado Brasileiro frente a microrganismos associados a infecções gastrintestinais, através do método de disco-difusão. Duas plantas típicas do cerrado foram selecionadas: *Stryphnodendron obovatum* e *Lafoensia pacari*. As folhas foram trituradas e reduzidas ao estado de pó, e para a extração de substâncias foi utilizado metanol 100% como solvente seguido pela proporção de 1 g e 2 g de pó vegetal 10 ml de solvente [extratos 1:10 e 2:10 (p/v)] e os microrganismos submetidos ao teste foram *Escherichia coli*, *Staphylococcus aureus* e *Klebsiella sp.* Os resultados demonstraram que *L. pacari* apresentou um melhor percentual de atividade bactericida pelo método de disco-difusão em todos os microrganismos testados com halos de inibição entre 15 e 20 mm em ambas as concentrações; *S. obovatum* apresentou apenas atividade antimicrobiana em culturas de *S. aureus* em ambas as concentrações com halos de inibição entre 12 e 16 mm. Ambas as espécies apresentam grandes possibilidades de serem úteis na luta contra bactérias multirresistentes, permitindo a investigação e o isolamento guiados por bioensaios, bem como a futura identificação de novos compostos bioativos. Conclui-se que é necessário preencher lacunas relacionadas ao conhecimento científico de compostos bioativos produzidos por espécies do bioma Cerrado brasileiro.

Palavras-chave: Cerrado brasileiro, bactericida, extratos, plantas medicinais, fitoterápicos

INTRODUCTION

The indiscriminate use of antibiotics, without a careful evaluation of their appropriate indications, can lead to the growth of resistant bacterial strains, that is, to select resistant microorganisms. Antibiotic resistance develops as a natural consequence of the bacterial population's ability to adapt. The indiscriminate use of antibiotics increases the selective pressure and the opportunity of the bacteria to be exposed to them, facilitating the acquisition of mechanisms of resistance (Santos 2004; Nicolini et al. 2008; Zaman et al. 2017). These mechanisms can be observed in the genus *Klebsiella* Cassettari et al. (2006), which reported the progression of antibiotic resistance in bacteria with the appearance of *Klebsiella pneumoniae* producing Extended Spectrum Beta-Lactamase (ESBL), class of enzymes that confer resistance to all cephalosporins. In 2011 in Germany an outbreak of Shiga-toxicogenic *Escherichia coli* was reported leading to a significant increase in the number of patients with hemolytic uremic syndrome and bloody diarrhea caused by this bacterial strain. *Escherichia coli* is a Gram-negative bacillus belonging to the family Enterobacteriaceae and it has been proven that the species has several strains multiresistant to current antimicrobials (Cassettari et al. 2006).

In an attempt to minimize, the problems related to bacterial resistance, new investigations for active principles in native plants have been increasing due to the investments in research of alternative medicines due to their therapeutic actions (Bakal et al. 2017). Phytochemical research is important, especially when complete chemical studies with species of popular interest are not available; its objective is to determine and quantify the chemical compounds, many of which exert several functions important to the survival of the plant, such as, for example, defense against pathogenic microorganisms and parasites. However, for the study of biological activities of plant extracts it is important to choose bioassays to detect the specific effects, which are simple, sensitive, and reproducible (Sousa et al. 2008; Giordani et al. 2015).

Regarding native medicinal plants, we highlight those belonging to the Cerrado, the second largest biome in Brazil, identified as one of the richest in biodiversity, and considered an important source of innovative plant molecules for several conditions, including infectious diseases (Guarim-Neto and Morais 2003). Among its various medicinal species consecrated by popular use, we can mention the genus *Lafoensia* of the family Lythraceae, popularly known as "dedaleiro", "louro-da-serra", "mangava-brava" (Santos et al. 2009). Its infused leaves are used as diaphoretic, the macerated shell in water

is used to treat ulcers and external wounds such as cicatrizante and the fruits of the plant are used to treat pneumonia (Rogerio et al. 2006). The genus *Stryphnodendron* of the family Fabaceae ("barbatimão"), in the popular medicine is used in the treatment of inflammatory processes, like cicatrizante, against diarrhea, sorghum and "braba" wound (Russo et al. 1990; Vasconcelos et al. 2004; Ramos et al. 2007).

