v. 3, n. 4, 2023

International Journal of Biological and Natural Sciences

INFLUENCE OF PHENOLOGICAL STAGES OF GROWTH AND SEASONALITY OF *Piptadenia gonoacantha* ON THE PRODUCTION OF NEW ANTIBACTERIAL AGENTS FOR HEALTH

Gisele Carvalho Mendes
Universidade Federal de Viçosa. Programa de Pós-Graduação Stricto Sensu em Ciências da Saúde do Departamento de Medicina e Enfermagem. Viçosa, Minas Gerais, Brasil.
https://orcid.org/0000-0001-6823-0783

Ana Luiza Dias dos Santos Melo
Universidade Federal de Viçosa. Departamento de Medicina e Enfermagem. Viçosa, Minas Gerais, Brasil.
https://orcid.org/0009-0001-0804-8565

Luísa Barbosa Ulhoa
Universidade Federal de Viçosa. Departamento de Medicina e Enfermagem. Viçosa, Minas Gerais, Brasil.
https://orcid.org/0009-0000-4404-8530

Catarina Maria Nogueira de Oliveira Sediyama
Universidade Federal de Viçosa. Programa de Pós-Graduação Stricto Sensu em Ciências da Saúde do Departamento de Medicina e Enfermagem. Viçosa, Minas Gerais, Brasil.
https://orcid.org/0000-0002-1185-5819

Marilane de Oliveira Fani Amaro
Universidade Federal de Viçosa. Programa de Pós-Graduação Stricto Sensu em Ciências da Saúde do Departamento de Medicina e Enfermagem. Viçosa, Minas Gerais, Brasil.
https://orcid.org/0000-0002-9495-0861

Helio Garcia Leite
Universidade Federal de Viçosa. Departamento de Engenharia Florestal. Viçosa, Minas Gerais, Brasil.
https://orcid.org/0000-0002-6357-9174

Haroldo Nogueira de Paiva
Universidade Federal de Viçosa. Departamento de Engenharia Florestal. Viçosa, Minas Gerais, Brasil.
https://orcid.org/0000-0002-8891-5373

Marcelo Barcellos da Rosa
Universidade Federal de Santa Maria. Departamento de Química. Santa Maria, Rio Grande do Sul, Brasil.
https://orcid.org/0000-0001-5959-0381

Camilo Amaro de Carvalho
Universidade Federal de Viçosa. Programa de Pós-Graduação Stricto Sensu em Ciências da Saúde do Departamento de Medicina e Enfermagem. Viçosa, Minas Gerais, Brasil.
https://orcid.org/0000-0001-9539-8653

Abstract: Background: Due to the increase in resistant microorganisms, the search for new antibacterial becomes urgent, and the standardization of collection and development of plant extracts is essential for therapeutic efficacy. The objective was to evaluate the influence of the four seasons of the year and the phenological growth stages of *Piptadenia gonoacantha*, on the antibacterial activity and toxicity against *Artemia salina*. **Methods**: *P. gonoacantha* leaflets were collected separately, during the four seasons and according to five tree diameter classes. Subsequently, they were dried, macerated in alcohol (80%) and concentrated in a rotary evaporator. By means of the diffusion test in agar wells, the antibacterial activity of the extracts was evaluated. Tests were performed against Gram negative and Gram positive bacteria. Toxicity evaluation was performed against *Artemia salina*. **Results**: By the method of diffusion in solid medium with perforation in agar, the extracts (500 mg/mL) showed antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas putida*, with the best results (inhibition halos ≥ 8.0 mm) observed, when compared to the other samples, in leaflets collected in winter, in trees with a trunk diameter between 5 and 10 cm. The MIC was 62.5 mg/ml, 15.6 mg/ml and 31.2 mg/ml for *S. aureus*, *S. pyogenes* and *P. putida*, respectively. The extracts were considered non-toxic against *Artemia salina*. **Conclusions**: It is concluded that the standardization of the harvest of *P. gonoacantha* leaves, in terms of seasonality and diametric class, is very relevant, since it influences the antibacterial action.

Keywords: Antimicrobials; Climate Seasons; Microbial Sensitivity Tests; Toxicit.

INTRODUCTION

The search for pharmacological actives with antimicrobial action has been growing as resistant microorganisms threaten the efficiency of available therapy[1]. Organizations such as the WHO (World Health Organization) and Sanitary Surveillance Agency warn about growth peaks in microbial resistance, highlighting the need for a change in behavior, in relation to the use of antimicrobial agents, both in civil society and for health, agricultural and livestock professionals. Therefore, actions must be taken to reduce the development of antimicrobial resistance, such as, for example, controlling the use of antibiotics, developing research to better understand the genetic mechanisms of resistance and continuing studies on the development of new drugs, whether synthetic or natural[2].

In recent years, several studies have been carried out in different countries to prove the effectiveness of plant extracts and phytochemicals, both with antimicrobial properties[2]. Therefore, quality control of herbal medicines is essential throughout the process of developing herbal medicine, as many plant species are sold without any quality guarantees, favoring, from the sale of counterfeit species, to inadequate storage during commercialization[3].

Thus, it is necessary to achieve a more accurate understanding of the improvement of new drugs of natural origin. For that, one must consider from the cultivation of plant species, to the optimization and standardization of methods of harvesting the plant material used in the manufacture of these formulations. Evidences indicate that the content and quality of phenolic compounds in plant tissues can be affected by many factors, such as genotype, environment, plant growth stage, harvest time, process conditions, storage and analysis methods[4-5].

Corroborating these data, a study carried out by Isah[6] demonstrates that under the influence of environmental factors, cellular physiological processes and plant development are affected, producing secondary metabolites that play a variety of roles in response to these changes. Seasonality is reported as a contributing factor to the variation in secondary metabolite production[7].

