Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Use of cerrado plants as an alternative in the control of bacterial contamination in the alcoholic fermentation process

Uso de plantas de cerrado como alternativa no controle da contaminação bacteriana no processo de fermentação alcoólica

M. C. Lima[a], K. S. Dias[a], G. G. Fonseca[b], R. S. R. Leite[c], M. Batistote[d] and M. F. Paz[a]*

[a] Universidade Federal da Grande Dourados – UFGD, Laboratório de Biotecnologia de Alimentos, Dourados, MS, Brasil
[b] University of Akureyri, School of Business Health and Science, Faculty of Natural Resource Sciences, Akureyri, Iceland
[c] Universidade Federal da Grande Dourados – UFGD, Laboratório de Processos Fermentativos e Enzimáticos – LEPFER, Dourados, MS, Brasil
[d] Universidade Estadual do Mato Grosso do Sul – UEMS, Centro de Pesquisa e Tecnologia em Recursos Naturais – CPTRN, Naviraí, MS, Brasil

Abstract

Bacterial contamination causes irreparable losses in the performance of alcoholic fermentation. Antibiotics are used to control these microorganisms, but they generate residues and cause microbial resistance. Today the only commercial product used by the mills is hops, but it is very expensive. As an alternative, the objective of this work was to evaluate the feasibility of using extracts from plants grown in the Cerrado for antimicrobial control during an alcoholic fermentation to replace antibiotics. Hydraethanolic extracts of leaves and essential oil of the following species were tested: *Schinus terebinthifolius* Raddi, *Serjania erecta*, *Serjania marginata*, *Campomanesia adamantium* and *Syzygium cumini*. Only the extract of Serjania marginata did not show any activity against the bacterium *Bacillus* sp. Both the essential oils as well as the hydroalcoholic extracts of *S. terebinthifolius* and *C. adamantium* and the extract of *S. erecta* showed antibacterial activity without harming the yeast, with potential to replace the hops.

Keywords: antibiosis, cerrado plants, contamination control, essential oil.

Resumo

A contaminação bacteriana provoca perdas irreparáveis no desempenho da fermentação alcoólica. Antibióticos são utilizados para controlar esses microrganismos, mas geram resíduos e causam resistência microbiana. Hoje o único produto comercial utilizado pelas fábricas é o lúpulo, mas é muito caro. Como alternativa, o objetivo deste trabalho foi avaliar a viabilidade da utilização de extratos de plantas cultivadas no Cerrado para controle antimicrobiano durante uma fermentação alcoólica em substituição a antibióticos. Foram testados extratos hidroetanólicos de folhas e óleo essencial das seguintes espécies: *Schinus terebinthifolius* Raddi, *Serjania erecta, Serjania marginata*, *Campomanesia adamantium* e *Syzygium cumini*. Apenas o extrato de *Serjania marginata* não apresentou atividade contra a bactéria *Bacillus* sp. Tanto os óleos essenciais como os extratos hidroetanólicos de *S. terebinthifolius* e *C. adamantium* e o extrato de *S. erecta* apresentaram atividade antibacteriana sem agredir a levedura, com potencial para substituir o lúpulo.

Palavras-chave: antibiose, plantas de cerrado, controle de contaminação, óleo essencial.

1. Introduction

Brazil is responsible for 1/3 of the world's sugarcane production (*Saccharum offificinarum* L.) and the second largest ethanol producer, in 2016, only behind the US (Fernandes et al., 2020; Rigotti et al., 2017). It currently has 418 sugar and alcohol mills, of which 73.20% are located in the central-south region comprising the state of SP, MG, GO, PR and MS (NovaCana, 2021).

The production of first generation ethanol from sugarcane is popular due to its high yields and low costs (Raza et al., 2019). However, it is important to understand the conditions of the process and factors that can affect production, such as environmental changes and bacterial contamination. Bacterial contamination reduces the sugar available for conversion to ethanol and essential micronutrients needed for optimal yeast growth, resulting in reduced ethanol production (Ceccato-Antonini 2021; Fernandes et al., 2020; Rich et al., 2018). Contamination reaching 107-108 CFU per mL causes these losses, with homofermentative lactobacilli being more inhibitory on yeast when present in an equal number of cells, and

*e-mail: mfpaz9@gmail.com; marcelopaz@ufgd.edu.br
Received: December 14, 2023 – Accepted: March 14, 2024

This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

heterofermentative lactobacilli being more deleterious due to their success in competing for sugars during fermentation (Basso et al., 2014).

A variety of antimicrobial agents are used to treat contamination, including antibiotics such as erythromycin, penicillin and tetracycline, but their cost is high (Rich et al., 2018). Nobre et al. (2007) have already raised the hypothesis that bacteria have the ability to acquire resistance to antibiotics, but a study demonstrating this in alcoholic fermentation has never been carried out. Despite the use of antibiotics to combat contaminant bacteria populations in Brazilian sugarcane mills, there are many market-based factors pressuring ethanol manufacturers to reduce the use of these substances. For example, yeast biomass, which is a by-product of the ethanol industry, has high added value as supplement for animal feed (Rigotti et al., 2017).

The problems with antimicrobial resistance are well known, and most antibiotics are used unnecessarily, both in disease treatment and in agriculture, resulting in the generation of resistant microorganisms (Laxminarayan et al., 2013; Béjar-Serrano et al., 2019; Venturieri et al., 2019). Waste and effluent from activities where antibiotics are used to control contamination may contain both these antimicrobials and resistant bacteria. Thus, they are potential environmental routes through runoff to the soil by fertilization resulting in the contamination of surface waters, harming the microbial dynamics in the soil, as well as reaching humans, animals raised for consumption and even wildlife (Laxminarayan et al., 2013; Yan et al., 2013).

The ethanol production industry is seeking alternatives to antibiotics due to their harmful effects on the environment. Plant-based antimicrobials like hops and bacteria are being considered as substitutes for large-scale use (Rich et al., 2018).

The present work aimed to evaluate the viability of using plants grown in the Cerrado that already showed evidence of antimicrobial activity, such as *Schinus terebinthifolius* Raddi, *Serjania erecta* Radlk, *Serjania marginata* Casar, *Syzygium cumini* Linnaeus and *Campomanesia adamantium* Cambess. for antimicrobial control during alcoholic fermentation in the sugar-energy production process.

