



Bioactivity of leaf extracts of *Podocarpus lambertii* Klotzsch ex Endl.: Antioxidant, antimicrobial, and antibiofilm action

Bioatividade de extratos foliares de *Podocarpus lambertii* Klotzsch ex Endl.: Ação antioxidante, antimicrobiana e antibiofilme

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Podocarpus lambertii Klotzsch ex Endl. is a native Brazilian species traditionally used in folk medicine; however, studies on its biological properties remain limited. This study evaluated the antioxidant, antimicrobial, and antibiofilm activities of leaf extracts obtained by dynamic maceration using ethanol, methanol, and acetone as extraction solvents. Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), while antibiofilm activity was assessed by crystal violet staining and the MTT reduction assay. At a concentration of 1.0 mg mL⁻¹, the methanolic (ME), acetone (AE), and ethanolic extracts (EE) exhibited DPPH radical scavenging activities of 94.93%, 93.99%, and 89.26%, respectively. All extracts exhibited antibacterial activity against the tested bacterial strains, with MIC values ranging from 0.39 to 12.5 mg mL⁻¹ and MBC values from 1.56 to 12.5 mg mL⁻¹. Concerning antibiofilm activity, the ME extract reduced the biomass of *Pseudomonas aeruginosa* biofilms, although biofilm cells remained highly viable (> 50%) after treatment. These results demonstrate that *P. lambertii* leaf extracts possess antioxidant and antibacterial activities, highlighting their potential as a source of bioactive compounds for future biotechnological and pharmacological applications.

Keywords: plant extracts, bioactive compounds, free radicals.

A espécie *Podocarpus lambertii* Klotzsch ex Endl. é nativa do Brasil e tradicionalmente utilizada na medicina popular; no entanto, os estudos sobre suas propriedades biológicas ainda são limitados. Este estudo avaliou as atividades antioxidante, antimicrobiana e antibiofilme de extratos foliares obtidos por maceração dinâmica utilizando etanol, metanol e acetona como solventes extratores. A atividade antioxidante foi determinada pelo ensaio de captura do radical livre 2,2-difenil-1-picril-hidrazil (DPPH). A atividade antimicrobiana foi avaliada por meio da determinação da Concentração Inibitória Mínima (CIM) e da Concentração Bactericida Mínima (CBM), enquanto a atividade antibiofilme foi avaliada pelos ensaios de coloração com cristal violeta e redução do MTT. Na concentração de 1,0 mg mL⁻¹, os extratos metanólico (EM), acetônico (EA) e etanólico (EE) apresentaram atividades de captura do radical DPPH de 94,93%, 93,99% e 89,26%, respectivamente. Todos os extratos apresentaram atividade antibacteriana frente às cepas bacterianas avaliadas, com valores de CIM variando de 0,39 a 12,5 mg mL⁻¹ e de CBM entre 1,56 e 12,5 mg mL⁻¹. Em relação à atividade antibiofilme, o extrato metanólico (EM) reduziu a biomassa dos biofilmes de *Pseudomonas aeruginosa*, embora as células do biofilme tenham permanecido com elevada viabilidade (> 50%) após o tratamento. Esses resultados demonstram que os extratos foliares de *P. lambertii* apresentam atividades antioxidante e antibacteriana, evidenciando seu potencial como fonte de compostos bioativos para futuras aplicações biotecnológicas e farmacológicas.

Palavras-chave: extratos vegetais, compostos bioativos, radicais livres.

1. INTRODUCTION

The diversity of bioactive compounds produced by plants is a key feature of plant metabolism and is associated with their ability to respond to abiotic and biotic interactions [1]. In addition to playing important roles in plant defense and protection, these compounds possess a wide range of therapeutic properties [2]. They include, for instance, secondary metabolites with antioxidant properties, such as flavonoids and polyphenols [3], antimutagenic and anticancer activities, such as alkaloids [4], antimicrobial effects, such as terpenoids [5, 6], and antidiabetic actions, also associated with alkaloids [7]. Owing to these properties, metabolites have broad applications in the pharmaceutical, food, cosmetic, agricultural, and various other industries [8].