In view of these data, this study aimed to evaluate the in vitro antibacterial action of the extract of *P. gonoacantha* leaves, harvested during the four climate seasons and at different phenological stages of growth, as well as to evaluate its toxicity against *Artemia salina* from de most promising stratum. These data will favor the standardization of the harvest time and validation of the biological activity of the species.

METHODS

PLANT HARVEST AND EXTRACT CHARACTERISTICS

The plant material was collected at the Centro Tecnológico de Viçosa – MG, with geographic coordinates 42° 51' W and 20° 42' S and an average altitude of 721m[8], in *Piptadenia gonoacantha* trees.

Three trees were selected for each *dbh* class (diameter to 1.3 m in height), in order to monitor the phenological stages of growth, namely: *dbh* 1 (5 to 10 cm), *dbh* 2 (10 to 15 cm), *dbh* 3 (15 to 20 cm), *dbh* 4 (20 to 25 cm) and *dbh* 5 (> 25 cm). In each diameter class, three trees were identified and selected for sampling.

Three units of leaf samples were taken from each tree. This procedure was repeated in the four seasons of the year. The extract was obtained from leaves and petioles of "Pau Jacaré" (*Piptadenia gonoacantha*) and the collection was equally repeated in the four climate seasons. After this collection, the

plant material was placed in a circulating air oven at 60ºC for three days and ground in a knife mill[9].

For the extracts preparation, powder from leaves and petioles was used, in a 1:5 ratio (25 g powder: 125 mL of 80 % v/v ethanol/ water solution with 0.3% citric acid), resulting in the concentration of 20% of dry extract (m/v). Then, the extract was subjected to the maceration process for 48 hours at room temperature. After this period, filtration was performed on filter paper, where the collected filtrate was stored at a temperature of 2 to 8°C, in an amber bottle protected from light.

The residue retained (cake) on the filter was subjected to extraction by maceration twice more with the same extracting solution. At the end of the process, the filtrates were subjected to the solvent removal process, which was carried out in a rotary evaporator at a temperature of 60°C with the aid of a vacuum pump[10].

ANTIBACTERIAL ACTIVITY

The microorganisms used in the tests were obtained from the Biochemistry Laboratory of the Department of Medicine and Nursing at the Federal University of Viçosa - MG. The bacterial strains used were: Gram positive *Staphylococcus aureus* (ATCC 29213) and *Streptococcus pyogenes* (ATCC 19615); Gram negative *Escherichia coli* (ATCC 14948), *Salmonella spp* (ATCC 14028) and *Pseudomonas putida* (WFCC 885).

The culture medium used in the tests was Mueller-Hinton, prepared according to the manufacturer's specifications. Bacteria cultures were maintained at 4°C in Mueller-Hinton. Before the tests, the strains were replicated to the aforementioned medium and incubated at 30 ± 2 ºC for 24 hours, with the process being repeated for another 24 hours. Bacterial suspensions were prepared from fresh cultures, with turbidity equivalent to McFarland Scale 0.5 (1.5×10^8 cells/mL)[11].

To carry out the antibacterial activity, 2 mL of the microorganism suspension (1.5×10^8 cells/mL) was poured into sterile Petri dishes with Mueller-Hinton agar. In each well, 20µL of the extracts and controls were deposited in the corresponding wells. After 24 hours incubation, in an oven at 30 ± 2 ºC, the diameter of the inhibition halo was measured in mm.

As a negative control, 80% ethyl alcohol in 0.3% citric acid was used, the same used for the preparation of the extracts, in addition to the positive control Erythromycin $0.33.10^{-3}$ mg/mL for the bacteria Gram positive, and Ciprofloxacin 0.41. 10^{-4} mg/mL for Gram negative bacteria.

The tests were performed in triplicate, with the average of the three measurements being considered as the final result of each extract, and a halo equal to or above eight mm in diameter was considered susceptible[12-13].

After the preliminary evaluation of the antibacterial activity of the strains, those that presented satisfactory results were selected to determine the minimum inhibitory concentration (MIC).

To perform the MIC, the extract that showed the best antibacterial activity in the screening was evaluated by the broth microdilution method. In other words, extracts prepared with material collected from trees of the first phenological stage of growth (*dbh* 1 - 5 to 10 cm) in the winter season were selected. A pool with triplicates of extracts classified as *dbh* 1 was prepared. Starting from the highest extract concentration (500 mg/ml), serial dilutions were prepared in sterile Mueller-Hinton medium, having a concentration range from 500 mg/ml to 3.9 mg/ml.

The preparation of Petri dishes with Mueller-Hinton agar and bacterial suspension followed the same methodology

used in the evaluation of antibacterial activity. Subsequently, 20 µL of each extract dilution was transferred to its respective wells, as well as the positive and negative controls. The plates were incubated at 30 ± 2 ºC for 24 hours. The evaluation of the antibacterial activity was done by observing the formation of inhibition halos around the standardized cavities. The MIC is considered as the highest dilution of *Piptadenia gonoacantha* extract (EPG), which can inhibit the growth of microorganisms[11].

TOXICITY OF *PIPTADENIA GONOACANTHA* EXTRACT

The toxicity evaluation of the hydro-alcoholic extract of *Piptadenia gonoacantha* (EPG) was carried out against *Artemia salina* Leach, based on the modified method proposed by Meyer at al[14]. The eggs of *Artemia salina* Leach were incubated (10 mg.100 mL-1) for 48 hours in NaCl solution (26 g/L), under artificial lighting and constant aeration at 26±1ºC, obtaining the hatching of the eggs and the formation of larvae in the metanauplius stage. Winter season *dbh* class 1 extracts were selected for this test.

Subsequently, the serial dilution of the EPG was performed with NaCl solution (26 g/L) at different concentrations (500.10^3 to $15.75.10^3$µg/ml). In an 18-well plate, 1mL of the different dilutions and 10 *Artemia salina* larvae were added to each well. After 24 hour incubation, the number of larvae that remained alive was evaluated.