In recent years, there has been a growing interest in the scientific community for medicinal plants and phytotherapy because they have therapeutic and economic potentials, especially target at the pharmaceutical industry that prospects for new products, with less undesirable effects than existing drugs (Santos et al. 2011). The most relevant factor for such growth is summarized in the evolution of scientific studies, highlighting the discovery of the efficacy of medicinal plants, especially those that are used by the population for therapeutic purposes, through the chemical and pharmacological studies (Cechinel Filho and Yunes 1998).

Therefore, this study aimed to evaluate the bactericidal activity of plant extracts produced from medicinal plants of the Brazilian cerrado against microorganisms generally associated with gastrointestinal infections by means of the Disk-diffusion method in agar.

MATERIALS AND METHODS

Plant Material

Leaves and branches were collected from two native plant species found in the Cerrado of the East Coast of the State of Mato Grosso do Sul: *Stryphnodendron obovatum* Benth. and *Lafoensia pacari* A. St.-Hil. The botanical material was collected in Jardim Santa Júlia and in the Campus of the Federal University of Mato Grosso do Sul, Unit II, in the city of Três Lagoas and the species identified by Professor PhD Maria José Neto. The specimens are deposited in the collection of the Herbarium, on the campus of the Federal University of Mato Grosso do Sul. The plant material was washed, sanitized with 2% sodium hypochlorite solution for 1 h and rinsed in distilled water; then it was packed in paper bags and dried in an air-circulating oven at 42 °C to eliminate humidity and stabilize the enzymatic content.

Obtaining extracts

To get the extracts, the leaves and branches were crushed in a knife mill and the vegetable powder subjected to the extraction by maceration process for ten days, at room temperature, in "amber" containers under light, static, with daily homogenizations (three times a day). The solvent used was 100% Methanol.

Two proportions of the extracts were

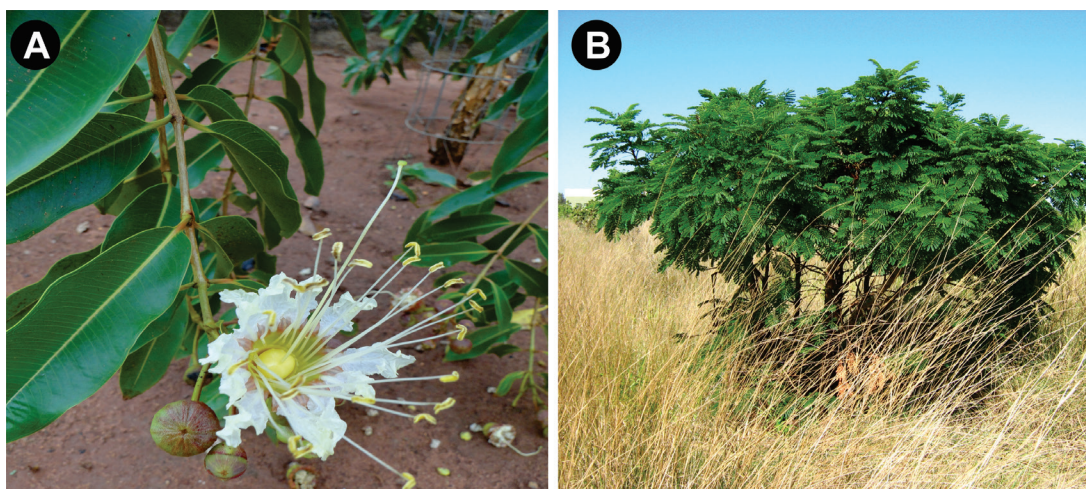


FIGURE 1. Cerrado species studied. In (A) *Stryphnodendron obovatum* Benth and (B) *Lajoensia pacari*. Font: Neto, 2014.

established: 1 g and 2 g of vegetable powder for 10 ml of solvent [extracts in the proportion 1:10 and 2:10 (p/v)], respectively, the mixtures remained at rest for 10 days. Thereafter, the solutions were filtered on Whatman n° 1 filter paper and evaporated under reduced pressure at 50 °C and 90 RPM for evaporation of the solvents and concentration of the samples by means of the Rotary Evaporator. For the analyses, the extracts were used in their concentrated form, reconstituted in a 2% DMSO (Dimethylsulfoxide) solution to obtain a concentration of 500 ml/ml as described by Rabanal et al. (2002).