The extraction of compounds depends on factors such as the method used and the polarity of the solvents employed, which directly influence the diversity and quantity of metabolites obtained [9]. Several metabolites are recognized for their antimicrobial activity and may offer mechanisms distinct from those of conventional antimicrobials, representing an alternative or complementary approach in the fight against resistant microbial strains [10, 11]. Furthermore, many metabolites have demonstrated potential to inhibit the formation of bacterial biofilms, a widespread resistance strategy observed in clinical, industrial, food, and water distribution system environments [12-14].

Among the species known for producing bioactive compounds, the genus *Podocarpus*, belonging to the family Podocarpaceae, stands out. *Podocarpus lambertii*, commonly known as "pinheiro-bravo," is a conifer native to Brazil, occurring in the Caatinga, Cerrado, Atlantic Forest, and Pampa phytogeographic domains [15]. Traditionally, species of the *Podocarpus* genus have been used to treat asthma, fever, venereal diseases, cholera, wounds, fractures, cough, and body odor [16]. In addition to their medicinal applications, these plants are also sources of tannins, waxes, industrial oils, and are used for ornamental and culinary purposes [17]. Previous studies involving different species of this genus, such as *Podocarpus elongatus*, *Podocarpus falcatus*, *Podocarpus henkelii*, and *Podocarpus latifolius* have demonstrated high antioxidant activity, including strong free radical scavenging capacity, β -carotene bleaching inhibition, and ferric reducing power, as well as antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi [18, 19].

In the specific case of *P. lambertii*, recent studies have reported the presence of several secondary metabolites, including tannins, alkaloids, steroids, flavonoids, and saponins, which are believed to contribute to its antioxidant, antimicrobial, and antibiofilm properties [20, 21]. These findings underscore the potential of *P. lambertii* as a valuable source of bioactive compounds for the development of alternative antimicrobial strategies with applications in biotechnology and pharmacology.

The antioxidant effect of plant-derived compounds is related to their ability to neutralize free radicals, for example, through the donation of hydrogen atoms or electrons [22, 23]. Moreover, compounds such as totarol, isolated from *Podocarpus totara*, have demonstrated the ability not only to inhibit the formation of bacterial biofilms but also to degrade already established biofilms, reinforcing the potential of the genus as a source of effective antimicrobial agents [24].

Currently, although several bioactive compounds have proven clinical efficacy, such as aspirin, taxol, linalool, eugenol, and cannabidiol [25, 26], a large number of plant species with pharmacological potential remains unexplored [27]. In this context, the present study aimed to evaluate the antioxidant, antimicrobial, and antibiofilm potential of leaf extracts from *P. lambertii* against foodborne pathogenic bacteria. This research seeks to contribute to the advancement of knowledge on native species, promoting the value of Brazilian biodiversity and encouraging the development of sustainable and innovative solutions for public health and industry.

2. MATERIAL AND METHODS

2.1 Collection and preparation of leaves

Leaf collection was carried out in the Paulo Gorski Ecological Park (24°57'51.61"S, 53°26'14.80"W), located in the municipality of Cascavel, Paraná, between August and October 2020. The botanical identification of the species *P. lambertii* was performed by the Herbarium of the State University of Western Paraná - UNIOESTE and registered under voucher number UNOP 10730. After collection, the leaves were dried in an oven at 35 °C for 7 days and ground using a Willey-type knife mill until reaching a particle size of less than 0.42 mm. The resulting powder was stored in hermetically sealed glass containers, protected from light, until used for extract preparation [28].

2.2 Preparation of organic extracts

Extract preparation was carried out according to the methodology proposed by Pandini et al. (2015) [29], with modifications. Three crude extracts were prepared separately using acetone, methanol and ethanol as extraction solvents. For each extraction, plant material was mixed with the respective solvent at a ratio of 10 g per 100 mL and maintained under agitation in a rotary shaker at 220 rpm for 24 h. After this period, each solution was centrifuged in Falcon-type conical tubes at 3800 rpm for 15 min and filtered through Whatman No. 1 filter paper. The filtrates were subjected to rotary evaporation for solvent removal and crude extract recovery. The resulting acetone extract (AE), methanol extract (ME) and ethanol extract (EE), were stored in a freezer (-20 °C) until further use in the experiments.

2.3 Antioxidant activity

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical reduction method [20, 30]. Sample absorbances were measured using a spectrophotometer at 515 nm. Methanol was used as a blank, 60 µM DPPH as the negative control, BHT (butylated hydroxytoluene) as the positive control, and DPPH concentrations ranging from 0 to 60 µM were used to generate the calibration curve. All assays were performed in triplicate.

