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# EVALUATION OF CYTOTOXITY AND ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACT OF EUGENIA ASTRINGES CAMBESS LEAVES

## *AVALIAÇÃO DA CITOTOXIDADE E ATIVIDADE ANTIOXIDANTE DO EXTRATO AQUOSO DAS FOLHAS DE EUGENIA ASTRINGES CAMBESS*

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## ABSTRACT

The genus *Eugenia* belongs to the Myrtaceae family, being one of the most important families due to its wide distribution throughout the Brazilian territory. Some species have antioxidant, antifungal, antiviral, bactericidal, and lowering triglyceride and cholesterol levels. In addition to terpenoids, phenolic substances such as tannins and flavonoids have been described in the genus *Eugenia*. It is worth mentioning that in recent decades, it has been observed that there is a growing interest in polyphenolic substances, as most of them protect biological systems against the negative effects of processes or reactions that lead to the oxidation of molecules or cellular structures. In view of the above, the objective of this work was to evaluate the in vitro antioxidant capacity using the ABTS radical capture method of aqueous extracts of *Eugenia astringens* leaves, obtain the $LD_{50}$ through the bioassay with *Artemia salina* Leach, determine the action of the isolated and combined aqueous extract with antibiotics against cultures of *Escherichia coli* BW9091 and AB 1157, evaluate the biological effects of the extract on the survival of cultures of the mutant *Escherichia coli* BW9091 against the action of oxidizing agents ($SnCl_2$ and $H_2O_2$) and compare with the wild species (*Escherichia coli* AB 1157), which has all efficient genetic DNA repair mechanisms. We can conclude that the preliminary analysis of the aqueous extract obtained a positive result for the presence of polyphenolic compounds, among which tannins stand out, which have several pharmacological activities already described in the literature; the bioassay with *Artemia salina* revealed that the extract presented high toxicity with $LD_{50}$ of 32.3 µg $mL^{-1}$ determined by linear regression analysis, with a correlation coefficient ($R^2$) equal to 0.9745 and straight line equation y = 0 .0125x+77.5926; the aqueous extract showed antioxidant activity, with a TEAC value of 3746.50 ± 210.71 µM trolox /g, determined by linear regression analysis with a correlation coefficient ($R^2$) equal to 0.9982, equation of the straight line y = 0.0002X + 0.0009, with a relative standard deviation of 5.62% between measures; the extract did not exhibit microbicidal action; the associations of the extract with antibiotics revealed an increase in the size of the halos and differences between them in the *E. coli* AB 1157 and BW9091 strains, however, these differences were not significant by the Tukey test; the aqueous extract in *E. coli* BW 9091, inhibited the oxidative capacity of stannous chloride and increased the oxidative capacity of hydrogen peroxide and in *E. coli* AB 11577, the extract promoted an increase in oxidative capacity, when associated with stannous chloride and oxygen peroxide.

**Keywords**: Leaves; aqueous extract; *Eugenia astringens* Cambess.; ABTS, $LD_{50}$; microbial and antioxidant activity.