2. Materials and Methods

2.1. Obtaining extracts and essential oils

The extracts and essential oils were obtained from cultivated and/or native Cerrado plants that showed evidence of antibacterial activity in previous works. The following species were used: *Schinus terebinthifolius* Raddi (Brazilian pepper) which was cited in the works of Nocchi et al. (2016) and Uliana et al. (2015), *Campomanesia adamantium* Cambess. (guavira) rated by Sá et al. (2018), *Serjania erecta* Radlk ("Cipó-cinco-folhas") quoted by Cardoso et al. (2013), *Serjania marginata* Casar ("Cipó-timbó") which was cited in the works of Périco et al. (2015) and Leitão et al. (2021) and *Syzygium cumini* Linnaeus (jambolan) quoted by Anas and Malik (2021), Singh et al. (2016), Yadav et al. (2011). The plants were collected at the Medicinal Plants Garden of the Federal University of Grande Dourados, located in Dourados in the state of Mato Grosso do Sul, Brazil (22°11'43.7"S and 054°56'08.5" W and altitude of 430 m) in the morning. A sample of each plant was identified by Dr. Arnildo Pott and an exsiccate was deposited at the Herbarium of the Federal University of the Mato Grosso do Sul, Brazil.

The fruits and leaves of *S. terebinthifolius* Raddi (voucher specimen No. 5566) were collected and the essential oils of the fruits and the hydroethanolic extract of the leaves were obtained. Leaves and fruits of *C. adamantium* (voucher specimen No. 5678) were collected, extracting the essential oils from both parts separately. From *S. erecta* (voucher specimen No. 4678), *S. marginata* (voucher specimen No. 5561) and *S. cumini* (voucher specimen No. 3494) the hydroethanolic extracts were obtained from the leaves. The essential oil extraction was performed by Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia and by Soxhlet apparatus with ethanol and concentrated in vacuum .

The collected leaves were washed and sanitized, dried in a circulation oven at 40 °C for approximately 48 h, in the portion of 158 g of *S. erecta*, 152 g of *S. marginata,* 60 g *S. teribinthifolius* and 83 g of *S. cumini*.

For hops, processed grains were used in a portion of 6.892 g. Being crushed, separately, with 250 mL of water and 250 mL of ethanol 95% P.A. Then it was stirred by 72 h, in a shaker. The extracts were filtered and concentrated in a rotary evaporator at 55 °C until completely dehydrated, then macerated to powder.

For each treatment, fresh fruits or leaves were dried at 60 °C in fixed bed dryers, with ascending air flow, consisting of removable trays, which had a screened bottom.

For extraction in the Clevenger apparatus, a portion of 100 g of *S. teribinthifolius* and 200 g of *C. adamantium* were crushed with 500 mL of water, and were later hydrodistilled by 4 h, adapted method of Pacheco et al. (2021).

For the Soxhlet system 140 g of crushed leaves and 200 g crushed fruits of *S. teribinthifolius, and* 200 g of crushed fruits of *C. adamantium* were used. The distillation process was carried out with hexane and lasted 4 h.

The excess solvent was removed on a rotary evaporator at 40 °C.

2.2. Microbiological analysis

2.2.1. Microorganisms

At the Microbiology Laboratory of the State University of Mato Grosso do Sul (UEMS), the antimicrobial effect of contaminating bacteria isolated from the fermentation process at the São Fernando Sugar and Alcohol Plant (USFAA), Dourados, MS, Brazil, was investigated. The species of bacteria belongs to the genus *Bacillus* and is genetically related to *Bacillus toyonensis, B. thuringiensis, B. cereus, B. proteolyticus*. The analysis method used was genetic distance based on the partial sequence of the 16S ribosomal RNA gene from the Multidisciplinary Center for Chemical, Biological and Agricultural Research (CPQBA) at UNICAMP.

The industrial yeast *S. cerevisiae* Pe-2 was kindly provided by the São Fernando Sugar and Alcohol Plant (USFAA), Dourados, MS, Brazil.

2.2.2. Inoculum preparation

The text describes the storage and activation process for bacteria and yeasts. The microorganisms were kept in sealed tubes in an ultra-freezer at -80 °C and were thawed when needed. To activate the bacteria, they were streaked on Man, Rogosa and Sharpe agar (MRS - Sygma-Aldrich) and incubated for 24 hours at 35 °C.

The process of activating yeast by streaking it on Yeast Extract Peptone Dextrose (YEPD) and incubating it for 48 hours at 28 °C. For tests with diffusion discs, both yeast and bacteria inocula were obtained by scraping the surface of the activation agar with a 0.85% aqueous solution, and the suspensions were adjusted to turbidity equivalent to the 0.5 McFarland standard.

2.2.3. Preparation of disk diffusion test

For both hydroalcoholic extracts and essential oils, stock solutions were prepared at a concentration of 0.1 gL^{-1}, a concentration equal to $1x10^{-1}$ gL^{-1} (called the 10^{-1} dilution), and then proceeded the preparation of other dilutions: 10^{-2} (0.01 gL^{-1}); 10^{-3} (0.001 $g.L^{-1}$); 10^{-4} (0.0001 $g.L^{-1}$) and 10^{-5} (0.00001 $g.L^{-1}$). The tests were conducted in petri dishes with a 1:10 dilution difference between the disks (Chaves et al., 2018).

For all dilutions tested, filter paper discs of 5 mm in diameter and with a weight of 80 g/m^2 and 0.2 mm thick were impregnated with 0.5 µL of essential oil or hydroethanolic extract followed by drying under study at 40 °C.

The discs with the preparations were applied at the inoculation 2.5 cm from the center of the plate in a circular shape (5 discs per plate). The inoculations were incubated over 24 h at 35 °C for bacteria and 30 °C for yeast. Once incubated, the uniform microbial lawn around the disks facilitated the formation of zones of complete and partial inhibition of bacterial growth. The findings were counted by measuring the diameter of the growth inhibition zones.

Analyzes were performed in triplicate. Hops extract was used as a positive control.

2.3. Statistical analysis

The given text discusses the analysis of variance of results using the F test at a 5% probability level. The means for plants grown in the Cerrado were compared using the Tukey test with a p-value of 0.05. The concentrations of essential oil and hydroethanolic extracts were adjusted to polynomial regression equations, with significant effects determined by the F test at a 5% probability level and coefficients of determination measured by the SISVAR 5.3 program.