Preliminary tests were conducted with the AE, EE, and ME extracts to determine the stabilization time and the dilutions required for the assays. For this purpose, 1 mg of extract was diluted in 1 mL of analytical grade methanol and vortexed for 1 minute. Subsequently, a 0.1 mL aliquot of the dilution was transferred to a test tube, to which 3.9 mL of DPPH solution was added, and the mixture was homogenized. Absorbance readings were taken using a spectrophotometer at 1-minute intervals until stabilization was achieved. Since the absorbance values obtained in the preliminary tests ranged between 80% and 100%, concentrations of 1.0 mg mL⁻¹, 0.75 mg mL⁻¹, 0.50 mg mL⁻¹, 0.25 mg mL⁻¹, and 0.10 mg mL⁻¹ were used to determine the antioxidant potential of the extracts. For this, 0.1 mL of each dilution and 3.9 mL of DPPH solution were pipetted into test tubes, which were then left to rest for the stabilization time determined in the preliminary tests: AE - 27 minutes, EE - 35 minutes, ME - 58 minutes.

Based on the absorbance values obtained, the mean, standard deviation, and IC₅₀ (the concentration of the sample required to reduce the initial DPPH radical concentration by 50%) were calculated. The antioxidant activity at different concentrations was determined using the following equation: $AA\% = \frac{A_0 - A_1}{A_0} * 100$ (where A₀ is the absorbance of the negative control and A₁ is the absorbance of the sample). To determine the IC₅₀, the following linear equation was used: $y = -a * x + b$ (where y = 50, -a is the slope, x is the unknown, and b is the intercept). Antioxidant activity data were analyzed using analysis of variance (ANOVA) in a 3 × 5 factorial, considering extract type (AE, EE and ME) and concentration (0.10, 0.25, 0.50, 0.75 and 1.0 mg mL⁻¹) as factors. When the interaction between factors was significant, the interaction was further decomposed, and means were compared using Tukey's test at a 5% significance level (p < 0.05).

BHT was used as a positive control and was not included in the factorial analysis. Statistical analyses were performed using Sisvar® software, version 5.8.

2.4 Antimicrobial activity

2.4.1 Microorganisms used and inoculum preparation

The microorganisms used were standard strains from the American Type Culture Collection (ATCC): *Citrobacter freundii* (ATCC 8090), *Enterobacter cloacae* (ATCC 13047), *Enterococcus faecalis* (ATCC 33186), *Listeria innocua* (ATCC 33090), *Listeria monocytogenes* (ATCC 1911), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* serovar Enteritidis (ATCC 13076), *Salmonella enterica* serovar Abaetetuba (ATCC 35640), *Staphylococcus aureus* (ATCC 6538), and *Staphylococcus epidermidis* (ATCC 12228). The strains were transferred from stock agar to BHI (Brain Heart Infusion Broth) and incubated in a bacteriological incubator for 18-24 h at 35 ± 2 °C. Subsequently, streak plating was performed on Nutrient Agar (NA) plates, which were incubated for another 18-24 h. Isolated colonies of each bacterial strain were suspended in 0.85% saline solution to standardize the inoculum according to the 0.5 McFarland scale.

2.4.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Antibacterial activity tests were conducted using the serial microdilution method, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (2018) [31] and the methodology described by Bandeira et al. (2024) [21]. Antibacterial activity was classified according to Pandini et al. (2015) [29] as high (≤ 12.5 mg mL⁻¹), moderate (25 mg mL⁻¹), low (50 mg mL⁻¹), or very low (≥ 100 mg mL⁻¹). All tests were performed in triplicate.

In 96-well plates, 150 µL of double-strength Mueller-Hinton Broth (MH 2×) was added starting from the second well of each column. In the first wells, 300 µL of the plant extract, previously diluted at a ratio of 0.20 g of extract, 0.5 mL of analytical grade methanol, and 0.5 mL of MH 2× broth, was added. After performing the microdilution in a range from 200 to 0.09 mg mL⁻¹, 20 µL of the bacterial inoculum was added to each well. Controls were prepared as follows: 20 µL of the inoculum and 150 µL of double-strength Mueller-Hinton Broth (MH 2×) for the negative control, 75 µL of analytical grade methanol, 75 µL of MH 2× broth, and 20 µL of the inoculum for the diluent control, and 150 µL of the extract solubilized in analytical grade methanol and MH 2× broth for the extract sterility control. For the positive control, a serial microdilution of the commercial antibiotic gentamicin (200 mg mL⁻¹) was used, along with 20 µL of the inoculum added to each well of the plate.