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***RESUMO***

*O gênero Eugenia pertence à família Myrtaceae, sendo uma das famílias mais importantes devido a sua ampla distribuição em todo o território brasileiro. Algumas espécies apresentam atividades antioxidante, antifúngica, antiviral, bactericida, redutora dos níveis de triglicerídeos e colesterol. Além de terpenóides, foram descritos no gênero Eugenia substâncias fenólicas tais como taninos e flavonoides. Vale ressaltar que nas últimas décadas, têm-se observado que há um crescente interesse por substâncias polifenólicas, já que a maioria delas protegem os sistemas biológicos contra os efeitos negativos dos processos ou reações que levam à oxidação de moléculas ou estruturas celulares. Diante do exposto o objetivo deste trabalho foi avaliar a capacidade antioxidante in vitro pelo método de captura de radicais ABTS dos extratos aquosos das folhas de Eugenia astringens, obter a $DL_{50}$ através do bioensaio com Artemia salina Leach, determinar a ação do extrato aquoso isolado e combinado com antibióticos frente a cultura de Escherichia coli BW9091 e AB 1157, avaliar os efeitos biológicos do extrato na sobrevivência de culturas da mutante Escherichia coli BW9091 contra a ação de agentes oxidantes ($SnCl_2$ e $H_2O_2$) e comparar com a espécie selvagem (Escherichia coli AB 1157), que apresenta todos os mecanismos genéticos de reparo de DNA eficientes. Podemos concluir que a análise preliminar do extrato aquoso obteve um resultado positivo para a presença de compostos polifenólicos, dentre estes destacam-se os taninos que possuem diversas atividades farmacológicas já descritas pela literatura; o bioensaio com a Artemia salina revelou que o extrato apresentou uma alta toxicidade com $DL_{50}$ de 32,3 µg $mL^{-1}$ determinada pela análise de regressão linear, com coeficiente de correlação ($R^2$) igual a 0,9745 e equação da reta y = 0,0125x+77,5926; o extrato aquoso apresentou atividade antioxidante, com valor TEAC de 3746,50 ± 210,71 µM trolox /g, determinada pela análise de regressão linear com coeficiente de correlação ($R^2$) igual a 0,9982, equação da reta y = 0,0002X + 0,0009, com um desvio padrão relativo de 5,62 entre as medidas; o extrato não exibiu ação microbicida; as associações do extrato com os antibióticos revelaram um aumento no tamanho dos halos e diferenças destes nas cepas E. coli AB 1157 e BW9091, contudo, essas diferenças não foram significativas pelo teste de Tukey; o extrato aquoso em E. coli BW 9091, inibiu a capacidade oxidativa do cloreto estanoso e aumentou a capacidade oxidativa do peróxido de hidrogênio e em E. coli AB 11577, o extrato promoveu o aumento da capacidade oxidativa, quando associados com o cloreto estanoso e o peróxido de oxigênio.*

***Palavras-chave:*** *Folhas; extrato aquoso; Eugenia astringens Cambess.; ABTS, $DL_{50}$; atividade microbiana e antioxidante.*

## 1 INTRODUCTION

In recent decades, it has been observed that there is a growing interest in species that have antioxidant properties. Several studies have demonstrated that the consumption of antioxidant substances in the daily diet can promote an effective protective action against reactive oxygen species (ROS) produced in cells by various physiological and environmental stimuli (Badke *et al.*, 2011; Silva *et al.* , 2020). Furthermore, the increase in reactive oxygen species is considered a risk factor and is involved in the occurrence of certain pathologies, such as cancer, autoimmune diseases, cataracts, neurodegenerative diseases, diabetes mellitus, cardiovascular diseases and chronic kidney disease (Silva *et al.* , 2020). Furthermore, it is likely that the neutralization of inflammatory and oxidizing factors by antioxidant substances will become a strong ally in combating cytokine release syndrome that affects some viral diseases, including COVID 19 (Zhang *et al.*, 2020; Brazil, 2012).