As a negative control, saline solution was used and, as a positive control, potassium dichromate (0.33 mM) in saline solution.

STATISTICAL DATA ANALYSIS

A descriptive statistical analysis was used to characterize the formation of halos in the sample units of the four seasons of the year and the Student's t test, for independent samples, was used to evaluate the effect of the size of the tree (dbh class) on the formation of the halo. This test was applied to each bacterium in each season of the year, considering a significance level of 5%, to evaluate the hypothesis of equality between the means of a dependent variable (parametric) estimated for two independent groups (categorical)[15].

The variable analyzed was the size of inhibition halos observed in five classes of phenological growth stages. The proportion of cases with formation of halos greater than or equal to 8 cm was determined for each bacterium in each season of the year. These proportions were calculated independently of the diameter class, which resulted in 45 observations for each bacterium in the autumn, summer and winter seasons, and 30 for each bacterium in the spring season. In order to complement the descriptive analysis of these proportions, the p-value of the Z test was calculated, which assumed population proportions of 0.05 and 0.95 in the null hypothesis. If the conditions $np \geq 5$ and $nq \geq 5$ were satisfied, from a non-stratified sample, in which the analyzed variable presented only two possible alternatives, in this case, success or failure, then, the binomial distribution of the sample proportions could be approximated by a normal distribution with mean $\mu = np$ e $\sigma = \sqrt{npq}$. In this case, the statistic $z = (\hat{p} - p)/\sqrt{pq/n}$ can be used to test the hypothesis H_0: p = e *versus* H1: p > Ø, where p is the proportion of "successful" cases (in this case halo > 8 mm), q = 1 – p, n is the sample size and Ø is the population proportion used in the null hypothesis. $\hat{p}$ indicates the sample proportion, that is, the ratio between the number of successes and the sample size. The halo size was measured in 45 sample units (n = 45) for each bacterium (*P. putida, S. aureus and S. pyodenes*) and season (spring, summer, autumn and winter). For each of these 12 cases, the proportions of cases in which the size of the halo was greater than

or equal to 8.0 mm (p) were calculated. Then the H0 hypotheses was tested: p = Ø versus H1: p > Ø, where p is the proportion of cases with halo >= 8 mm and Ø is the parametric reference, set from 0.05 to 0.95.

RESULTS

The antibacterial activity of the extract was tested on five different bacterial strains. The plant material was collected in triplicate in the four climate seasons and in five diameter classes (phenological stages of growth), resulting in 45 samples of extracts for the summer, autumn and winter seasons. In the spring season, only 30 samples of extracts were obtained because the collection was performed in duplicate.

Of the bacteria tested, *Escherichia coli* and *Salmonella spp* did not have their antibacterial action proven against the studied extract, as no inhibition halos were observed. On the other hand, the bacteria *Streptococcus pyogenes*, *Pseudomonas putida* and *Staphylococcus aureus* showed satisfactory inhibition halos, and thus were used for continuity of the presented tests (MIC and toxicity).

In the evaluation of the results, the extract under test showed satisfactory microbial activity in the different seasons of the year. The extract of the leaves extracted in autumn and summer, respectively, was less effective against the culture of *Streptococcus pyogenes*, whereas against *Pseudomonas putida* it was only 4% ineffective in autumn, that is, the antibacterial action does not depend on the season of the year that the leaves are collected.

On the other hand, it did not show efficiency against *S. aureus* bacteria, nether in the summer nor autumn seasons. The descriptive analyses, which describe and characterize the formation of halos in the samples for the four seasons of the year, are shown in Figure 1.

Regarding the diameter classes and the seasons, the results suggest that the extract with the best antibacterial activity would be the one whose leaves were harvested in winter with the trees in class 1 ($5 \leq dbh \leq 10$ cm), since they presented halos of inhibition ≥8.0 mm in greater quantity compared to the others.

Figure 1 contains the analyzes for each of the three bacteria evaluated, in each of the seasons. Figure 2 shows the average sizes of bacterial inhibition zones observed in five phenological stages of growth of *P. gonoacantha* in the four seasons and the results obtained with the application of the t test for independent samples. The *p*-values of the t test are shown in Table 1.

Considering a parametric value Ø = 50%, and that the hypothesis of the test is of nullity, that is, with the purpose of rejection, based on the reference line of 5 % of significance (dotted red line), it is verified that the null hypothesis is rejected for *Pseudomonas putida* and for *S. pyogenes*, in any season of the year (*p*-value ≤ 0.05). Therefore, *P. gonoacantha* leaves can be harvested at any season of the year. The rejection of H0 also occurred for *S. aureus* in the winter and spring seasons. Therefore, if the proportion of effectiveness cases (halo ≥ 8.0 mm) equal to or less than 50 % is not sufficient, the collection of biomass from "*Pau Jacaré*" leaves should be avoided in the autumn and summer seasons, in the case of production of extracts for therapies where there is colonization by *S. aureus* (Figure 3; Table 2).

The test was carried out to verify the minimum inhibitory concentration with the extract being collected in the winter season, of diametric class 1, due to the better antibacterial activity. The MIC of the extract against the three bacteria was defined as the lowest concentration of the extract that completely inhibited the growth of the bacteria, that is, in which there was no formation of an inhibition halo.

