

Effect of oxidation of the hydroalcoholic extract of *Myracrodruon urundeuva* All. (Anacardiaceae) on its antimicrobial activity

ABSTRACT

This study investigates the relationship between oxidation and antimicrobial activity of the hydroalcoholic extract of *Myracrodruon urundeuva*, a plant known for its traditional medicinal uses. The primary objective was to evaluate how the oxidation of bioactive compounds within the extract affects its antimicrobial efficacy against common pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. The extract was prepared following a standardized methodology, and its antimicrobial properties were assessed using disk diffusion assays over a 60-day period, with samples tested every 15 days to monitor changes in activity. The results revealed that the extract exhibited significant antimicrobial activity, particularly at higher concentrations (50% and 75%), with inhibition zones reaching up to 16.7 mm against *Staphylococcus aureus* by day 30. However, a notable decline in activity was observed between days 30 and 45, indicating a potential degradation of the extract's efficacy over time. Interestingly, lower concentrations (12.5% and 0.4%) showed a resurgence in inhibitory activity towards the end of the study, suggesting that certain oxidative processes may enhance the antimicrobial properties of specific compounds. The study highlights the complex relationship between oxidation and antimicrobial activity, demonstrating that certain compounds lose effectiveness, while others become more potent after oxidative modification. This finding is particularly relevant in the context of increasing bacterial resistance to conventional antibiotics, as it suggests that the oxidation of plant-derived compounds could be a viable strategy to enhance their antimicrobial properties. Overall, this research underscores the potential of *Myracrodruon urundeuva* as a natural alternative in combating resistant pathogens and emphasizes the need for further investigation into the mechanisms by which oxidation influences the activity of bioactive compounds in medicinal plants.

Keywords: *Myracrodruon urundeuva*, Antimicrobial activity, Oxidation, Hydroalcoholic extract, Bacterial resistance

1. INTRODUCTION

The Aroeira-do-Sertão, *Myracrodruon urundeuva* All. (Anacardiaceae), is a plant species that occurs in Paraguay, Argentina, Bolivia and Brazil [22]. It is widely used in the folk medicine of northeastern Brazil in the treatment of wounds and by midwives during the postpartum period, attributing such results to its healing properties [5, 11].

The compounds present in plant extracts made with Aroeira are the main ones responsible for their applicability, whether in antimicrobial, healing or antiulcerogenic activity [12, 25, 7]. However, factors like exposure to light, high temperatures, and time can alter their chemical components [19], leading to molecular changes and the formation of free radicals [6, 15].

In plant extracts, the presence of ROS can lead to the manipulation of bioactive components, such as flavonoids, terpenoids and alkaloids [9]. This oxidation process can alter the three-dimensional conformation of these organic molecules, resulting in the loss of their biological activity.

In addition, proteins present in plant extracts can undergo structural modifications, such as the formation of anomalous disulfide bridges, leading to denaturation [17]. These changes in molecular conformation can reduce the stability of plant extracts, compromising their quality and functionality in pharmaceutical applications.

Understanding how manipulation affects the activity of plant extract compounds helps predict the efficacy of products made from these extracts, especially in folk medicine.

In view of the increase in bacterial resistance to common bacteria and the scarcity of data in the literature on the oxidation of plant extracts, the present work specifically investigates how the antimicrobial activity of the hydroalcoholic extract of *M. urundeuva* varies as a result of the oxidation of its bioactive compositions.

2. METHODOLOGY

2.1 Obtaining the plant extract and dilution

To obtain the hydroalcoholic extract, 3.9g of the Aroeira leaves, after drying in a closed circulation oven at 65°C for 24h, were mixed with 500ml of a 35% hydroalcoholic solution. The mixture was then left to rest in a place protected from light and temperature for 24h. At the end of this period, the mixture was filtered in a vacuum pump and placed in a water bath until it reached a temperature of 80°C in order to remove the alcoholic part.

The extract was stored in an amber bottle at 4°C to prevent degradation by light and temperature. Every 15 days, a portion of the extract was diluted into seven concentrations (100%, 75%, 50%, 25%, 12.5%, 6.25%, 3.13%, and 0.4%) for antimicrobial testing, forming treatments from the highest concentration (T1) to the lowest (T7). The concentrations were obtained through serial dilution, being: 100%, 75%, 50%, 25%, 12.5%, 6.25%, 3.13% and 0.4%.