The 96-well plates were gently homogenized and incubated for 18-24 h. After this period, the Minimum Bactericidal Concentration (MBC) was determined by transferring 2 µL of the inoculum from each well to Mueller-Hinton Agar (MH) plates. Following the removal of the 2 µL aliquots, 20 µL of 0.5% TTC (2,3,5-triphenyltetrazolium chloride) was added to the 96-well plates to assess the Minimum Inhibitory Concentration (MIC). The 96-well plates (used for MIC determination) were incubated for 3 h; the absence of a red coloration in the wells after this period indicated inhibition of bacterial growth. The MBC plates were incubated for 18-24 h; the absence of growth after this period indicated bacterial cell death.

2.5 Antibiofilm activity

2.5.1 Evaluation of extract activity on bacterial biofilm

The assays were conducted using strains from the American Type Culture Collection (ATCC): *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa*

(ATCC 27853), *Listeria monocytogenes* (ATCC 1911), *Salmonella enterica* Enteritidis (ATCC 13076), and *Salmonella enterica* Abaetetuba (ATCC 35640). The positive control consisted of Tryptic Soy Broth (TSB) supplemented with 1% glucose and bacterial inoculum, while the diluent control was prepared using DMSO (dimethyl sulfoxide). The tests were performed according to the methodology described by Stepanović et al. (2007) [32] and Laskoski et al. (2024) [33], with modifications. The antibiofilm activity was assessed at MIC, 2× MIC, and 4× MIC concentrations for each bacterial strain. Biofilm biomass was quantified using 2% Crystal Violet staining, and cell metabolic activity was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Statistical analysis was performed using analysis of variance ANOVA and Tukey's test at 5% significance ($p < 0.05$), with the aid of Sisvar[®] software, version 5.8.

2.5.2 Formation and treatment of bacterial biofilm

For biofilm formation in 96-well microplates, 180 μL of TSB supplemented with 1% glucose and 20 μL of bacterial inoculum ($0.5 \times 10^5 \text{ CFU mL}^{-1}$) were distributed into each well, and the plates were incubated for 24 h. Subsequently, the culture medium was removed and the wells were washed with 1X PBS (Phosphate Buffered Saline), after which 150 μL of the treatments (EE, ME, and AE) at concentrations ranging from 200 to 1.56 mg mL^{-1} were added. The plates were then incubated for an additional 24 h. After this period, the medium was removed and the wells were washed three times with 1X PBS. For cell fixation, the wells were exposed to 150 μL of analytical grade methanol for 20 min. After complete air drying of the plates, staining procedures were performed to assess biomass and cell viability.

2.5.3 Biofilm biomass assessment using crystal violet

Each well of the plates was exposed to 150 μL of 2% Crystal Violet for 15 min, followed by gentle washing with running water until complete removal of the dye. Subsequently, 150 μL of 95% ethanol was added, and after 30 min, the contents were transferred to a new plate for optical density (OD) reading at 570 nm using a Biotek Epoch microplate reader.

The percentage of inhibition was calculated using the following equation: Inhibition percentage = $((\text{OD of the positive control} - \text{OD of the experimental group}) / \text{OD of the positive control}) \times 100$ [34] and classified as: low antibiofilm activity ($< 50\%$) and high antibiofilm activity ($> 50\%$) [12].

2.5.4 Assessment of biofilm cell viability using MTT

After dispensing 180 μL of TSB and 20 μL of MTT into each well of the plates, they were incubated for 2 h. Subsequently, after removing the medium, 150 μL of DMSO were added, and after 15 min, the contents were transferred to a new plate for optical density (OD) reading at 570 nm using a Biotek Epoch microplate reader.

The percentage of cell viability was determined using the equation: Cell viability = $(\text{OD experimental} / \text{OD positive control}) \times 100$ [35] and classified as: low metabolic activity ($< 50\%$) and high metabolic activity ($> 50\%$) [12].