Bacteria	*Dbh*					*Dbh*			
	Dbh	2	3	4	5	2	3	4	5
			Autumn				**Summer**		
P. putida	1	**0,0000**	**0,0163**	**0,0081**	**0,0000**	0,8375	0,5549	**0,0022**	**0,0003**
	2		**0,0036**	**0,0008**	0,0512		0,4955	**0,0052**	**0,0015**
	3			0,8568	0,0806			**0,0020**	**0,0001**
	4				**0,0226**				0,7909
S. aureus	1	**0,0041**	**0,0317**	0,3257	0,5086	0,3266	0,9259	0,2001	0,6289
	2		0,1649	0,0690	**0,0144**		0,2526	0,4220	0,0804
	3			0,1188	0,1147			0,1493	0,6915
	4				0,2396				**0,0443**
S. pyogenes	1	**0,0014**	**0,0194**	**0,0019**	**0,0001**	0,7543	0,9495	0,2667	0,9178
	2		0,1267	0,6906	1,0000		0,6904	0,1092	0,8731
	3			0,2235	0,0569			0,2531	0,8721
	4				0,6264				0,3202
			Winter					**Spring**	
		2	3	4	5	2	3	4	5
P. putida	1	0,3009	0,4829	**0,0169**	0,2158	0,6788	**0,0163**	0,3932	0,5350
	2		0,7028	0,1501	**0,0193**		**0,0005**	0,1281	0,1114
	3			0,0573	**0,0410**			0,0683	**0,0056**
	4				**0,0001**				0,6438
S. aureus	1	**0,0034**	0,1890	**0,0001**	0,1649	1,0000	0,4608	0,2167	**0,0033**
	2		0,1220	0,3844	**0,0351**		0,4608	0,2167	**0,0033**
	3			**0,0163**	0,7466			**0,0169**	**0,0000**
	4				**0,0016**				**0,0169**
S. pyodenes	1	**0,0032**	**0,0008**	**0,0007**	**0,0015**	**0,0007**	**0,0000**	**0,0001**	**0,0000**
	2		0,6900	0,8463	**0,0203**		0,2240	0,5078	0,0939
	3			0,7927	**0,0136**			0,5490	0,4770
	4				**0,0158**				0,2345

In bold are the cases with rejection of the null hypothesis for 5% of significance, ($p \leq 0.05$).

Table 1 – *p*-values for the t test for independent samples applied to evaluate the effect of growth stage (1 to 5) of *P. gonoacantha* on the size of the bacterial inhibition halo of *P. putida, S. aureus* and *S. pyogenes* in the four seasons of the year.

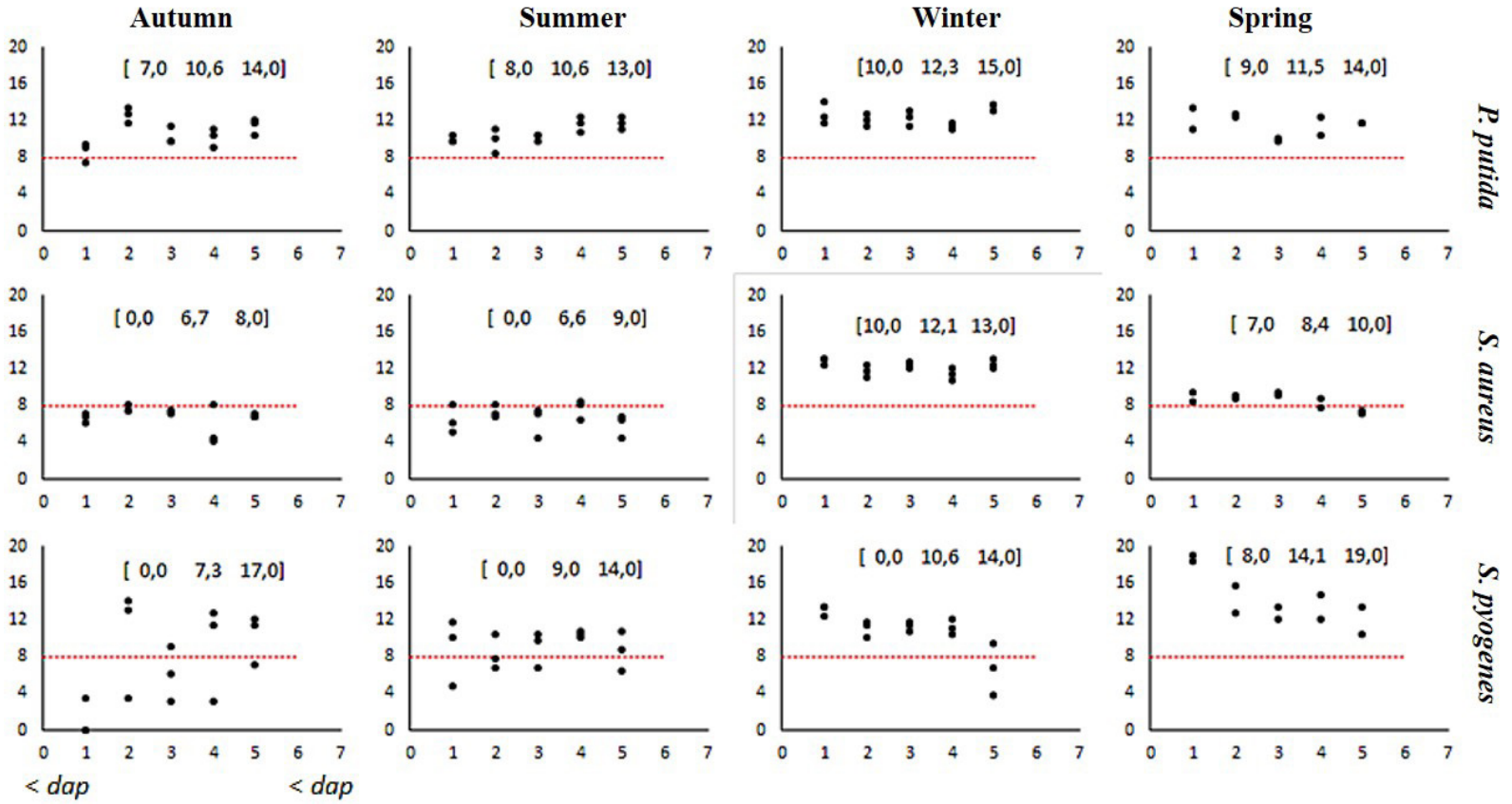

Figure 1 - Size of bacterial inhibition zones observed in five phenological growth stages (dbh) of *P. gonoacantha* in the four seasons. The line vectors indicate the minimum, average and maximum halo size values.

