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Antioxidant and antimicrobial properties of eucalyptus leaf extract obtained using pressurized ethanol

Propriedades antioxidantes e antimicrobianas do extrato de folhas de eucalipto obtidos com etanol pressurizado

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ABSTRACT
Eucalyptus leaves are a secondary product of wood production. The pressurized liquid extraction (PLE) technique is an attractive process due to its advantages. The objective of this work was to obtain extracts of *Eucalyptus saligna* leaves using the PLE technique, and to characterize different extraction time fractions in relation to bioactive compounds and antioxidant and antimicrobial activities. The extraction of *E. saligna* leaves was carried out in a jacketed extractor at 40 °C, pressure of 10.4 MPa, with a solvent flow of 2.5 mL.min-^{1}, using 95% ethanol. The system remained pressurized for 30 minutes in static extraction, followed by 180 minutes in dynamic extraction. The levels of total phenols, flavonoids, antioxidant activity and antimicrobial potential against *Staphylococcus aureus* and *Escherichia coli* were evaluated. The extraction showed an overall yield of 12.3% after 180 minutes. The total concentrations of phenols and flavonoids were 618.57 mgGAE.g^{-1} and 160.28 mgQE.g^{-1} respectively. The extracts showed good antioxidant and antimicrobial potential, with IC_{50} between 0.016 and 0.043 mg.mL^{-1}, and MIC against *S. aureus* and *E. coli* between 0.313 and 1.25 mg.mL^{-1}. PLE demonstrated efficiency in obtaining extracts of *E. saligna* leaves with bioactive properties.

Keywords: *Eucalyptus saligna*, bioactive properties, phenolic compounds, pressurized liquid extraction.

RESUMO
As folhas de eucalipto são um produto secundário da produção da madeira. A técnica de extração com líquidos pressurizados (PLE) é um processo atraente pela suas vantagens. O objetivo deste trabalho foi a obtenção de extratos de folhas de *Eucalipto saligna* pela técnica PLE, e a caracterização de diferentes frações do tempo de extração em relação a compostos bioativos e a atividades antioxidante e antimicrobiana. A extração das folhas de *E. saligna* foi realizada em um extrator encamisado a 40 °C, pressão de 10.4 MPa, com fluxo de solvente de 2.5 mL.min^{-1}, utilizando etanol a 95%. O sistema permaneceu pressurizado por 30 minutos em extração estática, seguido por 180 minutos em extração dinâmica. Foram avaliados os níveis de fenóis totais, flavonóides, atividade antioxidante e potencial antimicrobiano contra *Staphylococcus aureus* e *Escherichia coli*. A extração apresentou rendimento global de 12.3% após 180 minutos. As concentrações totais de fenóis e flavonoides foram 618.57 mgEAG.g^{-1} e 160.28 mgEQ.g^{-1} respectivamente. Os extratos apresentaram bom potencial antioxidante e antimicrobiano, com IC_{50} entre 0.016 e 0.043 mg.mL^{-1}, e CIM contra *S. aureus* e *E. coli* entre 0.313 e 1.25 mg.mL^{-1}. A PLE demonstrou eficiência na obtenção de extratos de folhas de *E. saligna* com propriedades bioativas.

Palavras-chave: Compostos fenólicos, *Eucalyptus saligna*, extração líquida pressurizada, propriedades bioativas.

1 INTRODUCTION

The genus *Eucalyptus*, belonging to the Myrtaceae Family, has about 800 species and subspecies and is known to be a renewable source of wood (ELANSARY et al., 2017). Eucalyptus

is of great importance in the Brazilian economic sector and can be used in the pulp and paper industry, furniture, fuel, biomass and bioenergy, poles, among others (TAVANTI et al., 2018).

Eucalyptus leaves are a secondary product, often left out, which ends up generating aspects unfavorable to the environment due to the lack of decomposers that feed on their leaves. The essential oils present in the leaves make it impossible for animals to consume, accumulating on the soil, which can impact aquatic ecosystems and soil renewal (DE VECHI & JÚNIOR, 2018). Therefore, the study of eucalyptus leaves in obtaining natural products with bioactive properties, such as extract and essential oils, is feasible and constitutes an alternative for producers of the species to use the leaf material after cutting the wood.

Some studies of essential oil and extracts from eucalyptus have reported acaricidal activity (SOUZA et al., 2016), bioactivity against *Zabrotes subfasciatus* (SAMPAIO et al., 2017), in addition to good antimicrobial and antioxidant properties (CARDOSO et al., 2019; MUHAMMED et al., 2018; SYUKRI et al., 2019). However, most research is related to essential oil, or the use of the wood for extraction instead of leaves.