The main chemical constituents with high antioxidant and anti-inflammatory capacities are: vitamins C, E and phytochemicals, such as carotenoids and polyphenols (Iddir *et al*., 2020; Silva *et al*., 2020). The Myrtaceae family has 6,000 species and about 140 genera (Wilson, 2011), being one of the most important families due to its wide distribution in the tropical and subtropical regions of the globe, with peaks of diversity in South America, Australia and Southeast Asia, in addition to some representation in Africa. (Proença *et al*., 2025**)**. The leaves are widely used in folk medicine due to their antioxidant, anti-inflammatory, healing, antiseptic and anti-diarrheal activities (Di Stasi, Hiruma-Lima, 2002). One of the largest representatives of the Myrtaceae family are species of the genus *Eugenia* (Proença *et al*., 2025). This genus is quite rich in phenolic compounds, such as tannins and flavonoids. Probably, due to the high content of phenolic compounds, species of the genus *Eugenia* have antioxidant activity (Stieven *et al*., 2009). Our interest in evaluating the antioxidant potential of *Eugenia astringens* Cambess. leaves is due to the relevance of evaluating this activity for human health, the availability of the species in the southeast region and the high concentration of antioxidant and phenolic substances in representatives of this family (De Morais Rodrigues *et al*., 2016), of the genre (Carvalho Junior *et al*., 2014; Magina *et al*., 2010) and the species (Leitão *et al*., 2014). Given the above, the objective of this work is to determine the preliminary phytochemical profile, according to Matos (1987); evaluate the *in vitro* antioxidant capacity using the ABTS radical capture method; obtain $LD_{50}$ through bioassay with *Artemia salina* Leach; determine the action of *E. astringens* extract isolated and combined with antibiotics against cultures of *Escherichia coli* BW9091 and AB 1157; evaluate the biological effects of the aqueous extract of *E. astringens* on the survival of cultures of the mutant *E. coli* BW9091 against the action of oxidizing agents ($SnCl_2$ and $H_2O_2$) and compare with the wild species, *E. coli* AB 1157, which presents all the genetic mechanisms of efficient DNA repair.

## 2 MATERIALS AND METHODS

Much of the study was carried out at the Chemical and Biological Analysis Laboratory (LAQB) of the Rio de Janeiro State University (UERJ), west zone campus, except for the evaluation of *in vitro* antioxidant capacity, using the $ABTS^+$ radical capture method, carried out at Embrapa Food Agroindustry of Barra de Guaratiba. The leaves of *Eugenia astringens* Cambess were collected in the morning (07:00) in the Marambaia restinga, located in the neighborhood of Guaratiba, in the municipality of Rio de Janeiro- RJ, with geographic coordinates 23°03'02.0"S 43°35 '42.3"W. The leaves of *Eugenia astringens* were collected in the early morning (07:00) from individuals belonging to a population located in the Restinga da Marambaia, in the Guaratiba neighborhood, municipality of Rio de Janeiro, RJ (23°03'02.0"S, 43°35'42.3"W). These individuals were identified in the field by Dr. Marcelo C. Souza. This same population had been previously studied by Dr. Souza in 2002 and a fertile specimen was documented in the Herbarium of the Department of Botany (RBR) of

the Federal Rural University of Rio de Janeiro (UFRRJ), through the voucher registered under number RBR00012191. All plant material used in the present study was collected by Dr. Marcelo C. Souza.

### *2.1 Obtaining the aqueous extract.*

The fresh leaves were weighed on a semi-analytical scale (class II Bel Mark 2500), obtaining a value of 56.37 g. Subsequently, the fresh leaves were ground in a blender. The aqueous extract was obtained by infusing the fresh leaves in a 500 mL beaker with hot distilled water (70 ºC). After 30 minutes, the tea (or aqueous extract) was filtered and stored in glass jars with screw caps wrapped in aluminum foil. The extract was frozen (24 hours) and then lyophilized (Lyophilizer, LIOTOP 220) at a temperature of - 80ºC until constant mass.

### *2.2 Preliminary phytochemical tests*

The aqueous extract of *Eugenia astringens* Cambess was subjected to a series of phytochemical characterization reactions of: phenols and tannins (reaction with ferric chloride/Dynâmica brand and comparison with an authentic tannic acid standard/ Sigma Aldrich brand); catechins (reaction with hydrochloric acid/ Dynâmica brand and heating), flavonols, flavonones, flavanonols and xanthones (reaction with granulated magnesium/ Vetec brand with hydrochloric acid/ Dynâmica brand), anthraquinones (reaction with toluene/Synth brand and ammonium hydroxide/brand Vetec), anthocyanins, anthocyanidins and flavonoids (hydrochloric acid/Dynâmica brand; potassium hydroxide/ Vetec) and leucoanthocyanidins, catechins and flavonones (hydrochloric acid/ Dynâmica brand; sodium hydroxide/ Vetec brand and heating). Second, Mattos (1997) observes the final reaction characteristic of each class of secondary metabolites makes it possible to qualitatively predict the presence or absence of these metabolites in the extracts evaluated.