3. Results and Discussion

3.1. Hydroethanolic extracts from leaves

Although the literature reports that the extract from *S. marginata* presents evident antimicrobial effects against *Helicobacter pylori, Staphylococcus aureus, Escherichia coli, Salmonella setubal,* and the yeast *Candida albicans* (Périco et al., 2015), the tests performed did not show inhibition against *Bacillus* sp. nor against the yeast *S. cerevisiae* Pe-2. A hypothesis raised in the literature is that bacteria of the genus *Bacillus* have resistance mechanisms to various stressful conditions related to a protein DPS ("*DNA-binding protein from starved cells*"). In fact, the presence of DPS increases bacterial survival from many stresses, including starvation, heat shock, oxidative stress, and iron overexposure (Karas et al., 2015). Altowayti et al. (2019), when studying arsenic adsorption mechanisms with *B. thuringiensis* attribute the resistance of the species to this mechanism given the similarity between this and the studied species.

This difference in inhibitory behavior between the bacteria studied by Périco et al. (2015) and in the present study shows the degree of resistance of the bacteria studied, justifying quite fully the need to study specific inhibitors for contaminating bacteria in alcoholic fermentation. It is noteworthy that the conditions of the fermentation process in plants is very stressful (Fernandes et al., 2020) and the selective pressures suffered by the microorganisms involved in these processes elevate them to a greater degree of resistance.

On the other hand, *S. erecta* showed a halo of inhibition in all tested dilutions.

The halos obtained with the extracts of *S. erecta* had the highest mean with 12 ± 0 mm, in the extract of *S. terebinthifolius* the highest mean was 10 ± 0 mm (Table 1). The extract of *S. marginata* did not show inhibitory activity. Considering that this genus, *Serjania*, has antimicrobial properties, it is possible to suppose that these results indicate activity only in the five-leaf vine species. Studies carried out found that different *Serjania* species have compounds with antibacterial and anti-inflammatory activity (Gomig et al., 2008).

Regarding the use of *S. cumini* extract, it was observed that the greatest inhibition halo was 16 ± 12.53 at the concentration of 0.1 $g.L^{-1}$ against bacteria. However, in yeasts, the halo was approximately 15.00 ± 3.54 L of inhibition, at the highest concentration of 0.01 $g.L^{-1}$ (Table 1). It can be said that the *S. cumini* extract would not be viable for industrial use, as the positive results observed, both in bacteria and yeast, make its use in the alcoholic fermentation process unfeasible.

The hydroethanolic extract extracted from the leaf of *S. terebinthifolius* showed a halo of inhibition only at the concentration of 0.1 $g.L^{-1}$, against the bacteria, when observing the halo in the Petri dish, it was absent (-), there was normal growth of yeasts in all the dilutions tested around the disc. In the test with the hydroethanolic extract extracted from the fruits of *S. terebinthifolius* against bacteria and yeasts, there was no halo of inhibition. Although some studies indicate antimicrobial activity against microorganisms (Nocchi et al., 2016), the bacterium *Bacillus* sp. and the yeast *S. cerevisiae* Pe-2 were resistant to the hydroethanolic extract of the fruit of *S. terebinthifolius*.

Nobre et al. (2007) studied the influence of the genus *Bacillus* and *Lactobacillus* in reducing the cell viability of the yeast *S. cerevisiae*, cultivating the bacteria *B. subtilis*, *B. coagulans*, *B. stearothermophilus*, *L. fermentum and L. plantarum* with the yeast *S. cerevisiae*, for 72 h under

Table 1. Means and standard deviation obtained from hydroethanolic extract of leaves of plants grown in the Cerrado against bacteria and yeasts.

Plant	Bacteria (b)/Yeast (y)		Concentration ($g.L^{-1}$)				
	Abs.	Pres.	$1x10^{-1}$	$1x10^{-2}$	$1x10^{-3}$	$1x10^{-4}$	$1x10^{-5}$
S. terebinthifolius	+	-	10 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
Leaf extract			0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y
S. Terebinthifolius Fruit extract	-	-	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
			0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y
S. cumini	+	+	16 ± 12.53b	12.89 ± 7.49b	4.67 ± 7.07b	0 ± 0 b	0 ± 0 b
Leaf extract			13.22 ± 2.33y	15.00 ± 3.54y	8.56 ± 8.47y	4.22 ± 6.33y	0 ± 0 y
S. marginata	-	-	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
Leaf extract			0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y
S. erecta	+	-	12 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
Leaf extract			0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y
Hop (reference)	+	-	24 ± 0b	24 ± 0b	24 ± 0b	24 ± 0b	24 ± 0b
			0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y

Absence of halo: (-); Presence of halo: (+); bacteria (b); yeast (y).

Table 2. Summary of analysis of variance for antimicrobial tests of hydroethanolic extracts from leaves of plants grown in the Cerrado.

Source	df	SS	MS	Fc	Pr > Fc*
Plant	2	3936.88	1968.44	610.90	≤0.001
Extract concentration	2	1536.88	768.44	238.48	≤0.001
Extract concentration	4	137.77	34.44	10.69	≤0.001
Residue	72	232.00	3.22		
Total	80				
CV (%)	20.89				
Overall Average	8.59				

df: degree of freedom; SS: sum of squares; MS: mean square; Fc: F calculated; Pr>Fc: Exact significance; CV: coefficient of variation.
*Significant at 5% of probability by the test F.

stirring at 32 ºC, with influence of acidity and pH in the culture media, resulting in loss of yeast viability only by *L. fermentum* e *B. subtilis*. Compared to the hydroethanolic extract of the fruit of the *S. terebinthifolius* the bacteria *Bacillus* sp. proved to be resistant.

According to the analysis of variance, there was a significant difference ($p \leq 0.05$) between the Cerrado plants and the doses of hydroethanolic leaf extracts tested (Table 2). According to Uliana et al. (2015) the extract from the leaves of *S. terebinthifolius* have strong antimicrobial activity against bacterial strains *S. aureus and E. coli*.

The hydroethanolic leaf extracts tested against the studied bacteria showed satisfactory results with the use of the plants *S. terebinthifolius*, *S. erecta* and *S. cumini* (Table 3).

The results of the test of means presented in Table 3 show that there was a significant difference ($p \leq 0.05$) in the concentrations of $1x10^{-1}$ $g.L^{-1}$ to $1x10^{-3}$ $g.L^{-1}$, where *S. cumini* stood out in relation to *S. terebinthifolius* and *S. erecta*, with a halo of inhibition statistically superior to the other plants studied, and among the concentrations tested, the Minimum Inhibitory Concentration (MIC) level was equal to $1x10^{-3}$ $g.L^{-1}$.

The variable halo of inhibition was adjusted to the linear regression model, with decreasing results as the dilution of the hydroethanolic extract of leaves increased, in both plants evaluated (Figure 1).