Solvent extraction is the most widespread process for obtaining bioactive compounds, where the main factors are the type of solvent, the temperature and the time of extraction. In addition, the extraction technique used also influences the efficiency of the process. Alternative techniques have been developed to improve efficiency and reduce environmental impact, such as microwave assisted extraction, ultrasound assisted extraction or pressurized fluid extraction (FERNÁNDEZ-AGULLÓ et al., 2015).

The concept of “green extraction” is linked to processes with low energy consumption, which use alternative solvents and renewable natural products. Within this context, pressurized liquid extraction (PLE) is considered a “more ecological process” when compared to conventional methods that require a greater amount of solvents for extraction (RUDKE et al., 2019).

The PLE technique is based on the use of solvents under conditions of high pressures and temperatures, but keeping the solvent in a liquid state throughout the extraction process. These conditions provide a higher extraction yield in a shorter process time due to the improvement of mass transfer kinetics. The high temperature increases the solubility of the compounds and decreases the viscosity and the surface tension of the solvent, improving the penetration in the matrix (HERRERO et al., 2013).

Considering the wide availability of eucalyptus leaf material and the possibility of using leaves as a source of natural products, the work aimed to obtain extracts of *E. saligna* leaves by the PLE technique, and to characterize different fractions of the extraction time in relation to bioactive

compounds and antioxidant and antimicrobial activities.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

E. saligna leaves were collected in Severiano de Almeida city, RS – Brazil (27°25'01"S and 52°03'19"W) during the winter and taken to Herbário Padre Balduíno Rambo of the Universidade Regional Integrada do Alto Uruguai e das Missões – URI Erechim for later identification. The leaves were dried in a dark chamber at room temperature (~22 °C) until constant weight, crushed in an industrial shredder and stored under refrigeration at -20 °C.

2.2 PRESSURIZED LIQUID EXTRACTION (PLE)

Extraction with pressurized ethanol (Neon, 95%) followed the methodology of Paes et al. (2014) with adaptations, where 20 g of sample were added in a jacketed extractor, with a volumetric capacity of approximately 75 mL. After closing the extractor, an ultra-thermostatic bath (Sppencer) was used to maintain the extraction temperature, which was 40 °C. To pressurized the system, an HPLC pump (Series III Isocratic HPLC Pump, Scientific Systems) was used with a solvent flow of 2.5 $mL.min^{-1}$ and a working pressure of 10.4 MPa, considering equipment limitations. The system remained pressurized for 30 minutes, characterizing static extraction; after this time, the extractor outlet valve is opened, starting the dynamic extraction that occurred for 180 minutes.

The extracts were collected in amber flasks at different times during the extraction. To determine the yield, the collection flasks were kept in a vacuum oven (Q819V2, Quimis) for 7 days at 50 °C for solvent evaporation. The yield was calculated by Eq. (1):

$$\text{Yield } (\%) = (M_E * 100) / M_A$$

where M_E is the mass of extract obtaining, and M_A is the mass of initial sample placed from the extractor.

After determination the yield, the extracts were diluted in ethanol (Neon, 95%) at concentration of 20 $mg.mL^{-1}$ and grouped according to the extraction times of 0-15, 15-30, 30-60, 60-120 and 120-180 minutes for further analysis.

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2.3 TOTAL PHENOLIC COMPOUNDS CONTENT

The determination of the total phenol content followed the methodology described by Singleton et al. (1999), based on the Folin-Ciocalteau spectrophotometric method, using Gallic acid as a reference standard. In a test tube, 0.5 mL of previously diluted sample, 2.5 mL of the Folin-Ciocalteau reagent (diluted 1:10, v/v) and 2 mL of 4% sodium carbonate (m/v) were added. The tubes were incubated for 2 hours in the absence of light. The readings were performed on a spectrophotometer (UV-1600, Pro-analysis) at 760 nm and the content of total phenolic compounds was calculated using a standard curve ($y = 0.0105x - 0.0058$, $R^2 = 0.999$) and expressed in Gallic acid equivalent ($mgGAE.g^{-1}$ dry extract).