3. RESULTS AND DISCUSSION

3.1 Antioxidant activity

In the antioxidant activity analysis, a significant interaction was observed between extract type and concentration, indicating that the effect of one factor depended on the level of the other. For the ME, all concentrations differed from each other. No differences were observed between the

concentrations of 0.25 and 0.50 mg mL⁻¹ for the EE, nor between the concentrations of 0.50 and 0.75 mg mL⁻¹ for the AE. Comparison among extracts at each concentration showed that the EE exhibited higher antioxidant activity at 0.25 and 0.75 mg mL⁻¹. At 0.10 and 0.50 mg mL⁻¹, the EE and AE showed similar antioxidant activities, both being higher than that of the ME. At 1.0 mg mL⁻¹, the methanolic extract exhibited higher antioxidant activity than the EE, whereas the AE did not differ from either of them. The lower IC₅₀ values of EE (0.35 mg mL⁻¹) and AE (0.43 mg mL⁻¹), compared with ME (0.58 mg mL⁻¹), indicate that lower concentrations of these extracts were required to reduce the initial DPPH concentration by 50% (Table 1).

Table 1: Antioxidant activity (%) of *Podocarpus lambertii* leaf extracts determined by the DPPH free radical scavenging assay.

Concentration (mg mL ⁻¹)	BHT	Ethanollic extract (EE)	Methanolic extract (ME)	Acetone extract (AE)
1.0	97.20 ± 0.31	89.26 ± 1.40 Ab	94.93 ± 0.54 Aa	93.99 ± 1.45 Aab
0.75	67.52 ± 1.64	82.59 ± 3.53 Ba	63.66 ± 2.93 Bb	65.24 ± 3.47 Bb
0.5	44.11 ± 0.39	57.93 ± 3.80 Ca	41.00 ± 2.39 Cb	62.64 ± 3.12 Ba
0.25	37.50 ± 1.10	55.99 ± 0.30 Ca	15.93 ± 2.01 Dc	38.99 ± 2.71 Cb
0.10	22.64 ± 1.04	21.62 ± 4.94 Da	2.71 ± 2.14 Eb	21.00 ± 1.74 Da
IC ₅₀	0.47	0.35	0.58	0.43

Mean ± standard deviation (n = 3). Different uppercase letters within the same column indicate significant differences among concentrations for the same extract, whereas different lowercase letters within the same row indicate significant differences among extracts at the same concentration, according to Tukey's test (p < 0.05).

The antioxidant activity observed for the ME was similar to that reported for methanolic leaf extracts of *P. elongatus*, *P. falcatus*, *P. henkelii*, and *P. latifolius*, whose antioxidant activities ranged from 14.18% to 97.94%, depending on the concentration evaluated. These extracts contained varying levels of phenolic compounds and flavonoids, and the authors suggested that the antioxidant activity may be related to the presence of these compounds, as well as diterpenes and biflavonoids [18].

In this context, studies have shown that methanolic extract of *P. lambertii* contains higher levels of total phenolics, flavonoids, tannins, and alkaloids than ethanolic and acetone extracts [20, 21]. However, it is important to emphasize that antioxidant activity is determined not only by the amount of these compounds but also by their synergistic interactions, chemical structure, and stability [36]. This may explain why the ethanolic extract exhibited higher antioxidant activity at intermediate concentrations, whereas the methanolic extract reached maximum antioxidant activity only at the highest concentration evaluated.

This behavior may be attributed to the antioxidant mechanisms of the phytochemicals present in the extracts. The presence of phenolic compounds in the extracts enables the inhibition of free radicals due to their redox properties, which support their action as reducing agents, hydrogen donors, and radical scavengers [37, 38]. In the case of flavonoids, free radical elimination occurs through hydrogen donation at the active hydroxyl group and their ability to chelate metal ions [39].

The plant's phenological stage and its interaction with the environment influence the synthesis and accumulation of metabolites, resulting in differences in the chemical composition of plant extracts [40]. Although the phenological stage was not recorded in the present study or in the studies cited, differences in environmental conditions throughout the different collection months may have influenced the metabolic response of the plants and, consequently, the phytochemical composition and antioxidant activity of the extracts. Additionally, factors such as compound complexity, the polarity of the solvent used in the extraction process [41], and the intrinsic characteristics of each extract also play significant roles in antioxidant potential [18].