### *2.3 Toxicological test*

The cytotoxic activity was evaluated using the lethality test against the microcrustacean *Artemia salina* (Meyer *et al.*, 1982). In a 1,000 mL fish aquarium, a solution of artificial seawater (pH≅ 8.5) was prepared to which *A. salina* eggs were added for the larvae to hatch. Acclimation consisted of temperature control (25 ºC ± 2) with constant aeration and lighting for 48 hours. Then, 10 nauplii of *A. salina* were transferred using a Pasteur pipette to test tubes, containing the lyophilized aqueous extract in dilutions ranging from 10, 100 and 1000 μg $mL^{-1}$. After adding the extract and nauplii, the test tubes were filled with artificial sea water to a volume of 1 mL. The test was carried out in triplicate samples, with live and dead animals being counted after 24 hours,

to determine the median lethal dose ($LD_{50}$). As it is a crustacean active in saline water, lack of movement and sedimentation are indicators of death. The interpretation of the results was in $LD_{50}$ values with percentage, individual and concentration of the tested compound, using the PROBIT program to obtain $LD_{50}$ as analysis and respective confidence intervals $p < 0.5$. The $LD_{50}$ was calculated using the PROBIT analysis method, using statistical software with 95% confidence (Mello, 2008). According to Meyer's methodology (1982), extracts, fractions or substances are classified as toxic when the $LD_{50}$ value is less than 1000 µg mL-1 and non-toxic when the $LD_{50}$ is greater than 1000 µg $mL^{-1}$.

### *2.4. Antioxidant activity*

The ABTS method used was described by Re *et al.* (1999) and modified by Kuskoski *et al.* (2005). Absorbance was measured using a Hewlett-Packard 8452 A spectrophotometer, 7 minutes after adding the sample. After preparing the $ABTS^{\bullet+}$ radical (7 mM - 0.03836 g of ABTS reagent dissolved in 10 mL of deionized water), a solution of potassium persulfate (2.45 mM - 10 mL of ABTS (Sigma Aldrich, USA) was prepared ) and 10 mL of persulfate mixed, homogenized and kept in an amber bottle for a minimum of 16 hours protected from light). To test the sample, an aliquot of 200 µL of the formed radical was pipetted and diluted in 10 mL of 96° P.A. ethanol. Absorbance measurements were carried out to certify the optical density at around 0.700 ± 0.02 and the standard curve was prepared with 0.0101g of the water-soluble analogue of vitamin E, Trolox (6-hydroxy-2,5,7,8-tetramethylchrome-2-carboxylic acid; Sigma Aldrich, USA), dissolved in 0.02 L deionized water (2018 mM= 0.002 mol/ L). Trolox concentrations were used to construct a calibration curve. The lyophilized aqueous extract of *E. astringens* (0.1023 g) was homogenized in deionized water, stirred for 30 minutes in a vortex tube shaker at 3300 rpm. 4 test tubes were used. The blank was placed in the first tube (it does not contain the analyte to be measured), in the second the sample, and in the others the sample and the radical. The 10 mL automatic pipette was used for the sample and the 5 mL automatic pipette for the radical. The time for adding the sample and the radical was 6 minutes, with an interval of 30 seconds between the addition of one and the other. 5 mL of the radical was added to the test tubes. The sample was pink in color. The white tube showed a spectrometer reading of 705 nm, while the tubes with the sample showed a negative reading, requiring a new analysis. The sample reading on the UV spectrophotometer was negative because of the high antioxidant activity of the extract. It was necessary to dilute the sample (1 mL of sample) in deionized water (10 mL). The process of introducing the radical into the tube and reading it on the UV spectrophotometer was repeated. All analyzes were performed in triplicate and results expressed as mean ± standard deviation. Results with a coefficient of variation (CV) below 10% were accepted. Differences between means at the 5% level ($p < 0.05$) considered significant.