2.4 FLAVONOID CONTENT

The determination of flavonoid content followed the methodology described by Garrido et al. (2013) with adaptations, using Quercetin as a reference standard. In a tube test were added 0.5 mL of previously diluted sample, 4.3 mL of 70% (v/v) ethanol, 0.1 mL of 10% (m/v) aluminum nitrate and 0.1 mL of 10% (m/v) potassium acetate. The samples were incubated for 40 minutes in the absence of light. The readings were performed on a spectrophotometer (UV-1600, Pro-analysis) at 415 nm and the flavonoid content was calculated using a standard curve ($y = 0.0054x - 0.0189$, $R^2 = 0.9999$) and expressed in Quercetin equivalents ($mgQE.g^{-1}$ dry extract).

2.5 ANTIOXIDANT ACTIVITY (DPPH)

The determination of antioxidant activity followed the methodology of Brand-Williams et al. (1995), based on the capture of the free radical DPPH (2,2 diphenyl 1-1-picrylhidrazyl). The extracts were diluted in ethanol in concentrations from 0.1 to 0.0025 $mg.mL^{-1}$ based on previous tests to identify the linear range of increasing activity. 2 mL of the dilution of the extracts were incubated with 2 mL of the DPPH 0.1 mM alcoholic solution for 30 minutes in the absence of light. The readings were performed on a spectrophotometer (UV-1600, Pro-analysis) at 515 nm, and the antioxidant activity was expressed in IC_{50}, calculated by linear regression.

2.6 ANTIMICROBIAL ACTIVITY (MIC)

The determination of antimicrobial activity followed the methodology described by Antunes et al. (2016) with adaptations, based on the dilution of extracts in 96-well micro-plates. This method allows the determination of the minimal inhibitory concentration (MIC), which is the lowest concentration of the extract capable of inhibiting bacterial growth. The micro-plates were sterilized

with 5% hypochlorite for 24 h, then washed with distilled water and subjected to UV light (254 nm) for 20 minutes.

For assays, 150 µL of LB medium (Luria Bertani) was added to each well, then 150 µL of extract (5 $mg.mL^{-1}$) was added to the first well, making serial dilutions in the other wells. All tests were performed in triplicate. 10 µL of inoculum at a concentration of approximately 10^8 $CFU.mL^{-1}$ was added to each well, reading was performed at 490 nm in a microplate reader (EL800, Bio-Tek Instruments) and incubated in an oven at 35 °C. After 24 hours, the absorbance readings was performed again. The bacterial used were *Staphylococcus aureus* (ATTC 25923) and *Escherichia coli* (ATTC 25922).

2.7 STATISTICAL ANALYSIS

The analysis of variance (ANOVA) of the data was performed using the software Statistica (StatSoft, Tulsa, USA), where the level of significance established was 5% ($p<0.05$). The comparison of means was performed by Tukey's test, the results are expressed in the form of mean ± standard deviation, and the analyzes were performed in triplicate.

3 RESULTS AND DISCUSSION

The *E. saligna* leaves extraction showed a mass yield of 2.46 g of extract for 20 g of initial sample, equivalent to 12.3% after 180 minutes of extraction. Until this time, a continuous increase in the extract mass has been identified, being that the extraction not reaching a maximum value. The content of phenolic compounds and flavonoids (Figure 1) during the extraction demonstrated the same behavior, which showed a total yield of 618.57 $mgGAE.g^{-1}$ and 160.27 $mgQE.g^{-1}$ of dry extract after 180 minutes, respectively.

One of the possible causes of the extraction does not reach a peak, is related to the particle size of the plant matrix. According to Migliato et al. (2011), the granulometric standardization of the ground material is important in the extraction process because particles with homogeneous dimensions increase the contact area between the solid material and the extractor liquid. In addition, reducing particle size using a knife or ball mill could assist with mass transfer, improving extraction yield in a shorter time.