3.2 Antimicrobial activity

The tests performed using the broth microdilution method indicated strong bacteriostatic and bactericidal activity ($\leq 12.5 \text{ mg mL}^{-1}$) of the three extracts against all ten standard strains (ATCC). The extract concentrations required for antibacterial action varied according to the solvents used in the extraction of the plant's bioactive compounds (Table 2).

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Podocarpus lambertii* leaf extracts.

MICROORGANISM	MIC/MBC (mg mL^{-1})		
	Acetone Extract (AE)	Methanolic extract (ME)	Ethanollic extract (EE)
Gram-positive			
<i>Enterococcus faecalis</i> (ATCC 33186)	6.25/12.5	12.5/12.5	6.25/12.5
<i>Listeria innocua</i> (ATCC 33090)	3.12/12.5	6.25/6.25	1.56/1.56
<i>Listeria monocytogenes</i> (ATCC 1911)	3.12/3.12	3.12/3.12	3.12/3.12
<i>Staphylococcus aureus</i> (ATCC 6538)	1.56/6.25	1.56/1.56	1.56/3.12
<i>Staphylococcus epidermidis</i> (ATCC 12228)	0.78/6.25	12.5/12.5	0.39/3.12
Gram-negative			
<i>Citrobacter freundii</i> (ATCC 8090)	1.56/1.56	3.12/3.12	1.56/1.56
<i>Enterobacter cloacae</i> (ATCC 13047)	3.12/3.12	12.5/12.5	3.12/3.12
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	1.56/1.56	3.12/12.5	1.56/1.56
<i>Salmonella enterica</i> Abaetetuba (ATCC 35640)	6.25/12.5	12.5/12.5	12.5/12.5
<i>Salmonella enterica</i> Enteritidis (ATCC 13076)	3.12/3.12	3.12/3.12	6.25/6.25

Classification according to Pandini et al. (2015) [29]: High ($\leq 12.5 \text{ mg mL}^{-1}$), Moderate (25 mg mL^{-1}), Low (50 mg mL^{-1}), Very low ($\geq 100 \text{ mg mL}^{-1}$).

EE and AE exhibited superior antimicrobial activity compared to ME, as indicated by MIC/MBC values of $0.39/12.5 \text{ mg mL}^{-1}$, $0.78/12.5 \text{ mg mL}^{-1}$, and $1.56/12.5 \text{ mg mL}^{-1}$, respectively. Among the most effective extracts, the EE of *P. lambertii* demonstrated greater efficacy against *L. innocua*, *S. aureus*, and *S. epidermidis*, while AE showed better results against *S. Abaetetuba* and *S. Enteritidis*. Regarding ME, its effect on *S. aureus* was greater compared to EE and AE, however, its efficacy against *E. faecalis*, *S. epidermidis*, and *E. cloacae* was markedly lower than that of the other two extracts.

Bagla et al. (2014) [42] reported that two biflavonoids and one flavone, isolated from the leaves of *P. henkelii*, exhibited MIC values ranging from 60 to $250 \mu\text{g mL}^{-1}$ against *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa*. Thus, compounds such as flavonoids, found in the AE, EE, and ME of *P. lambertii* [20, 21], may be among the bioactives responsible for the high antibacterial potential observed in our assays, as in addition to their antioxidant properties, these compounds are capable of inhibiting metabolic processes and affecting microbial permeability [43].

According to Abdillahi et al. (2008) [19], extracts of *P. latifolius*, *P. falcatus*, *P. henkelii*, and *P. elongatus*, obtained using petroleum ether, dichloromethane, and ethanol, demonstrated antibacterial activity against *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 11775), and *Klebsiella pneumoniae* (ATCC 13883). Specifically, the EE from the leaves of these species showed MIC values ranging from 0.098 to 0.52 mg mL^{-1} against *S. aureus* (ATCC 12600), whereas in our assays with *P. lambertii* against *S. aureus* (ATCC 6538), the MIC was 1.56 mg mL^{-1} .