Antimicrobial activity Inoculum Preparation

The microorganisms tested were *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella sp.*, isolated from human clinical cases provided by the Laboratory of Microbiology and Molecular Biology

of the Federal University of Mato Grosso do Sul. The cultures were suspended in sterile saline until adjustment of the turbidity to the scale of McFarland 10⁸UFC (colony forming units)/ml and the microbial inoculums standardized in a spectrophotometer, at wavelength of 580 nm.

Culture medium and disk diffusion test

For microbiological analysis *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella sp* strains were isolated in Petri dishes with solid MacConkey agar medium for *Klebsiella sp* and *E. coli* and Muller-Hinton agar for *S. aureus* (Figure 2) and thereafter all microorganisms were picked on Muller-Hinton agar.

For the determination of the inhibitory activity of the extracts the disc diffusion method was used, according to the protocol accepted by the FDA (Food and Drug Administration) and established by NCCLS (National Committee for Clinical Laboratory

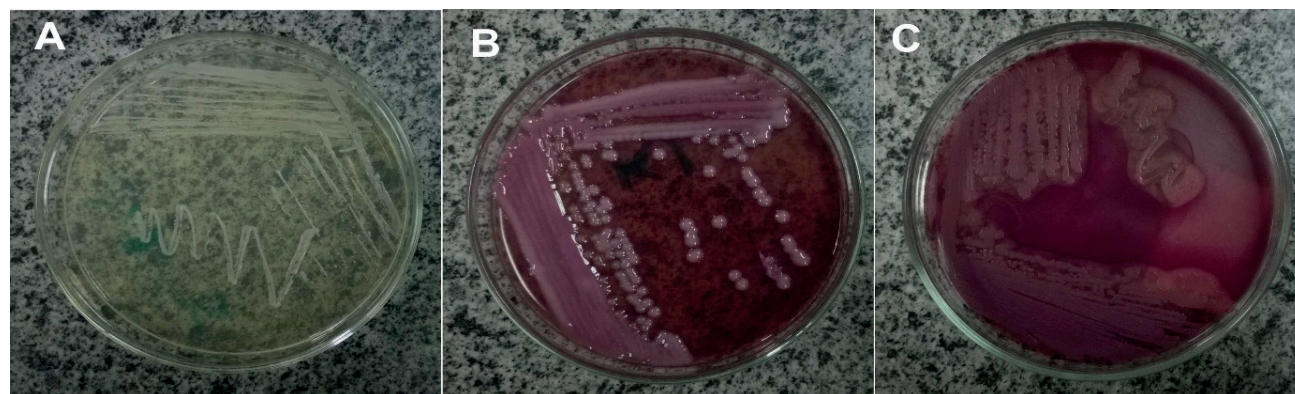


FIGURE 2. Culture media for isolation of bacterial colonies; In (A) *Staphylococcus aureus*; (B) *Klebsiella sp*; (C) *Escherichia coli*

Standards) and (Barry et al. 1991). The prepared bacterial suspension was used to inoculate the Müller-Hinton agar plates with the aid of sterile swab by the technique of seeding in streaks all over the surface of the medium. As suggested by Ostrosky et al. (2008), 10 µl of the solutions were applied to 6 mm diameter sterile filter paper disks at a concentration of 500 µg/ml. The discs were placed on the solid culture medium previously inoculated with microbial charges of 10⁸ UFC/ml. The arrangement of the discs was such that their distance to the side of the plate was greater than 15 mm and so as not to overlap the zones of inhibition. For the positive control, an antibiotic prescribed for the treatment of enterobacterial infections, Ciprofloxacin at 5 µg and for negative control, DMSO in the same proportion was used. The plates were kept in culture oven at a temperature of 37 °C ± 1 °C for 24 h. The inhibition halos produced were measured with the aid of a clear ruler.