### *2.5 Mycobiological assay*

The culture medium was prepared with nutrient agar in a suspension ratio of 28 g to 1 L. The medium was autoclaved at a temperature of 121 °C for 15 minutes at 1 atm. Then, 50 mL of the medium, still hot, was poured into the petri dishes, with the aid of a 25 mL graduated pipette and automatic pipettor, inside the laminar flow hood. The plates were kept inside the laminar flow hood until the medium solidified. Samples of *Escherichia coli* bacteria types AB 1157 and BW9091 were taken separately from the refrigerated stock (Glycerol 15% in TSB Broth - Soy Trypitone; Himedia®®, M 011 - 500 G). They were then placed separately in test tubes with lids containing 3 mL of tryptica-sein soy broth. The tubes were closed, homogenized and incubated in a bacteriological oven (Solab, Model SL-101, Brazil) at 37 °C for 24 hours. The activated bacteria were sown in a 90 mm petri dish, with the nutrient agar medium, previously prepared and solidified. Sowing was carried out with the aid of the bacteriological loop, inside the laminar flow cabinet.

The seeded plates were incubated in a bacteriological greenhouse. After 24h, the colony forming units (CFU) samples were collected with the aid of the loop and inserted into the tube containing physiological solution (0.9%) comparing with the turbidity standard on the McFarlande scale of index 0.5 equivalent to 1, $5x10^8$ UFC. $mL^{-1}$. Then, solutions of hydrogen peroxide, 0.9% saline solution, aqueous extract (50 mg/ mL), stannous chloride (5 mg/ mL) and amoxicillin (50 mg/ mL) were prepared in eppendorfs. in a biological safety cabinet (Pachane - Model Pa 400 - ECO), inoculation was carried out with the aid of a sterile swab soaked in the bacterial solution. The seeding was carried out gently in the form of streaks on the surface of the agar in three different directions, so as to cover the entire surface of the plate. Plate preparation began with the aid of tweezers, distributing the antibiotic discs and impregnated paper discs over the surface of the inoculated medium. The solutions were pipetted onto the discs and the plates were incubated in an oven at 36.5 ºC for 24 hours. The petri dishes, measuring 150 mm, were divided into 4 groups, all in triplicate. There are 3 groups with 5 discs and one group with 3 discs, totaling 23 discs (Table 1). To read the results, a ruler was used to measure halos in the Laborclin brand antibiogram. Statistical analysis with the results obtained was carried out with the aid of GraphPad (GraphPad Software, Inc., United States) and Tukey's correlative test, in order to determine significant statistical differences ($p \leq 0.01$).

## 3. RESULTS AND DISCUSSION

The extraction yield and determination of the antioxidant activity of the extracts depend on the type of solvent, due to the difference in antioxidant potentials and the polarity of the compounds (Andreo; Jorge, 2006). The use of different solvents to extract antioxidants has been used by several authors (Machado Silva, 2020; Mattos, 2013; Haminiuk *et al.*, 2011) to determine the best solvent to

be applied to each plant matrix. The chemical nature of antioxidants varies from the simplest molecules in the plant kingdom to highly polarized ones, just as their concentrations vary from plant to plant (Andreo; Jorge, 2006). The literature states that Wu and collaborators (2004), when evaluating the polyphenol content of fruits consumed in the United States, for example, observed that the hydrophilic fraction had a much higher quantity of these constituents than the lipophilic portion. Vieira and collaborators (2011), testing the efficiency of the extraction solvent, found that pure water presented better extraction power for phenolic compounds when compared to the hydroalcoholic solution (19%). The results obtained in the series of phytochemical characterization reactions (Mattos, 2013) provided important preliminary information regarding the production profile of secondary metabolites produced by the leaves of *Eugenia astringens.* The results of the tests obtained in the aqueous extract of *Eugenia astringens* (Table 2) suggest a strong presence of phenolic compounds (tannins and flavonoids) and the absence of saponins and terpenoids. It is worth mentioning that previous studies have proven the presence of phenolic substances in representatives of the family (De Morais Rodrigues *et al*., 2016), in the genus *Eugenia* (Carvalho Junior *et al*., 2014) and in the species *E. astringens* (Leitão *et al*., 2014).