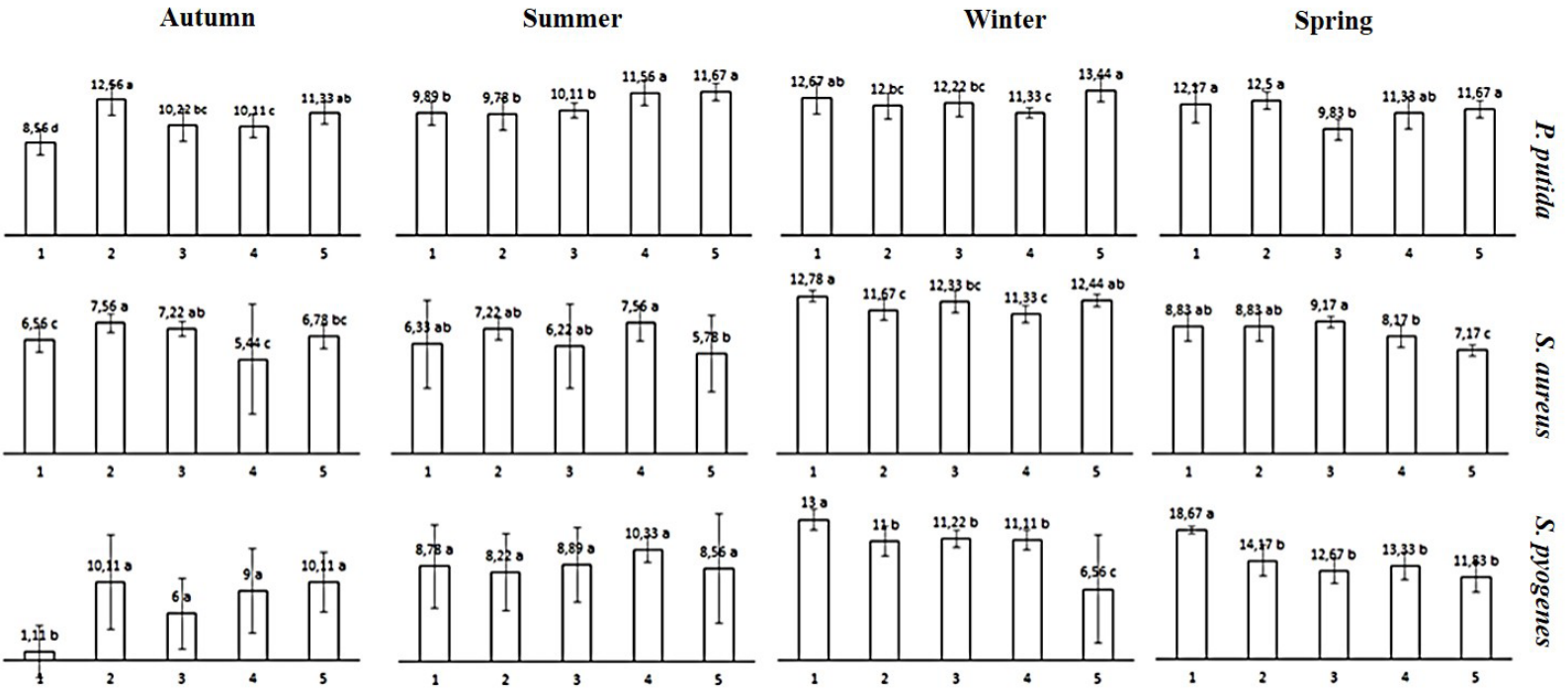

Figure 2 - Size of bacterial inhibition halos observed in five of *P. gonoacantha* growth phenological stages in the four seasons. Dbh classes 1 to 5 correspond, respectively, to 5 to 10 cm dbh, 10 to 15 cm, 15 to 20 cm, 20 to 25 cm and dp > 25 cm. Means with the same letter do not differ by the t test for independent samples ($p > 0.05$).