RESULTS

According to table 1, the results obtained evidenced the potential of microbial inhibition of the strains tested against the methanolic extracts of *L. pacari* and *S. obovatum*. Only *L. pacari* extracts at two concentrations (1:10 p/v and 2:10 p/v) showed activity against all bacterial species tested (Figure 3S) showing inhibition halos of 20, 14 and 21 mm against *S. aureus*, *E. coli* and *Klebsiella sp.* respectively. *S. obovatum* showed inhibition halos only for *S. aureus* (16 mm and 12 mm). Figure-3 shows the inhibition halos formed after the incubation period for the bacteria studied. *S. obovatum* extract only inhibited *S. aureus* cultures at two concentrations (1:10 p/v and 2:10 p/v) (Figure 3S).

The results obtained evidenced the potential of microbial inhibition of the strains tested against the methanolic extracts of *L. pacari* and *S. obovatum*. Figure 3 shows the inhibition halos formed after the incubation period for the bacteria studied. Only *L.*

pacari extracts at two concentrations (1:10 p/v and 2:10 p/v) showed activity against all bacterial species tested (Figure 3). *S. obovatum* extract only inhibited *S. aureus* cultures at two concentrations (1:10 p/v and 2:10 p/v) (Figure 3S).

In Table 1, it is possible to observe the values obtained in inhibition of bacterial growth that occurred in a non-dose-dependent manner for the studied microorganisms, reaching maximum inhibition with the concentration of 1:10 (p/v). In order to analyze the inhibitory effects of extracts, comparative tests were performed with the commercially produced drug ciprofloxacin, a drug commonly used to treat infections caused by *S. aureus*, *E. coli* and *Klebsiella sp.* *E. coli* showed greater sensitivity to Ciprofloxacin with 35 mm inhibition halos, *Klebsiella sp.*, the inhibition halo diameter was 32 mm and for *S. aureus*, 29 mm. When compared to the extracts of the studied species, ciprofloxacin showed greater inhibition potential, once the composition is defined for the treatment of the studied pathogens.

The solvent (DMSO 2%) used in the dilutions did not interfere in the obtained results, since it did not produce inhibition halos, demonstrating that it did not influence in the diameter of the zone of inhibition produced by the extracts. The extracts that did not present any type of antimicrobial activity when using the method of diffusion in agar, are probably have limitations because they have substances with low diffusibility in the culture medium.

DISCUSSION

Medicinal plants has been used as antimicrobial agents considering their composition and potential for inhibition, since there is a strong tendency to use when applied methodologies capable of proving the efficacy against the damages caused by pathogenic microorganisms. The in vitro antimicrobial activity tests showed that the application of traditional medicine represents a source of discovery for numerous drugs, as well

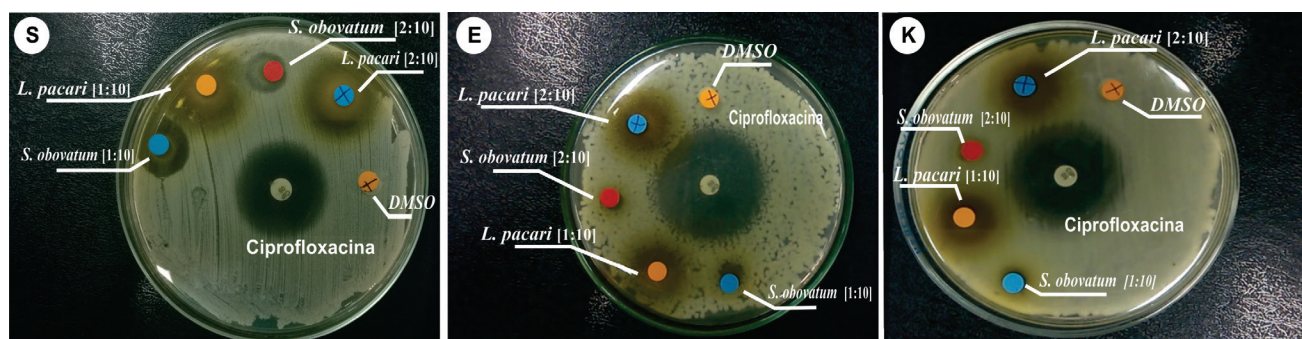


FIGURE 3. Inhibition zones produced by the plant extracts in the respective microorganisms where (S) *Staphylococcus aureus*, (E) *Escherichia coli* and (K) *Klebsiella sp.*

TABLE 1. Antimicrobial activity of methanolic extracts of medicinal plants of the cerrado selected on different bacterial species.