Note that the halo of inhibition values for *S. terebinthifolius* and *S. erecta* showed no halo of inhibition in the concentrations of $1x10^{-2}$ $g.L^{-1}$ and $1x10^{-3}$ $g.L^{-1}$, showing a significant difference between these plants in relation to the *S. cumini*, which obtained higher values in the inhibition halos according to the concentrations, in line with (MIC) of the antimicrobial activity tested in this work. The MIC is the Minimum Inhibitory Concentration, corresponding to the last dilution in which the presence of bacterial growth in the measured inhibitory halos of the different concentrations tested was not verified.

Table 3. Results of means of halo of inhibition of the antimicrobial activity of the hydroethanolic extracts of leaves against the tested bacteria.

Plants	Inhibition halo (mm)		
	$1x10^{-1}$	$1x10^{-2}$	$1x10^{-3}$
S. therebinthifolius	10.00 b	0.00 b	0.00 b
S. erecta	12.00 b	0.00 b	0.00 b
S. cumini	22.22 a	17.56 a	15.56 a

In the column, the same letters = no statistical significance, different letters = statistically significant.

Table 4. Summary of analysis of variance for antimicrobial tests of hydroethanolic extracts of *S. cumini* leaves against yeast.

Source	df	SS	MS	Fc	Pr > Fc*
Extract concentration	4	1316	329	76	≤0.001
Residue	40	174	4		
Total	44	1490			
CV (%)	19.54				
Overall Average	10.66				

CV: coefficient of variation; NS: not significant; df: degree of freedom; SS: sum of squares; MS: mean square; Fc: F calculated. Pr>Fc: Exact significance: *Significant at 5% of probability by the test F.

For the *S. cumini* plant there was a significant difference between the concentrations of the hydroethanolic leaf extract against yeast with a probability of 5% of significance (Table 4). Statistically this result indicates high variations.

Singh et al. (2016) when performing the phytochemical analysis of the compounds present in *S. cumini*, showed that the variation was significant and when studying the polyphenol compounds present, it was concluded that they exerted a strong antioxidant and antimicrobial activity against the microorganisms *Staphylococcus aureus*, *Klebsiella pneumonia* sub sp. *pneumoniae*, *E. coli* and the yeast *Candida albicans*, similarly to the results of this study, where *S. cumini* obtained halos of inhibition against industrial microorganisms, *S. cerevisiae* Pe-2, and *Bacillus* sp.

It can be said that despite the excellent results in tests with the *S. cumini*, it would be unfeasible for industrial use, as it presents levels of inhibition up to $1x10^{-4}$ g.L^{-1} against yeasts (Figure 2). Albuquerque et al. (2017), when evaluating the antimicrobial activity of the hydroalcoholic and alcoholic extracts from the leaves of some species of the Myrtaceae family, including the *S. cumini* against bacteria *S. aureus, S. pyogenes, P. aeruginosa, E. faecalis*, concluded that all extracts studied showed activity against strains of *S. aureus*. Martins et al. (2016) documented the antimicrobial effects against *Beauveria bassiana* fungus. This effect was ascribed to the existence of tannins, flavonoids, anthocyanins alkaloids, and other compounds which also exhibit established antibacterial properties.

The halo of inhibition as a function of the concentrations of the hydroethanolic extract of leaves of *S. cumini* against yeast, was adjusted to the decreasing linear regression model, since with the reduction of the tested extract

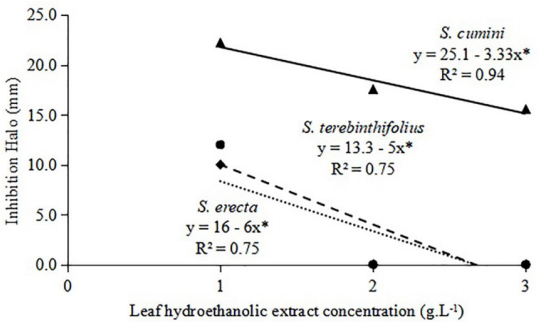

Figure 1. Antibacterial activity of hydroethanolic extracts from leaves of plants grown in the Cerrado against *Bacillus* sp. ▲ = *S. cumini*, ♦ = *S. terebinthifolius*, ● = *S. erecta* (1 = 0.1 g.L^{-1}; 2 = 0.01 g.L^{-1}; 3 = 0.001 g.L^{-1}).

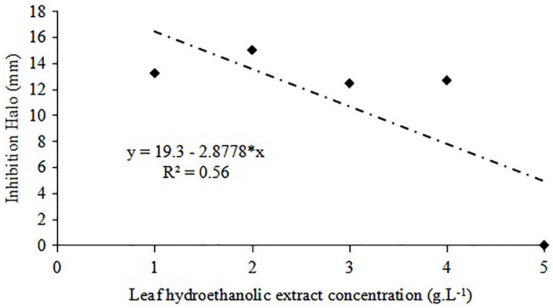

Figure 2. Antibacterial activity of the hydroethanolic extract of *S. cumini* leaves (♦) against yeast (1 = 0.1 g.L^{-1}; 2 = 0.01 g.L^{-1}; 3 = 0.001 g.L^{-1}, 4 = 0.0001 g.L^{-1}; 5 = 0.00001 g.L^{-1}).

concentrations there was a reduction in the size of the inhibition halo (Figure 2).

It is observed in Figure 2, an absence of inhibition halo in the concentration 10^{-5} of dilution of the hydroethanolic extract of the leaves of the *S. cumini* against yeast, with the results of inhibition halo size in the other extract concentrations being statistically higher than the dilution of 10^{-5}, not differing statistically from each other, showing results of 13.22 mm, 15 mm, 12.44 mm and 12.67 mm, respectively, in the dilutions of $1x10^{-1}$, $1x10^{-2}$, $1x10^{-3}$ e $1x10^{-4}$ g.L^{-1}. Thus, *S. cumini* extracts are also not viable for use in alcoholic fermentation.

Carvalho-Netto et al. (2015) clarify that contaminating microorganisms present in the industrial process, especially in distilleries, produce particles of cell coaggregation and adhere to equipment by flocculation of the yeast, harming the fermentation process and reducing the yield of bioethanol, which consists of the fermentation of sucrose present in sugarcane and yeast strains adapted from *S. cerevisiae,* lineage highly adapted to the process.

3.2. Essential oil from leaves and fruits of plants grown in the Cerrado

As for the essential oil of the fruit of the *guavira* species against bacteria, the formation of inhibition halos was present in all dilutions, being higher in the 10^{-1} g.L^{-1} dilution, which presented a halo of 33.33 ± 8.66 mm, surpassing the result of the largest halo present in the standard extract (hop extract), which obtained 24 ± 0 mm. It is important to compare the results with those of hop extract, as these have been used to replace antibiotics because hop extract products can significantly reduce the bacterial load at doses from 5.10^{-3} mg.L^{-1} (Rigotti et al., 2017; Maia et al., 2019).