2.2 Preparation of materials

Mueller-Hinton agar culture medium was sterilized in an autoclave at 121°C for 20 minutes, along with petri dishes, a Drigalisk loop, pipette tips and absorbent paper discs. The culture medium was then poured into the petri dishes in a vertical laminar flow hood to perform the antimicrobial test. The microorganisms selected for the antimicrobial test were *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 15442 and *Candida albicans* ATCC 4868.

2.3 Assessment of antimicrobial activity

The analyses were performed in triplicate for all microorganisms. The disk diffusion tests were performed starting on the day the extract was prepared, which was called day zero (D0), and every 15 days after the preparation of the extract until day sixty (D60), thus monitoring the oxidation of the extract.

In addition, two types of positive controls were used for each microorganism, for bacteria: Disks containing penicillin and disks soaked in 70% alcohol; for the fungus *C. albicans*: Disks containing Nystatin and disks soaked in 70% alcohol.

To perform the disk diffusion test, each microorganism strain was pipetted onto the culture medium, and spread using a Drigalisk loop until the surface was evenly inoculated. Sterile paper discs were soaked in the different extract concentrations and placed near the edge of the plate with slight pressure to ensure contact. Plates were then incubated at $\pm 37^{\circ}\text{C}$ for 24 hours [16].

After the incubation period of the plates, the inhibition halos formed by the discs containing the treatments and controls were measured using a caliper, and the results obtained in millimeters were used to form averages that were used to evaluate the results.

2.4 Statement of principal findings

The methodology described by Alves et al. [4] was used for the qualitative classification of the inhibition means obtained in each treatment. The results were categorized as susceptible (S), for inhibition zones greater than 12 mm; intermediate (I), for zones between 9 and 12 mm; and resistant (R), for zones less than 9 mm. The statistical analysis was performed using the Sisvar statistical software and the graphs were plotted in R 4.4.1 [20].

3. RESULTS AND DISCUSSION

The averages obtained from the antibiograms performed over the period were displayed in tables. The variation in the averages obtained over time was represented by means of line graphs for each species, for better visualization.

The treatments applied to the *C. albicans* species had a significant effect on the results from day 0 to 60 ($P=0.05$). The values are shown in Table 1 according to the concentration of the extract and the day on which the disk diffusion test was performed. The variation in these results over time can be seen in Figure 1.

Table 1. Means of the inhibition halos of *Candida albicans* obtained from the concentrations of the hydroalcoholic extract of *M.urundeuva*

Microorganism	Treatment	Days after the extract was prepared				
		0	15	30	45	60
<i>Candida albicans</i>	100%	10,3 (I)	10,2 (I)	11 (I)	11,73 (I)	12,13 (S)
	75%	9,33 (I)	10,13 (I)	10,87 (I)	9,37 (I)	10,7 (I)
	50%	9,33 (I)	9,4 (I)	9,6 (I)	8,33 (R)	10,8 (I)
	25%	7,2 (R)	8,53 (R)	8,13 (R)	7,3 (R)	10,33 (I)
	12,50%	7,37 (R)	7,5 (R)	7,27 (R)	7,03 (R)	9 (I)
	6,25%	4,33 (R)	4,52 (R)	3,23 (R)	4,97 (R)	8,4 (R)
	3,13%	2,33 (R)	2 (R)	2 (R)	0 (R)	4,83 (R)
Control	Nistatin	0 (R)				
	Alcohol 70%	7,73 (R)				

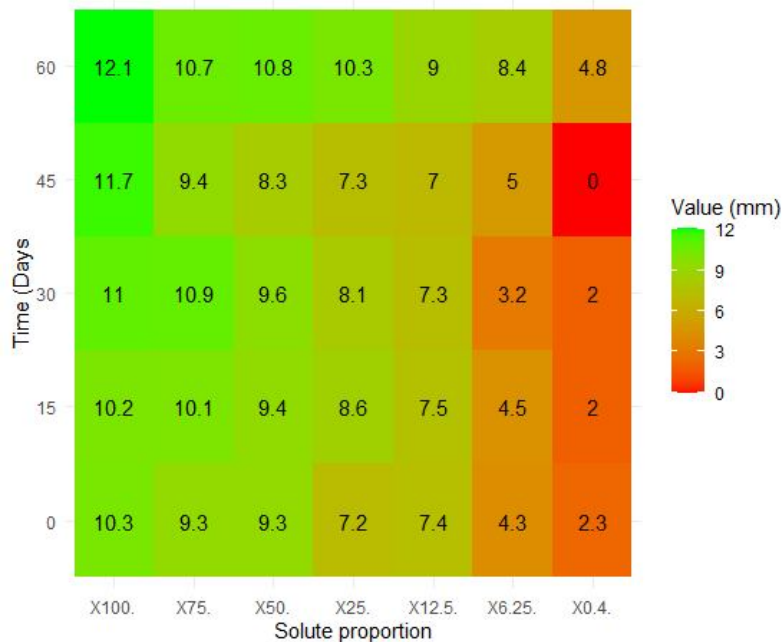


Fig. 1. Heat map of the variation of the mean inhibition halos of *Candida albicans* over time