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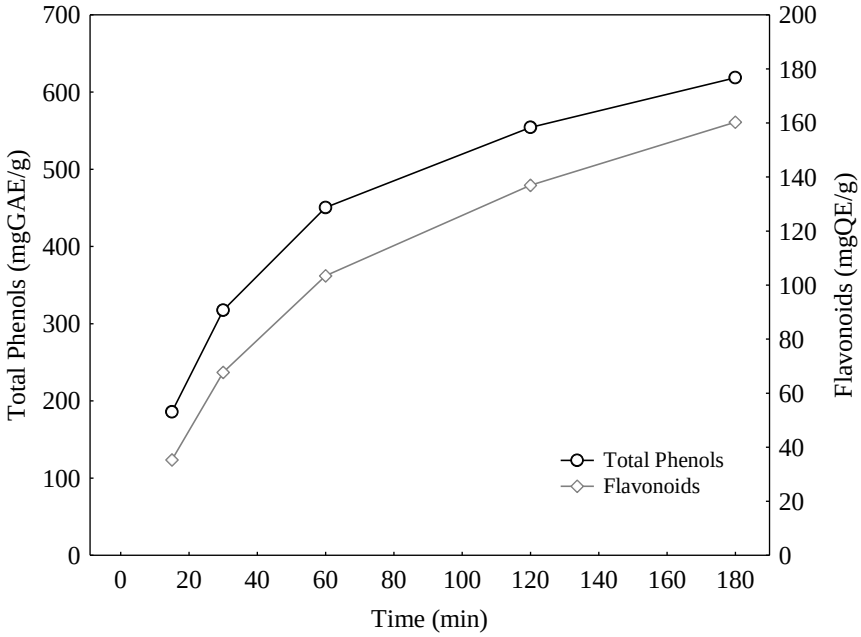
Figure 1. Effect of time on the yield of phenolic and flavonoid compounds.



In the study by Gharekhani et al. (2012), of microwave-assisted extraction and ultrasound-assisted extraction of phenols and flavonoids from *E. camaldulensis* leaves, it was also reported that the extraction of these compounds increases with increasing time and that microwave assisted extraction reached its highest point in 15 minutes. However, these extraction techniques are discontinuous, that is, it is carried out in the form of "batches", while in the PLE performed in this study, the solvent is renewed continuously. Therefore, the extraction of the compounds of interest is expected to occur continuously, since the solvent does not saturate during the mass transfer process.

According to Guerra et al. (2016), the operational variables temperature, time and solvent concentration have a significant effect on the extraction of phenolic compound. In addition to these, variables such as sample size, solvent flow and ecstatic extraction time also influence the extracts yield, as they interfere with the mass transfer rate. Soares et al. (2020) reported in their study that aqueous and hydroalcoholic extraction, at 50 and 70%, showed a higher content of phenolic compounds when compared to extraction using absolute ethanol.

The class of phenolic compounds can be divided into flavonoids, which include anthocyanins, flavonols and isoflavones and non-flavonoids, derived from phenolic acids (ROSSA et al., 2017). Phenolic compounds have hydroxyls (OH) at their ends, which interact with the hydrogen bond of ethanol, being easily separated by this solvent (ZHAO & ZHANG, 2014).

According to Table 1, the phenols content in the first fraction (0-15 minutes) of extraction was significantly higher than in the other times, with 186.10 mgGAE.g^{-1} of dry extract. Between the

fractions of 15-30 and 30-60 there was no significant difference in the phenol content, and in the final times there is a reduction in the extraction rate of this class of compounds, being that 60-120 and 120-180 minutes are significantly different from other times.

Table 1. Content of total phenols, flavonoids and antioxidant activity of *E. saligna* extracts.

Time (min)	Total phenols (mgGAE/g extract)	Flavonoids (mgQE/g extract)	IC_{50} (mg/mL)
0-15	186.10^{a} ±1.43	35.35^{ab} ±1.15	0.016^{c} ±0.003
15-30	131.33^{b} ±2.38	32.39^{b} ±1.07	0.023^{bc} ±0.002
30-60	133.24^{b} ±1.90	35.72^{a} ±1.21	0.025^{b} ±0.003
60-120	103.71^{c} ±2.47	33.50^{ab} ±1.29	0.026^{b} ±0.004
120-180	64.19^{d} ±2.97	23.31^{c} ±1.03	0.043^{a} ±0.004
Total	618.57	160.27	

The means ± standard deviation followed by equal letters in the columns indicate that there is no significant difference (Tukey's test, $p < 0.05$).

In relation to flavonoids, the extraction showed a similar yield throughout the extraction process, between 32.39 and 35.72 mgQE.g^{-1} of dry extract, with no significant difference until the time of 120 min. In 120-180 minutes the extraction rate of flavonoid compounds is reduced (23.31 mgQE.g^{-1} of dry extract), being significantly different from previous times.

Limam et al. (2020) in his study evaluated the phenolic content of 13 eucalyptus species through conventional extraction and obtained levels between 16.59 and 122.38 mgGAE.g^{-1} for phenolic compounds, and between 13.48 and 118.59 mgQE.g^{-1} for flavonoids, according to the species studied. These data are not close to those found in this study, since the extraction technique using pressurized liquids aims to maximize the extraction of these compounds in a shorter time and reduce the generation of waste.