Considering the differences in cell wall composition between Gram-positive and Gram-negative bacteria, EE and AE stood out in terms of inhibitory activity against

S. epidermidis, with MIC values of 0.39 mg mL⁻¹ and 0.78 mg mL⁻¹, respectively. Among the Gram-negative bacteria, the lowest MIC was observed for *C. freundii* and *P. aeruginosa*, both with MIC values of 1.56 mg mL⁻¹ for EE and AE. In the context of bacterial resistance, *E. faecalis* was the most resistant strain among the Gram-positive bacteria, with MIC/MBC values of 6.25/12.5 mg mL⁻¹ for both AE and EE, and 12.5/12.5 mg mL⁻¹ for ME. Among the Gram-negative bacteria, *S. Abaetetuba* was the most resistant, with MIC/MBC values of 6.25/12.5 mg mL⁻¹ for AE and 12.5/12.5 mg mL⁻¹ for both ME and EE.

The effects observed on the strains may be attributed, in part, to the presence of compounds such as terpenoids, which are commonly found in the genus *Podocarpus* [16]. The antimicrobial mechanisms of plant extracts against Gram-positive and Gram-negative strains are associated with the ability of their compounds to interact with cell wall structures, induce cytoplasmic membrane disruption, inhibit nucleic acid synthesis, trigger oxidative stress, and interfere with bacterial quorum sensing [44].

3.3 Evaluation of the activity of plant extracts on bacterial biofilm

The extracts were tested at concentrations corresponding to the MIC, 2×MIC, and 4×MIC for each bacterial strain, however, as no differences were observed, the results presented refer exclusively to the MIC. In the evaluation of biofilm biomass, the ME exhibited inhibitory activity of 50.67% against the biofilm formed by *P. aeruginosa*. Conversely, the EE, ME, and AE extracts stimulated cellular biomass production in the other tested strains (Figure 1).

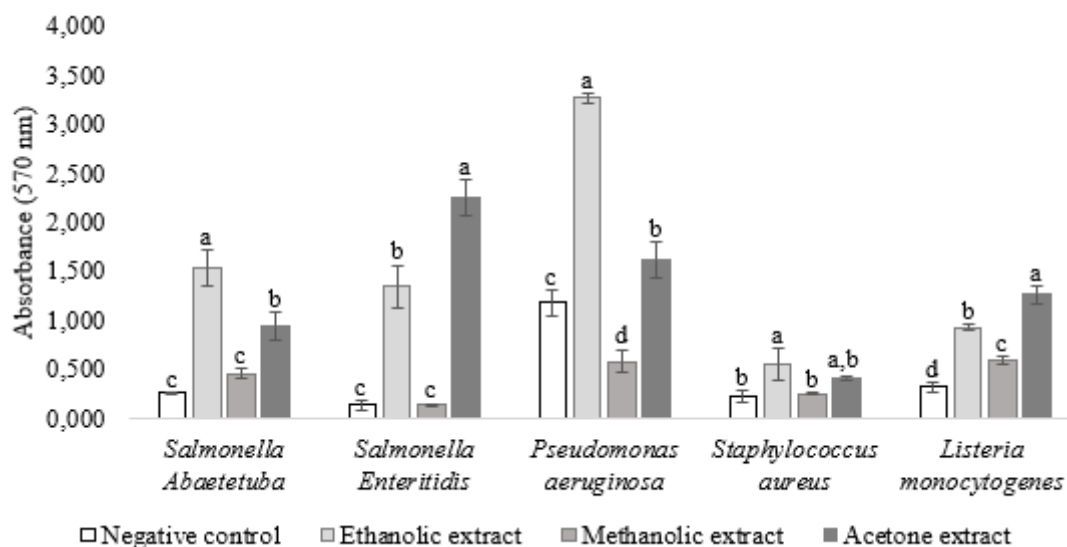


Figure 1: Antibiofilm activity of *Podocarpus lambertii* leaf extracts on the biomass of standard bacteria using the crystal violet assay. Optical density $\pm$ standard deviation. Different letters within the same group indicate significant differences. Tukey's test ($p < 0.05$).

Although this was not investigated in the present study, these responses may be associated with the subinhibitory action of compounds present in the extracts, which may have induced cellular stress. Therefore, future studies are needed to investigate whether the extracts contain compounds capable of modulating microbial metabolism and cellular homeostasis, as well as to elucidate the mechanisms underlying this response. If confirmed, such compounds may be explored as synergistic agents or adjuvants in combination with commercial antibiotics.

In addition to the increased biomass observed in most biofilms, the extracts did not reduce the number of metabolically active cells within the biofilms. All strains showed high cell viability ($> 50\%$) after treatment with the EE, ME, and AE extracts (Figure 2).