Plant extracts	Concentration	Microorganisms	Inhibition Zone (mm)
<i>L. pacari</i>	1:10	<i>S. aureus</i>	20
		<i>E. coli</i>	14
		<i>Klebsiela sp.</i>	21
<i>L. pacari</i>	2:10	<i>S. aureus</i>	20
		<i>E. coli</i>	15
		<i>Klebsiela sp.</i>	19
<i>S. obovatum</i>	1:10	<i>S. aureus</i>	16
		<i>E. coli</i>	0
		<i>Klebsiela sp.</i>	0
<i>S. obovatum</i>	2:10	<i>S. aureus</i>	12
		<i>E. coli</i>	0
		<i>Klebsiela sp.</i>	0
<i>Ciprofloxacin</i>	0,5 g	<i>S. aureus</i>	29
		<i>E. coli</i>	35
		<i>Klebsiela sp.</i>	32
DMSO 2%		<i>S. aureus</i>	0
		<i>E. coli</i>	0
		<i>Klebsiela sp.</i>	0

as research related to the search for therapeutic attributes brought by these, forming an important tool for scientific elucidation of healing properties (Gonçalves et al. 2011).

In the present study, the extracts obtained from *L. pacari* presented bactericidal action against all tested microorganisms. These results corroborate the findings of Porfírio et al. (2009) that 96.4% of the multiresistant bacteria strains used in the research (*P. aeruginosa* and *S. aureus*) isolated from patients with multiple infections demonstrated action bactericidal against *L. pacari* extract (methanolic extract, 200 mg) showing halos of growth inhibition up to 26 mm. Thus, there is the hypothesis that the extract of *L. pacari* presents substances useful in the fight against multiresistant bacteria.

However in this study it was not found that the proportion of dilution of vegetable powder (1:10 / 2:10) in methanolic solvent significantly interfered with the size of inhibition halos.

In a recent study proposed by Pereira et al. (2018) reinforce the popular use of *L.*

pacari leaves in the treatment of wounds. Its cicatrization activity is multidirectional and involves inhibition of the proliferative and anti-inflammatory phases, antioxidant and positive modulation of the remodeling phase that may be involved in different secondary metabolites, with emphasis on the ellagic acid, punicalin, punicalin, kaempferol, quercetin-3-O-xylopyranoside and quercetin-3-O-rhamnopyranoside.

The bactericidal properties of the *L. pacari* extracts were also evidenced by Lima (2006) *L. pacari* leaf extracts with minimum inhibitory concentration (MIC) in the range of 312.5 to 2500 mg/ml, 250 6 mg/ml, 625 mg/ml, and 1250 mg/ml, specifically against eight different Gram-positive *S. aureus*, Gram-negative *Proteus mirabilis* and acid-resistant bacilli *Mycobacterium phlei*, *M. fortuitum* and *M. smegmatis*. In addition chemical analysis revealed that crude extracts contained tannins, steroids, phenols, flavonoids, and triterpenes.

Although *S. obovatum* extracts showed bactericidal activity only in *S. aureus* culture the study

developed by Sanches et al. (2005) demonstrated the antioxidative and antifungal effects of the shell of this species with solvent of ethyl acetate, which showed antifungal activity moderate to the strains facing the strains of *C. albicans* and *C. parapsilosis*. However, Vasconcelos et al. (2004) conducted tests on the seeds of this species against *S. aureus* strains solvent ethanol and bactericidal activity not obtained. In the literature, there are few researches with the extracts of this species, however, in the traditional medicine the “Barbatimão” is widely used in the treatment of inflammatory and cicatrizantes processes

CONCLUSION

The researchers are still few and there are gaps regarding the scientific knowledge of bioactive compounds produced by the species of the Cerrado biome. We can conclude that the methanolic extracts of *Lafoensia pacari* are suitable for the search of substances with bactericidal activity. *Stryphnodendron obovatum* extracts require further investigation and use of other solvents.

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