The antioxidant potential of the *E. saligna* extracts analyzed at the different extraction times showed a small significant difference between fractions 0-15 min (0.016 mg.mL^{-1}), 120-180 min (0.043 mg.mL^{-1}) and the other times. The correlation of activity with the content of phenolic compounds (R = -0.939) and flavonoids (R = -0.928) indicates that the antioxidant effect is strongly linked to this class of compounds, which justifies the tendency to reduce activity over the extraction time.

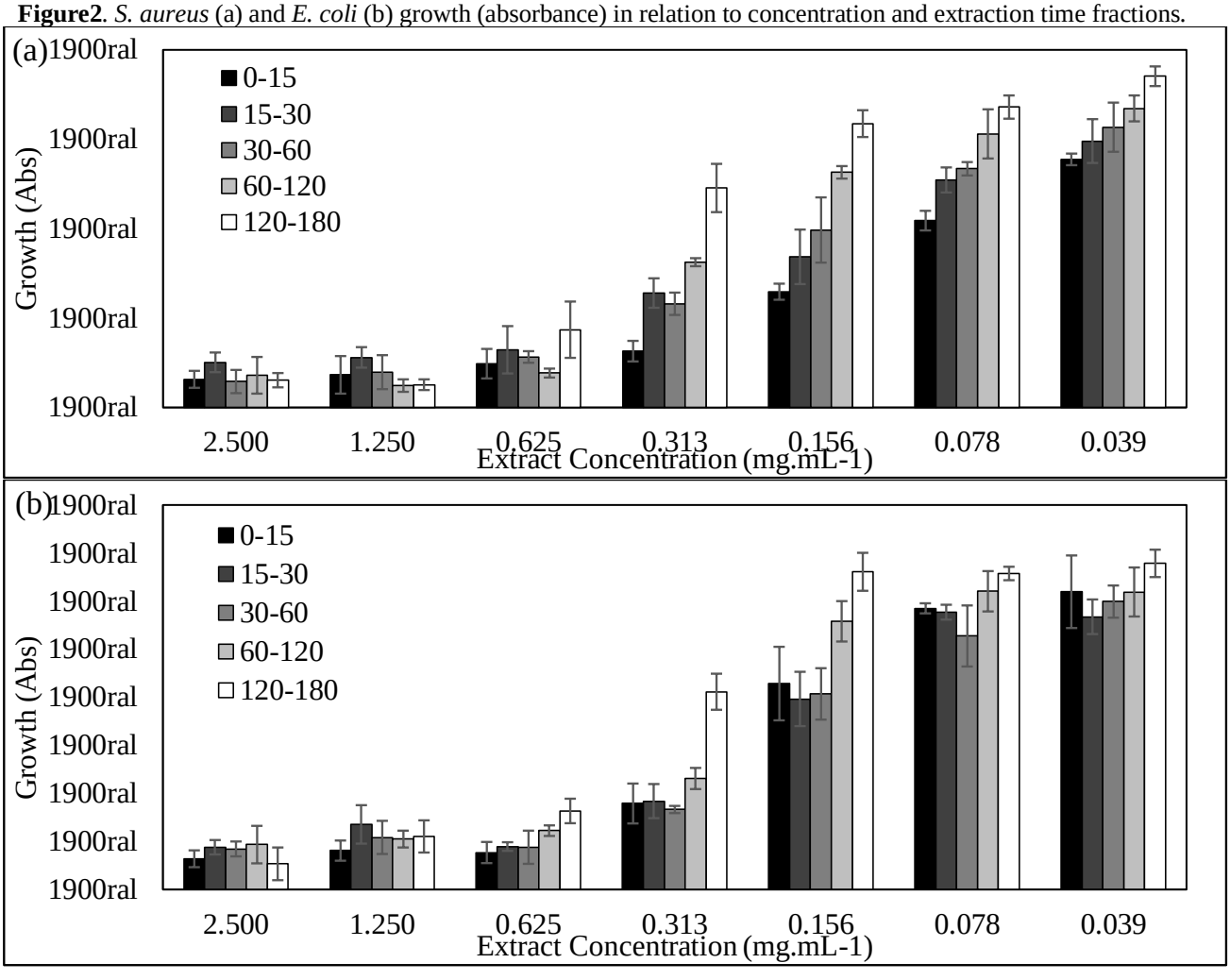
In the study by Bruzadelli et al. (2020) the aqueous extraction at 100 °C of *Mentha piperita* by decoction and infusion showed a greater extraction of phenolic compounds with the increase in the decoction time, however this increase did not influence the antioxidant potential of the extracts. The use of high temperatures in conventional techniques can cause the degradation of bioactive

compounds. An advantage of the PLE technique, when working with lower temperatures (40 °C), is to preserve this property of the extracts.