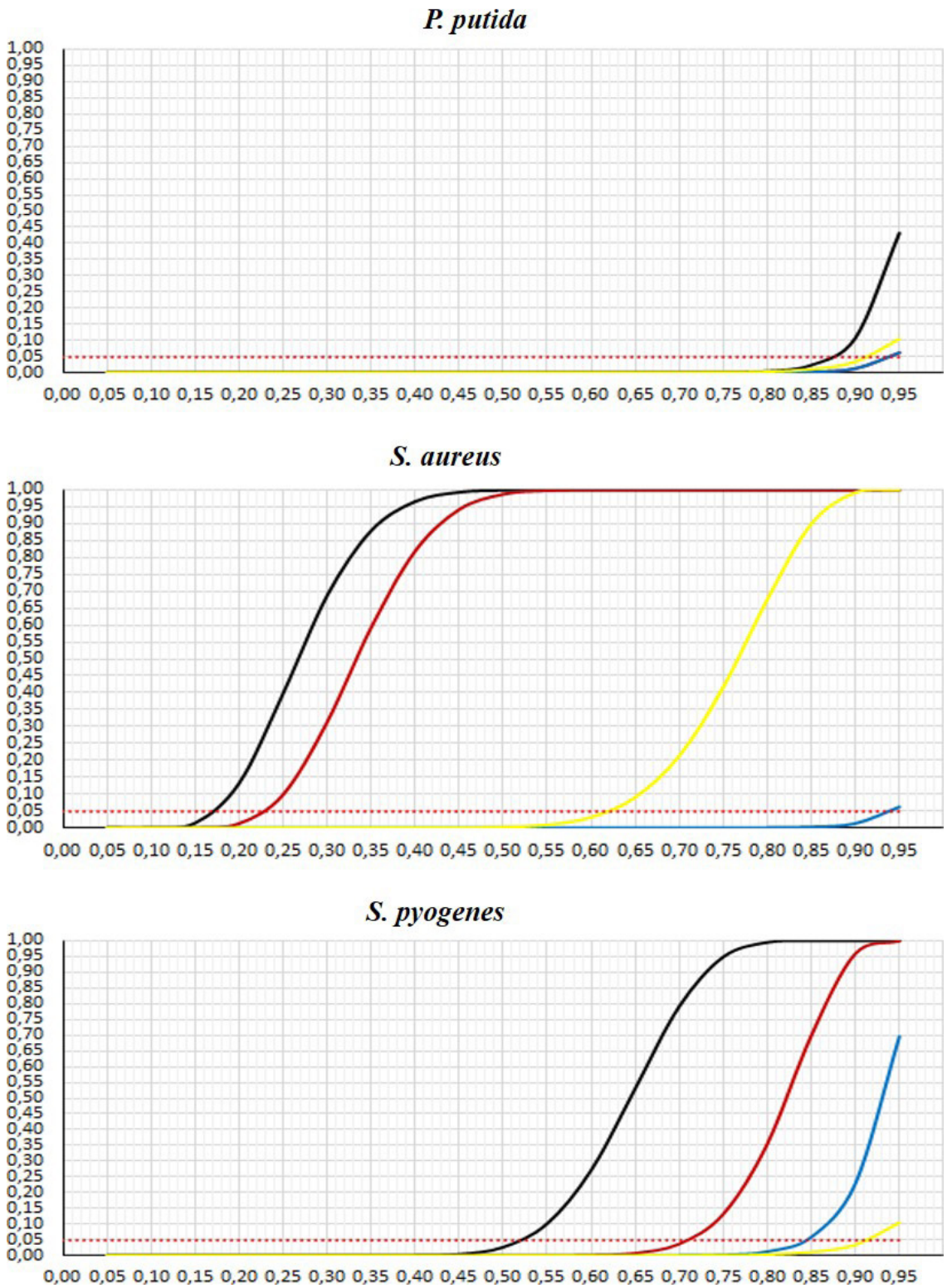

Figure 3 - Z test statistics for each combination of antibacterial activity against *P. putida*, *S. aureus* and *S. pyogenes* and the influence of the season. P-value estimates (y-axis) for hypothesis H0: p = Ø versus H1: p > Ø, where *p* is the proportion of cases with halo ≥ 8 mm and Ø is the parametric reference (x-axis). Dotted line is the significance level for H0 evaluated by the statistic $Z=(p-Ø)/\sqrt{pq/n}$. The colors of the curves indicate: autumn (black), summer (red), winter (blue), spring (yellow).

Bacteria	Climate Station	Fail (≤ 8 mm)	Success (> 8 mm)	Total	p	q
P. putida	Autumn	2	43	45	0,9556	0,0444
	Summer	0	45	45	1,0000	0,0000
	Winter	0	45	45	1,0000	0,0000
	Spring	0	30	30	1,0000	0,0000
S. aureus	Autumn	33	12	45	0,2667	0,7333
	Summer	30	15	45	0,3333	0,6667
	Winter	0	45	45	1,0000	0,0000
	Spring	7	23	30	0,7667	0,2333
S. pyogenes	Autumn	16	29	45	0,6444	0,3556
	Summer	8	37	45	0,8222	0,1778
	Winter	3	42	45	0,9333	0,0667
	Spring	0	30	30	1,0000	0,0000

Table 2 – Proportion of cases with halo > 8 mm (p) and of cases with halo ≤ 8 mm (q), for the bacteria *P.putida, S. aureus* and *S. pyogenes* in the four seasons.

Source: Search results.

The extract of *Piptadenia gonoacantha* showed a MIC equal to 62.5 mg/mL against *S. aureus bacteria*, 15.62 mg/mL for *S. pyogenes* and 31.25 mg/mL for *P. putida*. In addition, the toxicity of *Piptadenia gonoacantha* extract against *Artemia salina* was evaluated. Through the linear regression obtained by the percentage of dead larvae and the concentration of *Piptadenia gonoacantha* extract, an LD_{50} of $243.78.10^3$ µg/ml was estimated. Through the results, we can infer that the extract of the species under study does not present toxic values, since the value of the mean lethal dose for *Artemia salina* was below the reference limit, which is above 1000µg/mL[10]. The researched extract has an antibacterial effect at non-toxic doses, as the values are far below the lethal dose calculated in the toxicity test.

DISCUSSION

Piptadenia gonoacantha is a tree species of the Fabaceae family, with rapid leaf growth, native to the Atlantic Forest in southern and southeastern Brazil. This species is popularly known as "*Pau Jacaré*". In previous studies with extracts of the species, its growth inhibitory potential against microorganisms and anti-inflammatory action were verified[16].

In this work, Gram-positive and Gram-negative bacteria were studied, as part of the ongoing effort among scientists to find viable alternatives for the inhibition of antimicrobial action. Such an effort is necessary, since, in 2017, the WHO released a list of bacteria that urgently need new antibiotics[11].

In the last two decades, only two new classes of antibiotics have been developed and authorized by international health agencies and both are aimed at combating Gram- positive bacteria. The last class of antibiotics discovered to have an effect against Gram- negative bacteria was discovered in 1962[12]. Thus, studies like this are important for bringing new possibilities to traditional treatment, given that herbal medicines expand the therapeutic resources offered to patients, often, for a lower cost [12].