In yeasts, the halo formed was 0.1 mm, considered to be of little significance, as shown in (Table 5). Analysis of the essential oil of the species *C. adamantium* performed by Oliveira et al. (2016) showed antibacterial activity, the essential oil showed moderate activity against bacteria *Streptococcus mitis*, *Streptococcus mutans, Streptococcus sanguinis, Streptococcus sobrinus and Bacteroides fragilis.*

In the tests using the essential oil of the guavira leaves, the greatest inhibition halo was 16.00 ± 4.50 mm in the dilution 10^{-2} g.L^{-1} against bacteria. For yeasts the halo was negative in all dilutions, so there was no inhibition, a desirable characteristic for application in the sugarcane industry.

Table 5 presents the mean and standard deviation of each plant against the tested bacteria and yeasts, and compares the results in the different methods used in the production of essential oil. Where (+) presence of halo (-) is absence of halo in bacteria/yeasts. Values of 0 mean no halo.

As the results show, the EO of the fruit de *S. terebinthifolius* performed by the Soxhlet appliance had the best result with a halo that exceeded the size of the plate, with 120 ± 0 mm and 0 for yeast negative result (-) absence of halo. The same plant species did not obtain a positive result by the EO extracted from the leaf in the Clevenger apparatus. Salem et al. (2018) demonstrated good activity against the growth of *A. baumannii*, *P. aeruginosa*, *M. luteus,* and *S. aureus* using EO at 1.000 µg.mL^{-1} (equivalent to 1 g.L^{-1}).

The results obtained with the essential oil of the specie *S. terebinthifolius* fruits showed the greatest inhibition halo against the bacteria by the Soxlhet method, not being possible to measure them, except in the dilution 10^{-5} g. L^{-1}. When testing pure EO against bacteria, the halo was 44 mm (Table 5). When testing it against yeast, the result was negative, with no halo of inhibition, the yeast grew normally in the entire Petri dish.

The essential oil of fruits has a higher yield by the Soxlhet method and has antibacterial activity on both gram-negative and gram-positive bacteria (Barrales et al.,

Table 5. Means and standard deviation obtained from essential oils (EO) against bacteria and yeasts in different extraction methods.

Plant	Bacteria (b)/Yeast (y)		Concentration (g.L^{-1})				
	Abs.	Pres.	1x10^{-1}	1x10^{-2}	1x10^{-3}	1x10^{-4}	1x10^{-5}
S. terebinthifolius EO Sox Fruit	+	-	120 ± 0 b	120 ± 0 b	120 ± 0 b	120 ± 0 b	44 ± 2 b
			0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y
S. terebinthifolius EO Sox leaf	-	-	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
			0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y
S. terebinthifolius EO Cle leaf	-	-	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
			0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y
C. adamantium EO Cle Fruit	+	-	33.33 ± 8.66 b	23.33 ± 8.66 b	16.67 ± 10 b	13.33 ± 5b	10 ± 0b
			0.1 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y
C. adamantium EO Cle leaf	+	-	15.89 ± 4.68b	16.00 ± 4.50b	15.67 ± 0.50b	15.44 ± 5 b	3 ± 4.50b
			0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y
Hop (reference)	+	-	24 ± 0 b	24 ± 0 b	24 ± 0 b	24 ± 0 b	24 ± 0 b
			0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y	0 ± 0 y

EO cle: Essential oil Clevenger apparatus; EO Sox: Essential oil Soxhlet apparatus; Absence of halo: (-); Presence of halo: (+); bacteria (b); yeast (y).

Table 6. Summary of analysis of variance for antimicrobial testing of fruit essential oil (EO) *C. adamantium*.

Source	df	SS	MS	Fc	Pr > Fc*
Fruit EO concentrations	4	3083	770	14	≤0.001
Residue	40	2200	55		
Total	44	5280			
CV (%)	38.36				
Overall Average	19.33				

df: degree of freedom; SS: sum of squares; MS: mean square; Fc: F calculated; Pr>Fc: Exact significance; CV: coefficient of variation. *Significant at 5% of probability by the test F.

2015) as well as the hop extract used by plants in the countryside of São Paulo, Betatec hop products showed the effects of Isostab and Lactostab are quite effective against gram positive bacteria present in fermentation (LNF, 2017).

Thus, the use of essential oil can be considered for the control of sucroenergetic bacterial contamination in both species, *S. terebinthifolius and C. adamantium*, for presenting, in these times of crisis in the industrial sector, an alternative against the use of antibiotics, as natural biocides would result in cost reduction and would add less economic value, also helping to preserve the environment.

The search for natural biocides as a way to control contamination is increasing due to the growing number of bacterial resistance to various antibiotics, and medicinal plants represent an important source of obtaining new substances (Chen-Lung et al., 2012).

According to the analysis of variance, there was a significant difference ($p \leq 0.05$) between the concentrations of essential oil in the fruits of the *C. adamantium* (Table 6). This plant proved to be promising for presenting inhibition halos at all concentrations tested against contaminating bacteria in the alcoholic industrial fermentation process.

There was a decrease in the size of the inhibition halo with increasing dilution of the concentration of the essential oil of *C. adamantium* fruits, and the results were adjusted to the decreasing linear regression model (Figure 3). However, it is observed positively satisfactory values in the size of inhibition halos with the concentration levels of the essential oil of *C. adamantium* fruits against *Bacillus* sp. bacteria, which surpassed the results of the hop extract.

The results obtained by the analysis of variance demonstrate that there was a significant difference with a probability of (p5>0.05) significance in the samples of essential oil from the leaves of *C. adamantium* (Table 7). Studies of antimicrobial activity carried out with essential oils from plants are increasingly common as they are promising in the control of bacteria.

Sá et al. (2018) tested the antimicrobial activity of the essential oil of guavira leaves by hydrodistillation using the Clevenger apparatus, the phytochemical analysis showed new compounds, including the hexane fraction, antibacterial potential against bacteria, and the concentrated fraction of aqueous tannin and valoneic acid, against the yeasts.