The antioxidant potential in the elimination of DPPH free radicals from *E. saligna* leaf extracts evidenced in this study is satisfactory and is in accordance with the activity of ascorbic acid and eucalyptus (*E. urophylla* and *E. grandis*) extract reported by Li et al. (2020), whose IC_{50} values were 0.043 and 0.139 $mg.mL^{-1}$ respectively. Still, the antioxidant activity of the extracts obtained in this work corroborates the study by Limam et al. (2020), which obtained an IC_{50} between 0.0051 to 0.0216 $mg.mL^{-1}$ for methanol extracts from leaves of different eucalyptus species and also reported the correlation between the antioxidant activity of extracts with phenolic content.

In this study, both for *S. aureus* and *E. coli*, two pathogenic bacteria, Gram-Positive and Gram-Negative, respectively, partial inhibition of microbial growth by *E. saligna* extracts was verified in the tested concentrations, as shown in Figure 2. In addition, a tendency to decrease antimicrobial activity with increased extraction time can be observed.

Figure2. *S. aureus* (a) and *E. coli* (b) growth (absorbance) in relation to concentration and extraction time fractions.



According to Andrews (2001), the minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial capable of inhibiting the growth of a microorganism. For *S. aureus*, the extract fraction of 0-15 minutes showed the lowest MIC of 0.313 $mg.mL^{-1}$, with no significant difference between the concentrations of 2.5 to 0.313 $mg.mL^{-1}$, but significantly different from the other fractions in the same concentration (Tukey's Test, $p < 0.05$), while in the fractions of 15-120 minutes the MIC is 0.625 $mg.mL^{-1}$. For *E. coli* the MIC is 0.625 $mg.mL^{-1}$ for the fractions of 0-120 minutes and, for the two microorganisms tested, the MIC for the 120-180 minute fraction is 1.25 $mg.mL^{-1}$.

It is possible to observe the influence of different extraction time fractions on the inhibitory effect of microbial growth. From the concentration of 0.625 $mg.mL^{-1}$, there is an increase in growth with an increase in the extraction time. This indicates that over time the extraction of compounds with antimicrobial activity decreases, increasing the MIC values.

Pereira et al. (2014) evaluated the antimicrobial properties of *E. globus* extracts against *Pseudomonas aeruginosa,* where MIC values between 0.625 and 2.5 $mg.mL^{-1}$ were obtained, very similar to the results reported in the present study. Behbahani et al. (2013) determined the MIC of 4.0 and 32.0 $mg.mL^{-1}$ for *S. aureus* and *E. coli*, respectively, of the ethanol extract of *E. camaldulensis* leaves obtained by conventional technique.

The resistance of microorganisms to antimicrobial agents is related to cell morphology. Gram-negative bacteria have a phospholipid membrane that makes the cell wall impermeable to lipophilic solutes and selective to hydrophilic solutes, which hinders the action of antimicrobial molecules (LUÍS et al. 2014).

In terms of application of eucalyptus leaf extract, Li et al. (2020) describes that dietary supplementation with extracts of polyphenols from eucalyptus leaves can promote the quality of chicken meat and the intestinal health of chickens and other animals. Moreover, the *E. saligna* leaf extract has potential for application in the food or chemical industry as natural antioxidant and antimicrobial agents, as they have proven to be good inhibitors of free radicals and pathogenic microorganisms important to public health.

4 CONCLUSION

In this work, fractions were obtained at different times of extraction of *E. saligna.* leaves with antioxidant and antimicrobial properties, through the technique of extraction with pressurized liquid. The extract showed an overall yield equivalent to 12.3%, total phenolic compounds content of 618.57 $mgGAE.g^{-1}$ and flavonoids content of 160.27 $mgQE.g^{-1}$.

All extraction fractions showed good antioxidant activity (IC_{50} between 0.016 and 0.043 $mg.mL^{-1}$), but with a tendency to decrease over time, showing a correlation with the content of total phenols and flavonoids. The *E. saligna* leaf extract showed antimicrobial potential against *S. aureus* and *E. coli*, with MIC between 0.313 and 1.25 $mg.mL^{-1}$. There was a tendency to decrease the antimicrobial activity in relation to the extraction time.