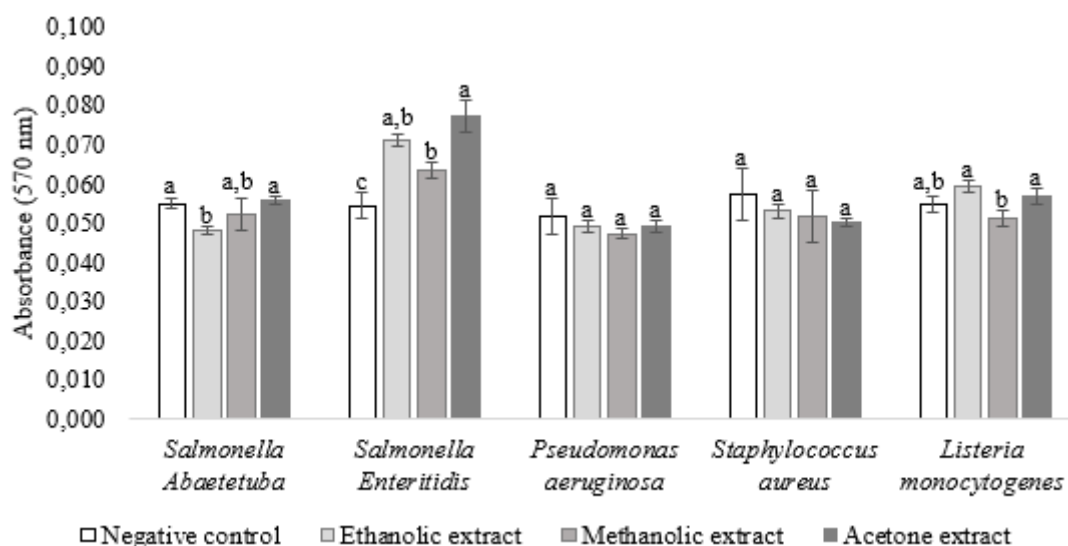


Figure 2: Antibiofilm activity of *Podocarpus lambertii* leaf extracts on bacterial cell viability using the MTT assay. Mean optical density $\pm$ standard deviation. Different letters within the same group indicate significant differences. Tukey's test ($p < 0.05$).

Although ME reduced the biomass of the biofilm formed by *P. aeruginosa*, it did not exhibit antibiofilm potential when cell viability was assessed. Similar results were reported by Bandeira et al. (2024) [21], who observed a comparable behavior with acetone, hexane, and ethyl acetate extracts of *P. lambertii*.

The transition from the planktonic to the sessile form resulting from biofilm formation endows bacteria with distinct properties, including increased resistance to high concentrations of antimicrobial compounds [45]. In this form, antimicrobial resistance can be up to 1000 times higher [46, 47], which may explain the discrepancy between the high antimicrobial activity (MIC and MBC) and the low antibiofilm activity on viability and microbial biomass, due to the limited effectiveness of the extracts in inhibiting bacterial biomass growth.

The extracellular polymeric substance (EPS) matrix is one of the features that hinders biofilm disruption, and although bioactive compounds exhibit antimicrobial activity and are capable of inhibiting microbial quorum sensing, an increase in biofilm formation or resistance to stress may still occur due to metabolic adaptations [48-50].

Compounds found in plant extracts may inhibit cell adhesion and EPS synthesis during biofilm formation, as well as disrupt pre-formed or mature biofilms [51]. However, as the compounds responsible for the biological effects observed in the present study remain unknown, further studies are needed to identify and quantify these compounds and elucidate their mechanisms of action during biofilm formation.

In this context, several studies report that plant extracts have the ability to inhibit biofilm formation, particularly when they act at the early stages of the cell adhesion process. However, the inhibition or reduction of already established biofilms represents a significantly greater challenge [52-54], as bioactive compounds face increased difficulty in penetrating the dense extracellular matrix that protects them [55].

4. CONCLUSION

The leaf extracts of *P. lambertii* exhibited antioxidant activity and antimicrobial action against various bacterial strains; however, they did not demonstrate antibiofilm activity. This study provides novel data on the bioactive potential of *P. lambertii*, suggesting its possible application in the control of pathogenic bacteria in sanitary and industrial contexts.

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