Analyzing Figure 4, it is observed that the size values of the inhibition halo were adjusted to the decreasing linear regression model with the increase in the dilution

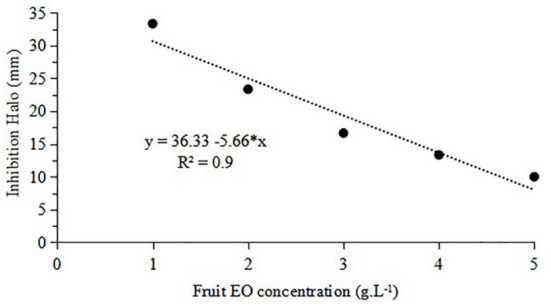

Figure 3. Antimicrobial activity of essential oil from *C. adamantium* fruits (●) against *Bacillus* sp. (1 = 0.1 g.L^{-1}; 2 = 0.01 g.L^{-1}; 3 = 0.001 g.L^{-1}, 4 = 0.0001 g.L^{-1}; 5 = 0.00001 g.L^{-1}).

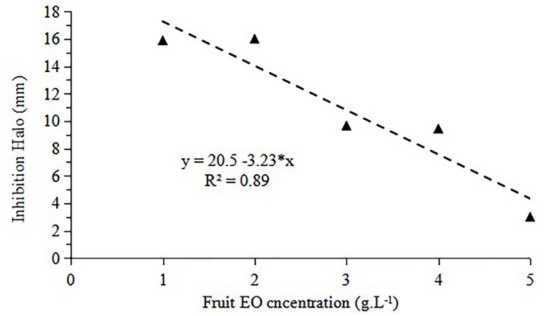

Figure 4. Antibacterial activity of the essential oil of *C. adamantium* leaves (▲) against the *Bacillus* sp. (1 = 0.1 g.L^{-1}; 2 = 0.01 g.L^{-1}; 3 = 0.001 g.L^{-1}, 4 = 0.0001 g.L^{-1}; 5 = 0.00001 g.L^{-1}).

of the concentration of the essential oil in the leaves of the *C. adamantium*. The halo of inhibition values range from 15.89 mm for 10^{-1}, 16 mm for 10^{-2}, 9.67 mm for 10^{-3}, 9.44 mm for 10^{-4} and 3 mm for 10^{-5}.

In the study by Santos et al. (2019) with the multiresistant bacteria *Acinetobacter baumannii* when evaluating the action of the essential oil of unripe and ripe fruits, they found that the *S. terebinthifolius* has antimicrobial activity extracted from the ripe fruits of the plant against this bacteria, confirming its promising potential with *C. adamantium* in the control of these multiresistant bacteria, which are also present in the sugar-energy industrial process.

Table 7. Summary of analysis of variance for antimicrobial tests of essential oil (EO) from *C. adamantium* leaves.

Source	df	SS	MS	Fc	Pr > Fc*
Leaf EO concentration	4	1052	263	21	≤0.001
Residue	40	503	13		
Total	44	1555			
CV (%)	32.84				
Overall Average	10.8				

df: degree of freedom; SS: sum of squares; MS: mean square; Fc: F calculated; Pr>Fc: Exact significance; CV: coefficient of variation. *Significant at 5% of probability by the test F.

4. Conclusion

Given the above, it can be concluded that the hydroethanolic extracts of the leaves of the species *S. terebinthifolius* and *S. erecta,* and the essential oils of the fruits of *S. terebinthifolius* and *C. adamantium* can be a viable alternative for the control of bacterial contamination. The hydroethanolic extracts of the *S. marginata* did not show antimicrobial activity and *S. cumini* extract promoted the inhibition of bacterial and yeast growth, therefore it would be unfeasible for the proposed objective, as it inhibits yeast growth. The extracts of the *S. terebinthifolius*, *S. erecta* and *C. adamantium* can be used as an alternative to the use of hop extract, as they promote the reduction of economic costs in the industrial sector, helping to preserve the environment. The use of natural extracts obtained from native species can be an alternative to hop extract. The control of bacterial contamination in fermentation from antibiotics is already prohibited in some countries because it causes resistance in bacterial strains. The sugarcane industry uses hop extract as an alternative, but the high cost makes it unfeasible for this sector.

References

ALBUQUERQUE, F.H.C., SOARES, K.S. and OLIVEIRA, M.A.S., 2017. Atividade antimicrobiana in vitro dos extratos aquosos, hidroalcoólicos e alcoólicos das folhas de espécies da família Myrtaceae frente à cepas de bactérias de interesse. *Revista de Ciências Médicas e Biológicas*, vol. 16, no. 2, pp. 139-145. http://doi.org/10.9771/cmbio.v16i2.17989.

ALTOWAYTI, W.A.H., ALGAIFI, H.A., BAKAR, S.A. and SHAHIR, S., 2019. The adsorptive removal of As (III) using biomass of arsenic resistant *Bacillus thuringiensis* strain WS3: characteristics and modelling studies. *Ecotoxicology and Environmental Safety*, vol. 172, pp. 176-185. http://doi.org/10.1016/j.ecoenv.2019.01.067. PMid:30708229.

ANAS, M. and MALIK, A., 2021. Impact of sodium alginate packaging film synthesized using *Syzygium cumini* seed extract on multi drug resistant *Escherichia coli* isolated from raw buffalo meat. *Indian Journal of Microbiology*, vol. 61, no. 2, pp. 137-150. http://doi.org/10.1007/s12088-021-00923-9. PMid:33927455.

BARRALES, F.M., REZENDE, C.A. and MARTÍNEZ, J., 2015. Supercritical CO_2 extraction of passion fruit (*Passiflora edulis* sp.) seed oil assisted by ultrasound. *The Journal of Supercritical Fluids*, vol. 104, pp. 183-192. http://doi.org/10.1016/j.supflu.2015.06.006.

BASSO, T.O., GOMES, F.S., LOPES, M.L., DE AMORIM, H.V., EGGLESTON, G. and BASSO, L.C., 2014. Homo- and heterofermentative lactobacilli differently affect sugarcane-based fuel ethanol fermentation. *Antonie van Leeuwenhoek*, vol. 105, no. 1, pp. 169-177. http://doi.org/10.1007/s10482-013-0063-6. PMid:24198118.

BÉJAR-SERRANO, S., DEL POZO, P., DE LA VARGA, M.F. and BENLLOCH, S., 2019. Multidrug-resistant bacterial infections in patients with liver cirrhosis in a tertiary referral hospitalInfecciones por bacterias multirresistentes en pacientes cirróticos en un hospital terciario. *Gastroenterologia y Hepatologia*, vol. 42, no. 4, pp. 228-238. http://doi.org/10.1016/j.gastrohep.2018.07.017. PMid:30342782.