Control: Nistatin = 0mm; Alcohol 70%= 7.73mm

It was observed that the size of the inhibition halos and the concentration of the extract follow a directly proportional relationship, where the higher the concentration, the greater the halo measurement. As shown in Figure 1, all concentrations exhibited increased antibacterial activity from day 45 to day 60. The most notable change was observed at the 0.4% concentration, where inhibition increased from 0 mm on day 45 to 4.8 mm on day 60.

The 6.25% concentration also showed a gradual increase in activity between days 30 and 60. This suggests that at lower concentrations, oxidation may improve antimicrobial activity, possibly by preserving or enhancing the bioactive compounds.

In general, the graph shows that long-term oxidation increases the antimicrobial activity of the extract, since when observing the averages obtained at each concentration on day 0, they increased on day 60. The fluctuations were more pronounced at lower concentrations, indicating that these concentrations were more affected by oxidative changes over time.

With regard to the bacteria *S. aureus*, the results obtained were significant from day 0 to 60 ($P=0.05$) and demonstrate that on day 30 the concentration of 100% had a significant increase in inhibition (Figure 2), going from 13.37 mm on day 15 to 22.1 mm (Table 2).

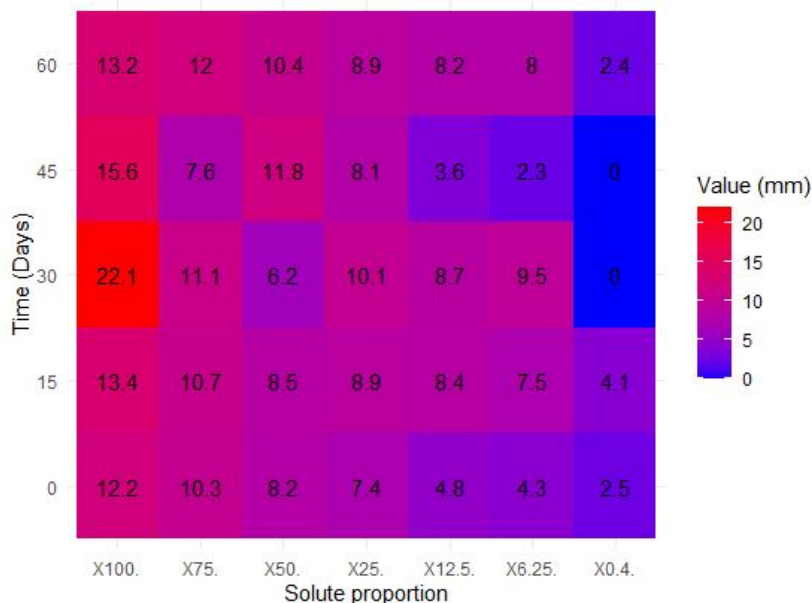


Fig. 2. Heat map of the variation of the mean inhibition halos of *Staphylococcus aureus* over time
Control: Penicilin = 2.13mm; Alcohol 70%= 0mm

Table 2. Means of the inhibition halos of *Staphylococcus aureus* obtained from the concentrations of the hydroalcoholic extract of *M.urundeuva*

Microorganism	Treatment	Days after the extract was prepared				
		0	15	30	45	60
<i>Staphylococcus aureus</i>	100%	12,23 (S)	13,67 (S)	22,13 (S)	15,6 (S)	13,19 (S)
	75%	10,27 (I)	10,7 (I)	11,13 (I)	7,57 (R)	12 (I)
	50%	8,24 (R)	8,5 (R)	6,2 (R)	11,8 (I)	10,43 (I)
	25%	7,4 (R)	8,93 (R)	10,13 (I)	8,13 (R)	8,93 (R)
	12,50%	4,77 (R)	8,37 (R)	8,73 (R)	3,6 (R)	8,2 (R)
	6,25%	4,33 (R)	7,57 (R)	9,53 (I)	2,3 (R)	8 (R)
	3,13%	2,5 (R)	4,17 (R)	0 (R)	0 (R)	2,37 (R)
Control	Penicilin	2,13 (R)				
	Alcohol 70%	0 (R)				

Regarding the categories, it is noted that both controls presented results below 9 mm, meaning that the bacteria were resistant to them. The 100% concentration remained with an average inhibition level above 12 mm throughout the experiment. The treatment with 75% of the extract maintained intermediate inhibition stability, unlike the treatment with 50%, in which the bacteria showed resistance during the first 30 days of the experiment and soon after ended with intermediate inhibition.