The extraction technique using pressurized liquid demonstrated efficiency in the separation of bioactive compounds from eucalyptus leaves and advantages such as the reduction of time and volume of solvent when compared to conventional techniques. In this study was demonstrated that *E. saligna* leaves are a source of phenolic and flavonoid compounds with good bioactive properties for industrial applications.

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DECLARATION OF CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

ANDREWS, J. M. Determinação de concentrações inibitórias mínimas. **Jornal de quimioterapia antimicrobiana**. v.48 n. suppl 1, p.5-16, 2001. doi: 10.1093/jac/48.suppl_1.5

ANTUNES, T. V.; FREITAS, R. F.; & ROYO, V. A. Avaliação da atividade antimicrobiana do extrato hidroalcoólico das folhas de *Petiveria alliaceae* L.(Phytolacaceae). **Revista da Universidade Vale do Rio Verde**, v.14, n.1, p.448-457, 2016. doi: 10.5892/ruvrd.v14i1.2525

BEHBAHANI B. A. et al. Effect of aqueous and ethanolic extract of *Eucalyptus camaldulensis* L. on food infection and intoxication microorganisms "in vitro". **Journal of Paramedical Sciences**, v.4, n.3, p.89-99, 2013. doi: 10.22037/jps.v4i3.4666

BRAND-WILLIAMS, W.; CULIVER M.E.; & BERSET, C. Use of a free radical method to evaluate antioxidant activity. **LWT – Food Science and Tecnology**, v.28, n.1, p.25-30, 1995. doi: 10.1016/S0023-6438(95)80008-5

BRUZADELLI, R. F. D. et al. Composição química, atividade antioxidante e qualidade microbiológica do extrato aquoso de Mentha piperita, de acordo com o tempo de decocção. **Brazilian Journal of Development**, v. 6, n. 4, p. 17165-17171, 2020. doi: 10.34117/bjdv6n4-039

CARDOSO R. C. et al. Potencial antimicrobiano do óleo da folha de *Eucalyptus urograndis* frente *Stafilococus aureus*. **Id on Line Revista Multidisciplinar e de Psicologia**, v.13, n.43, p.989-1002, 2019. doi: 10.14295/idonline.v13i43.1573

DE VECHI A.; & JÚNIOR C. A. O. M. Aspectos positivos e negativos da cultura do Eucalipto e os efeitos ambienteis do seu cultivo. **Revista Valore**, v.3, n.1, p.495-507, 2018. doi: 10.22408/reva312018101495-507

ELANSARY H. O. et al. In vitro antibacterial, antifungal and antioxidant activities of *Eucalyptus* spp. leaf extracts related to phenolic composition. **Natural product research**, v.31, n.24, p.2927-2930, 2017. doi: 10.1080/14786419.2017.1303698

FERNÁNDEZ-AGULLÓ, A.; FREIRE, M. S.; & GONZÁLEZ-ÁLVAREZ, J. Effect of the extraction technique on the recovery of bioactive compounds from eucalyptus (*Eucalyptus globulus*) wood industrial wastes. **Industrial Crops and Products**, v.64, p.105-113, 2015. doi: 10.1016/j.indcrop.2014.11.031

GARRIDO, G.; ORTIZ, M.; & POZO, P. Fenoles y flavonoides totales y actividad antioxidante de extractos de hojas de *Lampaya medicinalis* F. Phil. **Journal of Pharmacy & Pharmacognosy Research**, v.1, n.1, p.30-38, 2013. ISSN: 0719-4250.

GHAREKHANI, M.; GHORBANI, M.; & RASOULNEJAD, N. Microwave-assisted extraction of phenolic and flavonoid compounds from *Eucalyptus camaldulensis Dehn* leaves as compared with ultrasound-assisted extraction. **Latin American applied research**, v.42, n.3, p.305-310, 2012. ISSN 0327-0793.