In the list released by the WHO, one of the bacteria in this study, *Staphylococcus aureus*, is classified as a high priority for the discovery of new antibiotics. In other words, the literature suggests that it is difficult to find effective antibiotics for this bacterium. This is corroborated by the results found here, given that it was the bacterium in which the extract was successful in only one season of the year[12]. Corroborating the difficulty of finding antibiotics effectively against *Staphylococcus aureus*, the results of this work identified this bacterium as the most demanding for the production of an extract that is effective in antimicrobial action.

The results for *S. aureus* are corroborated by the work[17], who showed the effectiveness of different formulations containing extracts of *Piptadenia gonoacantha* in inhibiting this bacterium. It is important to highlight that the bacteria analyzed in this work, which were sensitive to the extract studied, are the cause of skin infections. Therefore, considering that there was considerable antimicrobial inhibition, the evidence reported by[18,19], corroborate the results found here.

O bioactive constituents most recognized for their antimicrobial effects are phenolic compounds, flavonoids, tannins, terpenoids and quinone compounds[20]. Among them, rutin stands out, a flavonoid, which is widely found in nature, including in considerable amounts in the leaves of *P. gonoacantha*. This compound has several biological effects reported in the literature, such as: antioxidant, anti-inflammatory, antibacterial, antiviral, vasodilatory and cytoprotective activity [10,21,22.]

Several works are being carried out in search of pharmacological compounds and natural alternatives with antimicrobial activity for the treatment of injuries and healing, exploring the diversity of plants and their therapeutic potential [23-26]. The evaluated the antibacterial activity of the extract of Qualea parviflora Mart. ("*Pau-terra*") and extract of *Rosmarinus officinalis* L. ("*Rosemary*") [27]evaluated *Caryophyllus aromaticus* L. ("*Cloves*"), both studies verifying the capacity against Gram-negative and Gram-positive strains. Both studies showed the inhibitory capacity of these extracts on the growth of the strains evaluated. Corroborating that, new drugs originated from nature, must be considered as important therapeutic Options [28].

Results were found for the effectiveness of *Piptadenia gonoacantha* extracts against the bacteria *S. pyogenes* and *P. putida*. There are studies that show a satisfactory inhibitory concentration of these bacteria using medicinal plants, such as the use of *Passiflora edulis*, which showed antibacterial activity against the bacterium *S. pyogenes* [29], and *Passiflora foetida*, which showed good antibacterial results both against *P. putida* and *S. pyogenes* [30].

Regarding the diameter class established for leaf harvesting, the results indicate class 1 (5 to 10 cm) as being the most efficient in inhibiting bacterial growth. That is, younger trees with lower heights are indicated, which in turn would facilitate management during sustainable harvesting. If it were produced on a large scale, it would not have to wait a long time for collection, considering that little growth time, reduced planting space, in addition to rapid natural regeneration, are important characteristics for industrial scale and reforestation with the species[13].

Previous studies with *P. gonoacantha* extracts, after phytochemical analyses, found that the rutin flavonoid was among the compounds with the highest concentration in the extract. Evaluating the influence of seasonality, the summer season had the highest concentration of rutin and the winter season the lowest concentration of hydroalcoholic extracts (80%) of *P. gonoacantha*[9].

International Journal of Biological and Natural Sciences ISSN 2764-1813 DOI 10.22533/at.ed.813342328045

The toxicity of the extract was also evaluated, where it presented results within the expected standards, since the test against *Artemia salina* shows values below 500µg/mL for toxic substances, from 500µg/mL to 1000µg/mL for moderate toxicity and above 1000µg/mL for non-toxicity. The analyzed material is in this third category, suggesting a non-toxic product. Other studies with the species *P. gonoacantha* also ensure the low toxicity of the extract and formulations based on Pau Jacaré leaves [31].

The results regarding the action of *Piptadenia gonoacantha* in the different seasons of the year, as well as the most suitable diameter class for harvesting the leaves for the extract are the main contributions of this work, as it is a study with an unprecedented approach. In addition, bacterial strains of *P. putida* were used, which have few studies evaluating the action of medicinal plants in their inhibition.

CONCLUSION

Extracts from leaves of *Piptadenia gonoacantha* showed antibacterial action against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas putida*. However, it did not reveal positive results against strains of *Escherichia coli* and *Salmonella spp*. The most favorable season for harvesting is winter, with *S. aureus* being the bacterial species in which the effect of seasonality was most relevant. Among the diameter classes evaluated, the most favorable was the first, where the trees had 5 to 10 cm of dbh. This demonstrates the possibility of harvesting in a shorter period of time, and in small spaces, favoring large-scale production.

REFERENCES

1. Kebede T, Gadisa E, Tufa A. Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. PLoS One. 2021;16(3):1-16.

2. Nascimento GG, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. 2000;31(4):247-56.

3. Carvalho CAD, Silva MBD, Oliveira TGD, Lima JDM, Rosa MBD. Estudo espectrométrico de diferentes estágios fenológicos da *Brassica oleracea* var. *capitata*. Braz J Pharmacog. 2008;18(2):249-57.

4. Vagiri M, Conner S, Stewart D, Andersson SC, Verrall S, Johansson E, et al. Phenolic compounds in blackcurrant (*Ribes nigrum* L.) leaves relative to leaf position and harvest date. Food Chem. 2015;172:135-42.

5. Bujor OC, Ginies C, Popa VI, Dufour C. Phenolic compounds and antioxidant activity of lingonberry (*Vaccinium vitis-idaea* L.) leaf, stem and fruit at different harvest periods. Food Chem. 2018;252:356-65.

6. Isah T. Stress and defense responses in plant secondary metabolites production. Biol Res. 2019; 52(39):1-25.

7. Gobbo-Neto L, Lopes N P. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quím Nova. 2006; 2021:30(2):374-81.

8. Souza LFT, Fernandes Filho EI, Faria MM. Compartimentação geomorfológica e mapeamento digital de solos no município de Viçosa–MG. Rev Geonorte. 2014;5(16):110-4.