For the other treatments, although the bacteria showed resistance to them, it is noted that, as the oxidative process of the extract components progresses, the average inhibition zone of the treatments increases when comparing the results from day 0 with day 60, indicating that in the long term, the oxidation of the components was also effective in increasing the inhibitory potential of the extract. The action of the extract against the species *P. aeruginosa* was also significant from day 0 to 60 ($p < 0.05$) and presented a similar effect of significant increase in inhibition on day 30 in relation to *S. aureus* in relation to the 100% concentration of the extract (Figure 3).

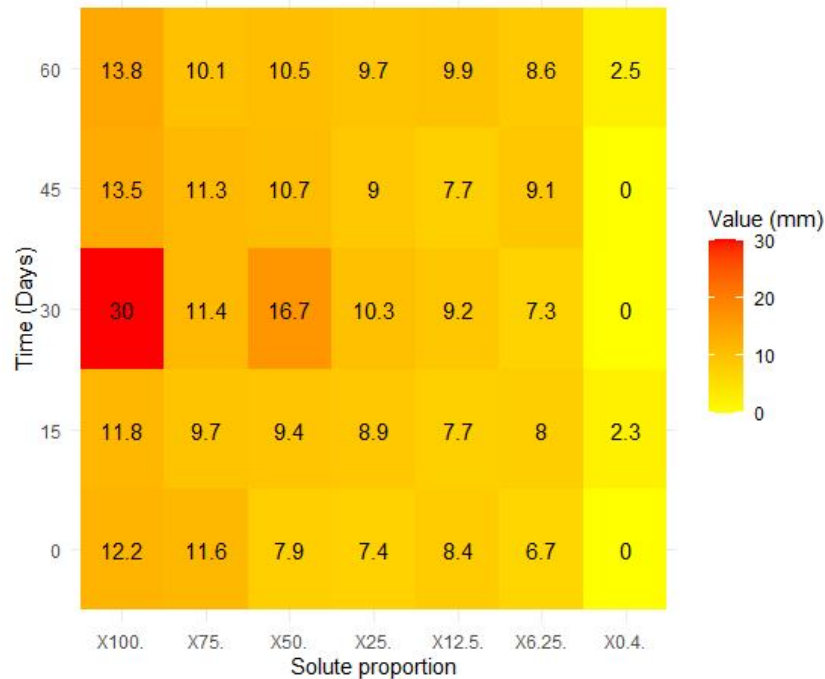


Fig. 3. Heat map of the variation of the mean inhibition halos of *Pseudomonas aeruginosa* over time
 Control: Penicilin = 2.13mm; Alcohol 70% = 5.43mm

It is observed that the 50% concentration also showed an increase in inhibition on day 30, going from 9.41 mm to 16.7 mm (Table 3), while it decreased in the period between day 30 and 45 and remained constant until day 60. In contrast, the lowest concentrations such as 12.5% and 0.4% had a slight increase in their inhibitory activity between day 45 and day 60, while the others had a relatively stable progression.

Table 3. Means of the inhibition halos of *Pseudomonas aeruginosa* obtained from the concentrations of the hydroalcoholic extract of *M.urundeuva*

Microorganism	Treatment	Days after the extract was prepared				
		0	15	30	45	60
<i>Candida albicans</i>	100%	12,2 (S)	11,75 (I)	25,9 (S)	13,53 (S)	13,8 (S)
	75%	11,6 (I)	9,7 (I)	11,4 (I)	11,33 (I)	10,13 (I)
	50%	7,87 (R)	9,41 (I)	16,67 (S)	10,7 (I)	10,47 (I)
	25%	7,4 (R)	8,92 (R)	10,33 (I)	9,03 (I)	9,67 (I)
	12,50%	8,43 (R)	7,72 (R)	9,17 (I)	7,7 (R)	9,9 (I)

	6,25%	6,73 (R)	8,03 (R)	7,27 (R)	8,13 (R)	8,57 (R)
	0,40%	0 (R)	2,3 (R)	0 (R)	0 (R)	2,47 (R)
Control	Penicilin	2,13 (R)				
	Alcohol 70%	5,43 (R)				

Regarding the categorization of the results, the bacteria remained stably susceptible to the extract at a concentration of 100%. However, the extract demonstrated intermediate inhibition at a concentration of 75%. Since the bacteria became susceptible to the extract at a concentration of 50% on day 30, this may indicate the formation of one or more compounds that are harmful to microorganisms, since this inhibition peak was also strongly observed at a concentration of 100% for *P. aeruginosa* and *S. aureus*. In addition, it is possible to observe that treatments between 50% and 0.4% had an increase in the mean inhibition, when comparing days 0 and 60.