The toxicity was determined using the adapted Meyer (1982) methodology. This study found that the aqueous extract of *Eugenia astringens* leaves had a $LD_{50}$ of 32.3 µg. $mL^{-1}$, determined by linear regression analysis, with a correlation coefficient ($R^2$) of 0.9745 and the straight line equation y = 0.0125x + 77.5926 (Machado Silva, 2020). Meyer (1982) considers substances with $LD_{50}$ values below 1000 µg $mL^{-1}$ to be toxic. Amarante and collaborators consider both organic extracts and aqueous extracts to have non-toxic values, that is, with low toxicity when the $LD_{50}$ is greater than 500 µg/mL; moderate for $LD_{50}$ between 100 and 500 µg/ mL; and very toxic when the $LD_{50}$ is less than 100 µg/ mL. *Artemia salina* is a microcrustacean that has been widely used to express the toxicity of an extract (Ruiz *et al*., 2005). The *A. salina* Leach lethality assay was developed to detect active compounds in plant extracts, but it can be used to express the preliminary toxicity of an extract (Meyer *et al*., 1982). Several studies have demonstrated a correlation between toxicity on *A. salina* and antifungal, viruscidal, antimicrobial, parasiticidal, trypanocidal activities, among others (Pisutthanan *et al*., 2004). McLaughlin *et al*., (1993) used this bioassay in the preliminary evaluation of plant extracts known to be antitumor. They found that substances known to be antitumor showed a positive toxic correlation in the lethality assay in *A. salina* Leach. Arcanjo *et al*., (2012) for example, found that the ethanolic extract (98%) of *E. uniflora* leaves showed bioactivity against *A. salina* of 288.45 µg. $mL^{-1}$. The literature cites several uses of *Eugenia uniflora* L. in Brazilian folk medicine. Most of this biological activity is restricted to the aerial parts. The main biological activities found were anti-giardial (Brandelli *et al*., 2009), antimicrobial (Coelho-de-Souza *et al*., 2004), and hepatopancreas toxicity in fish (Fiuza *et al*., 2009). Abrantes (2017) cited that a 50% aqueous ethanol solution of *E. florida* DC. leaves. does not present bioactivity against *A. salina* at $LD_{50}$ of 2500 µg. $mL^{-1}$ (Abrantes, 2017).

It is likely that the minority concentration of phenolic compounds in this species contributed to the non-toxicity of the extract.