9. Carvalho CA, Santana GS, Amaro MOF, Franco AJ, Pinto R, Zatti RA, et al. Antinociceptive and anti-inflammatory effects of hydroalcoholic extract of leaves of *Piptadenia gonoacantha* (MART.) Macbr. in experimental animal models. Ci. E Nat. 2014;36:775-81.

10. Franco AJ, Pereira CG, Silva KV, Almeida GFG, Amaro MOF, Caldeira EAC, et al. Antimicrobial activity of dermocosmetic formulations based on *Piptadenia gonoacantha*. Ci e Nat. 2021;43:1-31.

11. National Committee for Clinical Laboratory Standards (NCCLS). Reference method for broth dilution antifungal susceptibility testing of yeasts, Approved Standard M7-A6, 2nded., 2003.

12. Parekh J, Chanda S. *In vitro* Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turk J Biol. 2007; 31(1):53-8.

13. Santos SC, Ferreira FS, Rossi-Alva JC, Fernandez LG. In vitro antimicrobial activity of the extract of *Abarema cochliocarpos* (Gomes) Barneby & Grimes. Braz J. Pharmacogn. 2007;17(2):215-9.

14. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DEJ, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982;45(05):31-4.

15. Darski C, Kuhl CP, Terraciano PB, Nienov OH. Desfechos quantitativos: amostras independentes. In: Capp E, Nienov OH, editors. Bioestatística quantitativa aplicada. Porto Alegre: UFRGS; 2020. pág. 125-156.

16. Carvalho CA, Santana GS, Amaro MOF, Lima LM, Pires FB, Pra VD, Cardoso AS, et al. Aspectos químicos e atividade antibacteriana de *Piptadenia gonoacantha* (Fabaceae). Ci e Nat. 2014;36:731-44

17. Paiva LC, Silva KV, Almeida GFG, Caldeira EAC, Cruz CESG, Salgado PO et al. *Piptadenia gonoacantha*-based natural dermocosmetic: a clinical trial. Ci e Nat. 2021;43:1-31.

18. Franco AJ, Pereira CG, Silva KV, Almeida GFG, Amaro MDOF, Caldeira EAC et al. Antimicrobial activity of dermocosmetic formulations based on *Piptadenia gonoacantha.* Ci e Nat. 2021;43:1-31.

19. Barbier F, Timsit JF. Risk stratification for multidrug-resistant bacteria in patients with skin and soft tissue infection. Curr Opin Infect Dis. 2020;33(2):137-45.

20. Couto DS, Costa Perinoti LCS, Silva Felix AM, Figueiredo RM. Não conformidades no processo de administração de antimicrobianos: revisão integrativa. Saúde Colet. 2021;11(64):5702-13.

21. de Araújo M; Moreira FRANCO TE, Alberto TG, Sobreiro MA, Conrado MA, Priolli DG, et al. Enzymatic de-glycosylation of rutin improves its antioxidant and antiproliferative activities. Food Chem. 2013;141:266-73.

22. Enogieru AB, Haylett W, Hiss DC, Bardien S, EE Okobi. Rutin as a Potent Antioxidant: Implications for Neurodegenerative Disorders. Oxid Med Cell Longev. 2018;2018:6241017.

23. Souza DR, Rodrigues ECAMS. Plantas medicinais: indicação de raizeiros para o tratamento de feridas. RBPS. 2016;29(2)197-203.

24. Ramalho MP, Santos SLF, Castro NM, Vasconcelos LMO, Morais ICO, Pessoa CV. Plantas medicinais no processo de cicatrização de feridas: revisão de literatura. Rev. Expr. Catól. Saúde. 2018; 3(2)64-70.

25. Baldé MA, Emmy T, Mohamed ST, An Matheeussen, Paul C, Louis M, et al. Antimicrobial investigation of ethnobotanically selected guinean plant species. J. Ethnopharmacol. 2020;263,113232.

26. Franczak DD, Carreto R, Santana VTP, Souza GS. Atividade Antibacteriana dos Extratos Etanolícos do Caule e da Folha de *Qualea parviflora* Mart.(Pau-terra). Uniciências. 2019;23(1)43-7.

27. Guimarães CC, Ferreira TC, Oliveira RCF, Simioni PU. Atividade antimicrobiana *in vitro* do extrato aquoso e do óleo essencial do alecrim (*Rosmarinus officinalis* L.) e do cravo-da-índia (*Caryophyllus aromaticus* L.) frente a cepas de *Staphylococcus aureus* e *Escherichia coli*. R. Bras. Bioci. 2017;15(2)83-9.

28. Soares CRP, Oliveira-Júnior JB, Firmo EF. First report of a blaNDM-resistant gene in a *Klebsiella aerogenes* clinical isolate from Brazil. Rev Soc Bras Med Trop. 2021;54:(e02622020).

29. Silva SR, Souza FM, Espinheira MJCL. Avaliação da atividade antibacteriana do óleo essencial das sementes de *P. edulis* Sims frente às bactérias Gram Positivas e Gram Negativas.Rev Mult Psic.2019;3(43):1003-17.

30. Mohanasundari C, Natarajan D, Srinivasan K, Umamaheswari S, Ramachandran A. Antibacterial properties of *Passiflora foetida* L. a common exotic medicinal plant. Afr J Biotechnol. 2007;6(23):2650-3.

31. Fausto MC, Nonato IA, Fausto GC, Pinto R, Valente FL, Carvalho LM et al. Anthelmintic potential and in vivo acute toxicity study of *Piptadenia gonoachanta* leaf aqueous extract. J App Pharm Sci. 2017;7(10):111-17.