GUERRA, A. P.; DOS SANTOS GARCIA, V. A.; & DA SILVA, C. Otimização da extração de compostos fenólicos da casca de manga (*Tommy atkins*) utilizando processo assistido por ultrassom. **E-xacta**, v.9, n.1, p.103-110, 2016. doi: 10.18674/exacta.v9i1.1783

HERRERO M. et al. Compressed fluids for the extraction of bioactive compounds. **TrAC Trends in Analytical Chemistry**, v.43, p.67-83, 2013. doi: 10.1016/j.trac.2012.12.008

LI W. et al. In vitro and in vivo antioxidant activity of eucalyptus leaf polyphenols extract and its effect on chicken meat quality and cecum microbiota. **Food Research International**, v.136, p.109302, 2020. doi: 10.1016/j.foodres.2020.109302

LIMAM H. et al. Phenolic composition and antioxidant activities of thirteen Eucalyptus species cultivated in north east of Tunisia. **Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology**, p.1-18, 2020. doi: 10.1080/11263504.2020.1762791

LUÍS Â. et al. Stumps of *Eucalyptus globulus* as a source of antioxidant and antimicrobial polyphenols. **Molecules,** v.19, n.10, p.16428-16446, 2014. doi: 10.3390/molecules191016428

MIGLIATO K.F. et al. Planejamento experimental na otimização da extração dos frutos de

Syzygium cumini (L.) Skeels. **Química Nova**, v.34, n.4, p.695-699, 2011. doi: 10.1590/S0100-40422011000400024

MUHAMMED, D.; DADA, E. O.; & ALO, A. A. Antibacterial property of ethanolic leaf extract of *Eucalyptus citriodora* hook on clinical and typed isolates of *Escherichia coli*. **South Asian Journal of Research in Microbiology**, v.2, n.1, p.1-8, 2018. doi: 10.9734/sajrm/2018/v2i129234

PAES J. et al. Extraction of phenolic compounds and anthocyanins from blueberry (*Vaccinium myrtillus* L.) residues using supercritical CO_2 and pressurized liquids. **Journal of Supercritical Fluids**, v.95, p.8-16, 2014. doi: 10.1016/j.supflu.2014.07.025

PEREIRA V. et al. Antibacterial activity and synergistic effects between *Eucalyptus globulus* leaf residues (essential oils and extracts) and antibiotics against several isolates of respiratory tract infections (*Pseudomonas aeruginosa*). **Industrial Crops and Products**, v.52, p.1-7, 2014. doi: 10.1016/j.indcrop.2013.09.032

ROSSA U. B. et al. Influência da luminosidade e fertilizantes nos teores de metilxantinas e compostos fenólicos em folhas de erva-mate. **Ciência Florestal**, v.27, n.4, p.1365-1374, 2017. doi: 10.5902/1980509830217

RUDKE A. R. et al. Optimization of green PLE method applied for the recovery of antioxidant compounds from buriti (*Mauritia flexuosa* L.) Shell. **Food Chemistry**, v.298, p.125061, 2019. doi: 10.1016/j.foodchem.2019.125061

SAMPAIO A. A. et al. Bioatividade dos extratos aquosos de folhas de pimenta malagueta e eucalipto sobre *Zabrotes subfasciatus* em grãos de fava. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v.12, n.2, p.198-203, 2017. doi: 10.18378/rvads.v12i2.4695

SINGLETON, V. L.; ORTHOFER, R.; & LAMUELA-RAVENTOS, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagente. **Methods of Enzymology**, v.299, p.152-178, 1999. doi: 10.1016/S0076-6879(99)99017-1

SOARES, A. A. et al. Comparison of total phenolic content and antioxidant activity of different extracts of *Agaricus blazei* Murril. **Brazilian Journal of Development**, v. 6, n. 3, p. 13561-13573, 2020. doi: 10.34117/bjdv6n3-281

SOUZA L. P. et al. Composição química e toxicidade do óleo essencial de eucalipto sobre o ácaro-rajado. **Comunicata Scientiae**, v.7, n.4, p.486-493, 2016. doi: 10.14295/cs.v7i4.1502

SYUKRI, D. M.; NWABOR, O.; & ERGANTARA, R. I. Preliminary phytochemical, antioxidants, and antibacterial properties of eucalyptus aqueous leaf extract against *Streptococcus pyogenes*. **In IOP Conference Series: Earth and Environmental Science, IOP Publishing**, v.305, n.1, p.012067, 2019. doi: 10.1088/1755-1315/305/1/012067

TAVANTI R. F. R. et al. Eficiência da adubação boratada no desenvolvimento de mudas de eucalipto. **Revista de Ciências Agrárias Amazonian Journal of Agricultural and Environmental Sciences**, v.61, 2018. doi: 10.22491/rca.2018.2862

ZHAO S.; & ZHANG, D. Supercritical CO_2 extraction of Eucalyptus leaves oil and comparison

with Soxhlet extraction and hydro-distillation methods. **Separation and Purification Technology**, v.133, p.443-451, 2014. doi: 10.1016/j.seppur.2014.07.018