The oxidation process of phytochemical compounds is complex and involves many variables. However, according to the results obtained, it is possible to infer that oxidation has a degree of potentiation of the antimicrobial activity of the bioactive compounds of the extract, this idea being reinforced by the work of Ye et al. [28], in which the authors show that oxidation provided an increase in the antimicrobial activity of the components present in grape seeds against the bacterium *Staphylococcus aureus*.

When observing the results obtained with *Pseudomonas aeruginosa*, it was clear that penicillin was not effective against the microorganism, since this bacterium is capable of producing different types of β -lactamases such as AmpC (ampicillinase) [14] which is capable of hydrolyzing and inactivating beta-lactam antibiotics such as penicillin [8], demonstrating the resistance of these microorganisms to common antibiotics.

Regarding the results for *Staphylococcus aureus*, it was observed that the susceptibility of this microorganism to higher concentrations of the extract was very high, especially on day 30, similar to what occurred with *P. aeruginosa*. This is due to the fact that, although *S. aureus* has several virulence factors, such as the formation of a biofilm to protect the bacteria from antibiotics and the immune system, the use of sortase-A to promote colonization in the host, and others [27, 21, 18], the bacteria are not accustomed to dealing with the diversity of bioactive compounds from plant products, since they are capable of interfering in the formation of some virulence factors of this bacteria [27].

The phytochemical composition of the aroeira tree includes compounds such as dimeric chalcones, saponins, hydrolyzable tannins, catechins, triterpenes, alkaloids, and flavonoids, which, in humans, are what attribute the application of the plant in folk medicine [13, 10, 26]. The most typical mechanism of action of phytochemical compounds is certainly the alteration of the plasma membrane of the microorganism [3]. However, the present study did not observe the level of alteration of the extract compounds caused by oxidation, but it was possible to see that the oxidation of these compounds was capable of enhancing the capacity of the compounds to weaken the microorganisms tested, either by attacking the plasma membrane or rendering other virulence mechanisms useless.

Since the 100% concentration for the bacteria *S. aureus* and *P. aeruginosa*, on day 30, was capable of promoting an inhibition classified as very susceptible ($>18\text{mm}$) [4], it is possible to infer that up to a certain degree of degradation of the bioactive compounds there is an enhancement of their antimicrobial capacity for both gram-positive and gram-negative individuals. Regarding *Candida albicans*, there is not much data on the hydroalcoholic extract of *M. urundeuva* with this fungus, however, antimicrobial tests involving extracts from the bark of this plant are very abundant and show the effectiveness of the extract in inhibiting the growth of *C. albicans* by preventing the formation of biofilms and germ tubes [2].

In addition, some components when combined with nystatin can potentiate the inhibition of the growth of *C. albicans* by reducing the amount of nystatin needed to inhibit the growth of this fungus [2].

The results obtained from treatments with aroeira extract reinforce the idea that products of plant origin can be an alternative against antibiotic resistance by these bacteria [23], since the bioactive compounds with antimicrobial properties in plants are diverse.

The oxidation of plant compounds is capable of promoting reactions that are even more intriguing than expected. Taking quercetin, a metabolite present in the aroeira tree [1], for example, the oxidation of this component promotes an even greater capacity to neutralize reactive oxygen species (ROS) than the non-oxidized component [24].

These findings provide insights into the mechanisms by which oxidized compounds combat pathogens, as plants produce these bioactive molecules to defend against phytopathogenic bacteria and fungi in their natural environment.

4. CONCLUSION

A better understanding of the diversity of compounds with antimicrobial activity present in plants, combined with the fact that oxidation of these compounds can increase this activity, becomes a key point for the development of future treatments against these pathogens by combining existing drugs with compounds of plant origin. Despite the results

obtained, there is still little data in the literature on the mechanisms of action of phytochemical compounds after being oxidized and up to what point of degradation becomes acceptable for inhibition by the compounds to remain susceptible.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. It was used the ChatGPT, version GPT-4 model GPT-4 Turbo source provided by OpenAI. The inputs used were "Are there any problems with punctuation and spacing in this abstract?", "point out long sentences that can be shortened for better reading and cohesion"

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