The results obtained in this work in relation to the antioxidant activity by the ABTS method were expressed as total antioxidant capacity equivalent to Trolox (TEAC). It was found from the results that the aqueous extract of *E. astringens* presents antioxidant activity, with a TEAC value of 3746.50 ± 210.71 µM trolox/g, determined by linear regression analysis with a correlation coefficient ($R^2$) equal to 0.9982, equation of the line y = 0.0002x + 0.0009, with a relative standard deviation of 5.62% between the measurements. It was then observed that this extract presented a high capacity to donate its electrons and act as excellent antioxidants. The ABTS method evaluates total antioxidant activity through the capture of the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical. This method can be used in water-soluble and fat-soluble samples and demonstrates excellent stability, being one of the fastest among the antioxidant activity tests and the one that offers the most reproducible results with several absorption maxima (Sucupira *et al*., 2012). De Souza (2013) evaluated the acidified ethanolic extracts extracted at high temperature from the leaves of the myrtaceae *Psidium guajavara* L. (guava), *Campomanesia xanthocarpa* O. Berg (guabiroba), *Eugenia uniflora* L. (pitanga), *Myrciaria cauliflora* (Mart.) O. Berg. (jabuticaba) and *Psidium cattleianum* Sabine (araça). All Myrtaceae showed antioxidant activity superior to that found in *Eugenia astringens.* The highest antioxidant activity among the leaves analyzed in this study was found in the guabiroba leaf (13,439.45 ∓ 2,750.86 µg trolox/g fresh weight), and the lowest value was observed in the araça leaves (5018.1 ∓ 425.82 µg trolox/g fresh weight). The solvent probably contributed to the extraction of these phenolic compounds. Silva (2011), working with guabiroba pulps (*Campomanesia xanthocarpa* O. Berg.) stored on different days, obtained 109.9 µM trolox/g of pulp in 30 days for the hydroalcoholic extract. Kuskoski (2003), evaluating the antioxidant activity of the baguaçu fruit (*Eugenia umbelliflora* Berg. = *Eugenia astringens* Cambess.) native to the state of Santa Catarina, Brazil, found a TEAC of 3.08 µM. $g^{-1}$ of fresh fruit and Sellappan; Akoh; Krewer (2002) found that the TEAC values of blueberry (*Vaccinium myrtillos* L.) and zarzamora (*Rubus fruticosus* L.) were 0.8 and 3.8 µM. $g^{-1}$ of fresh fruit, respectively. Fetter *et al*., (2009) working with pitanga fruits (*Eugenia uniflora* L.) at different stages of ripening, obtained antioxidant activity ranging from 4502.57±353.14 to 13668.41±200.87 µg trolox/g of fresh weight. It is worth mentioning that although the antioxidant activity of the aqueous extract of *Eugenia astringens* leaves is not superior to that found in other Myrtaceae (Colelho-de-Souza, 2013; Felter *et al*., 2009), it presents an antioxidant activity in the ABTS radical capture test, superior to some traditional fruits usually consumed by the population due to their antioxidant activity (Kuskoski, 2003).

The results of the disk diffusion tests for the *E. coli* BW9091 and AB1157 strains were obtained from calculations of the average diameters of the halos formed (Table 1). No inhibition halo was observed around the impregnated disks for any of the concentrations of the crude extract of

*E. astringens* tested (Table 1). Holetz *et al.* (2002) observed that the hydroalcoholic extract of the leaves of *Eugenia uniflora* showed moderate antimicrobial action against the bacteria *Staphylococcus aureus* and *Escherichia coli*, also evidenced by De Queiroz *et al.* (2015), who obtained insufficient activity, in popular bottled preparations of *Eugenia florida* against *Escherichia coli*. The crude extract of *Eugenia uniflora* also did not show antimicrobial action against *Escherichia coli* in the studies carried out by Coelho-de-Souza *et al.*, (2004). The results of this study demonstrate that there was a difference in the formation of the inhibition halo of the strain when associated with the extract and the antibiotic Norfloxacin (Table 1). It is worth remembering that the *E. coli* AB1157 strain is wild-type and presents all efficient DNA repair genetic mechanisms. *E. coli* BW 9091 is a mutant of the xthA gene, whose product, exonuclease III, acts in the repair of oxidative DNA lesions in the exponential growth phase. It was observed that for the *E. coli* AB1157 strain, the mean of the association of the extract with the antibiotic Norfloxacin was slightly higher than the mean of the isolated antibiotic.