CARDOSO, C.A.L., COELHO, R.G., HONDA, N.K., POTT, A., PAVAN, F.R. and LEITE, C.Q.F., 2013. Phenolic compounds and antioxidant, antimicrobial and antimycobacterial activities of *Serjania erecta* Radlk. (Sapindaceae). *Brazilian Journal of Pharmaceutical Sciences*, vol. 49, no. 4, pp. 775-782. http://doi.org/10.1590/S1984-82502013000400017.

CARVALHO-NETTO, O.V., CARAZZOLLE, M.F., MOFATTO, L.S., TEIXEIRA, P.J.P.L., NORONHA, M.F., CALDERÓN, L.A.L., MIECZKOWSKI, P.A., ARGUESO, J.L. and PEREIRA, G.A.G., 2015. *Saccharomyces cerevisiae* transcriptional reprograming due to bacterial contamination during industrial scale bioethanol production. *Microbial Cell Factories*, vol. 14, pp. 13. http://doi.org/10.1186/s12934-015-0196-6. PMid:25633848.

CECCATO-ANTONINI, S.R., 2021. *Microbiologia da fermentação etanólica:* Fundamentos, avanços e perspectivas. São Carlos: EdUFSCar, 213 p.

CHAVES, M.R.V., OLIVEIRA, G.M.G., JOSÉ NETO, M., NEVES, F.M.L. and BARBOSA, I.M.L., 2018 [viewed 14 December 2023]. Potencial fungicida de plantas medicinais do Cerrado da Costa Leste do Estado de Mato Grosso do Sul. *Revista Saúde e Meio Ambiente* [online], vol. 6, no. 1, pp. 71-81. Available from: https://periodicos.ufms.br/index.php/sameamb/article/view/5660

CHEN-LUNG, H., PEI-CHUN, L. and YU-CHANG, S., 2012. Composition and antimicrobial activities of the leaf essential oil of *Machilus zuihoensis* from Taiwan. *Revista Brasileira de Farmacognosia*, vol. 22, no. 2, pp. 277-283. http://dx.doi.org/10.1590/S0102-695X2011005000213.

FERNANDES, A.M.O., GARCIA, N.F.L., FONSECA, G.G., LEITE, R.S.R. and DA PAZ, M.F., 2020. Evaluation of the fermentative capacity of *Saccharomyces cerevisiae* CAT-1 and BB9 strains and *Pichia kudriavzevii* BB2 at simulated industrial conditions. *Indian Journal of Microbiology*, vol. 60, no. 4, pp. 494-504. http://doi.org/10.1007/s12088-020-00891-6. PMid:33087999.

GOMIG, F., PIETROVSKI, E.F., GUEDES, A., DALMARCO, E.M., CALDERARI, M.T., GUIMARÃES, C.L., PINHEIRO, R.M., CABRINI, D.A. and OTUKI, M.F., 2008. Topical anti-inflammatory activity of *Serjania erecta* radlk (sapindaceae) extracts. *Journal of Ethnopharmacology*, vol. 118, no. 2, pp. 220-224. http://doi.org/10.1016/j.jep.2008.03.017. PMid:18513901.

KARAS, V.O., WESTERLAKEN, I. and MEYER, A.S., 2015. The DNA-binding protein from starved cells (Dps) utilizes dual functions

to defend cells against multiple stresses. *Journal of Bacteriology*, vol. 197, no. 19, pp. 3206-3215. http://doi.org/10.1128/JB.00475-15. PMid:26216848.

LAXMINARAYAN, R., DUSE, A., WATTAL, C., ZAIDI, A.K.M., WERTHEIM, H.F.L., SUMPRADIT, N., VLIEGHE, E., HARA, G.L., GOULD, I.M., GOOSSENS, H., GREKO, C., SO, A.D., BIGDELI, M., TOMSON, G., WOODHOUSE, W., OMBAKA, E., PERALTA, A.Q., QAMAR, F.N., MIR, F., KARIUKI, S., BHUTTA, Z.A., COATES, A., BERGSTROM, R., WRIGHT, G.D., BROWN, E.D. and CARS, O., 2013. Antibiotic resistance-the need for global solutions. *The Lancet. Infectious Diseases*, vol. 13, no. 12, pp. 1057-1098. http://doi.org/10.1016/S1473-3099(13)70318-9. PMid:24252483.

LEITÃO, M.M., RADAI, J.A.S., MACORINI, L.F.B., FRAGA, T.L., VIEIRA, S.C.H., CARDOSO, C.A.L. and KASSUYA, C.A.L. 2021. *Serjania marginata* Casar. hydroalcoholic extract reduced cytokine and inflammatory parameters in experimental models of inflammation and infection in mice. *Research Square*, pp. 1-21. Preprint. http://doi.org/10.21203/rs.3.rs-283967/v1.

LNF, 2017 [viewed 14 December 2023]. *Derivados de lúpulo* [online]. Bento Gonçalves: LNF. Available from: https://lnf.com.br/downloads/derivados_lupulo.pdf

MAIA, N.J.L., CORRÊA, J.A.F., RIGOTTI, R.T., DA SILVA-JUNIOR, A.A. and LUCIANO, F.B., 2019. Combination of natural antimicrobials for contamination control in ethanol production. *World Journal of Microbiology & Biotechnology*, vol. 35, no. 10, pp. 158. http://doi.org/10.1007/s11274-019-2734-6. PMid:31595344.

MARTINS, C., ALVES, L. and MAMPRIM, A., 2016. Effect of plant extracts and a disinfectant on biological parameters and pathogenicity of the fungus *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Cordycipitaceae). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 76, no. 2, pp. 420-427. https://doi.org/10.1590/1519-6984.17914.

NOBRE, T.P., HORII, J. and ALCARDE, A.R., 2007. Viabilidade celular de *Saccharomyces cerevisiae* cultivada em associação com bactérias contaminantes da fermentação alcoólica. *Food Science and Technology (Campinas)*, vol. 27, no. 1, pp. 20-25. http://doi.org/10.1590/S0101-20612007000100004.

NOCCHI, S.R., COSTA, G.F.M., NOVELLO, C.R., RODRIGUES, J., LONGHINI, R., MELLO, J C.P., DIAS FILHO, B.P., NAKAMURA, C.V. and NAKAMURA, T.U., 2016. In vitro cytotoxicity and anti-herpes simplex virus type 1 activity of hydroethanolic extract, fractions, and isolated compounds from stem bark of *Schinus terebinthifolius* Raddi. *Pharmacognosy Magazine*, vol. 12, no. 46, pp. 160-164. http://doi.org/10.4103/0973-1296.177903.

NOVACANA, 2021 [viewed 14 December 2023]. *As usinas de açúcar e etanol no Brasil* [online]. Available from: http://www.novacana.com/usinas_brasil

OLIVEIRA, J.D., ALVES, C.C.F., MIRANDA, M.L.D., MARTINS, C.H.G., SILVA, T.S., AMBROSIO, M.A.L.V., ALVES, J.M. and SILVA, J.P., 2016. Content, chemical composition and antimicrobial and antioxidant activities of the essential oil from leaves of *Campomanesia adamantium* submitted to different drying methods. *Revista Brasileira de Plantas Medicinais*, vol. 18, no. 2, pp. 502. http://doi.org/10.1590/1983-084X/15_206.

PACHECO, L.A., ÉTUR, E.M., SHEIBEL, T., BUHL, B., WEBER, A.C., KAUFFMANN, C., MARCHI, M.I., FREITAS, E.M. and HOEHNE, L., 2021. Caracterização química e atividade antimicrobiana de *Campomanesia aurea* contra três cepas de *Listeria monocytogenes*. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 81, pp. 69-76. http://doi.org/10.1590/1519-6984.219889. PMid:32130285.

PÉRICO, L.L., VIEIRA, S.C.H., BESERRA, F.P., SANTOS, R.C., WEISS, M.B., RESENDE, F.A., RAMOS, M.A.S., BONIFÁCIO, B.V., BAUAB, T.M., VARANDA, E.A., GOBBI, J.I.F., ROCHA, L.R.M., VILEGAS, W. and LIMA, C.A.H., 2015. Does the gastroprotective action of a medicinal plant ensure healing effects? An integrative study of the biological effects of *Serjania marginata* Casar. (Sapindaceae) in rats. *Journal of Ethnopharmacology*, vol. 172, pp. 312-324. http://doi.org/10.1016/j.jep.2015.06.025. PMid:26099637.

RAZA, G., ALI, K., HASSAN, M.A., ASHRAF, M., KAHAN, M.T. and KAHAN, I.A., 2019. Sugarcane as a bioenergy source. In: M.T. KAHAN and I.A. KAHAN, eds. *Sugarcane biofuels: status, potential, and prospects of the sweet crop to fuel the world*. Switzerland: Springer, pp. 3-19. http://doi.org/10.1007/978-3-030-18597-8_1.

RICH, J.O., BISCHOFF, K.M., LEATHERS, T.D., ANDERSON, A.M., LIU, S. and SKORY, C.D., 2018. Resolving bacterial contamination of fuel ethanol fermentations with beneficial bacteria: an alternative to antibiotic treatment. *Bioresource Technology*, vol. 247, pp. 357-362. http://doi.org/10.1016/j.biortech.2017.09.067. PMid:28954248.

RIGOTTI, R.T., CORRÊA, J.A.F., MAIA, N.J.L., CESARO, G., ROSA, E.A.R., MACEDO, R.E.F. and LUCIANO, F.B., 2017. Combination of natural antimicrobials and sodium dodecyl sulfate for disruption of biofilms formed by contaminant bacteria isolated from sugarcane mills. *Innovative Food Science & Emerging Technologies*, vol. 41, pp. 26-33. http://doi.org/10.1016/j.ifset.2017.01.007.

SÁ, P., CHAUL, L.T., ALVES, V.F., FIUZA, T.S., TRESVENSOL, L.M.F., VAZ, B.G., FERRI, P.H., BORGES, L.L. and PAULA, J.R., 2018. Phytochemistry and antimicrobial activity of *Campomanesia adamantium*. *Revista Brasileira de Farmacognosia*, vol. 28, no. 3, pp. 303-311. http://doi.org/10.1016/j.bjp.2018.02.008.

SALEM, M.Z.M., EL-HEFNY, M., ALI, H.M., ELANSARY, H.O., NASSER, R.A., EL-SETTAWY, A.A.A., EL SHANHOREY, N., ASHMAWY, N.A. and SALEM, A.Z.M., 2018. Antibacterial activity of extracted bioactive molecules of *Schinus terebinthifolius* ripened fruits against some pathogenic bacteria. *Microbial Pathogenesis*, vol. 120, pp. 119-127. http://doi.org/10.1016/j.micpath.2018.04.040. PMid:29704984.

SANTOS, C.T.C., AZEVEDO, M.M.R., SILVA, C.B., ROCHA, T.J.M., SANTOS, A.F. and PIRES, L.L.S., 2019. Comparação da atividade entre óleos essenciais de frutos verdes e maduros de *Schinus terebinthifolius* Raddi sobre isolados de *Acinetobacter baumannii* multirresistentes. *Diversitas Journal*, vol. 4, no. 1, pp. 285-291. https://doi.org/10.17648/diversitas-journal-v4i1.638.

SINGH, J.P., KAUR, A., SINGH, N., NIM, L., SHEVKANI, K., KAUR, H. and ARORA, D.S., 2016. In vitro antioxidant and antimicrobial properties of jambolan (*Syzygium cumini*) fruit polyphenols. *Lebensmittel-Wissenschaft + Technologie*, vol. 65, pp. 1025-1030. http://doi.org/10.1016/j.lwt.2015.09.038.

ULIANA, M.P., FRONZA, M., SILVA, A.G., VARGAS, T.S., ANDRADE, T.U. and SCHERER, R., 2015. Composition and biological activity of brazilian rose pepper (*Schinus terebinthifolius* Raddi) leaves. *Industrial Crops and Products*, vol. 83, pp. 235-240. http://doi.org/10.1016/j.indcrop.2015.11.077.

VENTURIERI, V.R., MASUKAWA, I.I. and NEVES, F.S., 2019 [viewed 14 December 2023]. Antimicrobial susceptibilty of urine culture bacterial isolates from a teaching hospital in Brazil. *Arquivos Catarinenses de Medicina* [online], vol. 48, no. 1, pp. 155-172. Available from: https://revista.acm.org.br/index.php/arquivos/article/view/458

YADAV, S.S., MESHRAM, G., SHINDE, D., PATIL, R., MANOHAR, S.M. and UPADHYE, M.V., 2011. Antibacterial and anticancer activity of bioactive fraction of *Syzygium cumini* L. seeds. *Hayati Journal of Biosciences*, vol. 18, no. 3, pp. 118-122. http://doi.org/10.4308/hjb.18.3.118.

YAN, C., YANG, Y., ZHOU, J., LIU, M., NIE, M., SHI, H. and GU, L., 2013. Antibiotics in the surface water of the Yangtze Estuary: Occurrence, distribution and risk assessment. *Environmental Pollution*, vol. 175, pp. 22-29. http://doi.org/10.1016/j.envpol.2012.12.008. PMid:23313734.