**Table 1.** Scheme of plates and discs and average halo size of strains *Escherichia coli* BW9091 e AB1157. Sodium chloride (NaCl), stannous chloride ($SnCl_2$) and hydrogen peroxide, ($H_2O_2$).

| Circuit Board and Disk Diagram | | | Average of the halos of the strains (mm) ± Standard Deviatio | |
|---|---|---|---|---|
| **Plates** | **Disc** | **Solution** | ***E. coli* BW9091** | ***E. coli* AB1157** |
| **1** | 1 | 24mL de NaCl 0,9 | 0,00 ± 0,0 | 0 ± 0,0 |
| | 2 | 24µL of extract | 0,00 ± 0,0 | 0 ± 0,0 |
| | 3 | 18mL of extract | 0,00 ± 0,0 | 0 ± 0,0 |
| | 4 | 12mL de amoxicillin | 39,33 ± 1,5 | 43,3 ± 2,8 |
| | 5 | 12mL de amoxicillin + 12mL of extract | 40,67 ± 1,1 | 49 ± 1,7 |
| **Plates** | **Disc** | **Solution** | | |
| **2** | 6 | Chloramphenicol | 27,33 ± 2,3 | 37,5 ± 2,5 |
| | 7 | Chloramphenicol* + 12mL of extract | 28 ± 3,4 | 36,6 ± 2,8 |
| | 8 | 24mL de amoxicillin | 36,67 ± 3,0 | 54,3 ± 5,1 |
| | 9 | Norfloxacin | 21 ± 1,0 | 16 ± 5,9 |
| | 10 | Norfloxacin + 12mL of extract | 15 ± 2,0 | 16,6 ± 10,5 |
| **Plates** | **Disc** | **Solution** | | |
| **3** | 11 | Ampicillin | 22,33 ± 4,6 | 40,6 ± 1,1 |
| | 12 | Ampicillin + 12mL of extract | 25 ± 0,0 | 38,3 ± 1,5 |
| | 13 | 24mL L of $SnCl_2$ | 18,67 ± 1,1 | 14 ± 2,0 |
| | 14 | 12mL of $SnCl_2$ | 17,33 ± 2,5 | 11 ± 1,0 |
| | 15 | 8mL of $SnCl_2$ | 10,67 ± 1,1 | 8 ± 1,0 |
| **Plates** | **Disc** | **Solution** | | |
| **4** | 16 | 12mL $SnCl_2$+ 12 mL of extract | 16,00 ± 5,2 | 11,6 ± 0,5 |
| | 17 | 8 mL of extract | 0 ± 0,0 | 0 ± 0,0 |
| | 18 | 12 mL of $H_2O_2$ + 12mL of extract | 34,67 ± 5,0 | 53,3 ± 2,8 |
| | 19 | 12mL of $H_2O_2$ + 12mL $SnCl_2$ | 26,00 ± 6,0 | 50 ± 1,0 |
| | 20 | 8mL of $H_2O_2$ + 8 mL $SnCl_2$+ 8mL of extract | 25,33 ± 2,3 | 45,3 ± 5,0 |
| **Plates** | **Disc** | **Solution** | | |
| **5** | 21 | 8mL of $H_2O_2$ | 23,33 ± 2,0 | 45,3 ± 5,0 |
| | 22 | 12mL of $H_2O_2$ | 28,67 ± 0,5 | 54 ± 6,5 |
| | 23 | 24mL of $H_2O_2$ | 50,00 ± 0,0 | 66,6 ± 5,7 |

For the *E. coli* BW9091 strain, the mean of the association of the extract with Norfloxacin was lower than that of the isolated antibiotic. Norfloxacin is an antibiotic with a broad spectrum of antibacterial activity against Gram-positive and Gram-negative aerobic pathogens, indicated for the treatment of urinary tract infections, gastroenteritis, gonorrhea and typhoid fever (Ferreira *et al.*, 2016). The literature states (Ferreira *et al.*, 2016) that the fluorine atom in position 6 of this antibiotic provides greater potency against Gram-negative organisms and the piperazine nucleus in position 7 is responsible for the antipseudomonas activity. It inhibits the synthesis of bacterial deoxyribonucleic acid and is bactericidal. Three specific events are attributed to norfloxacin in *Escherichia coli* cells at the molecular level (Ferreira *et al.*, 2016). They are: inhibition of DNA gyrase, which catalyzes the ATP-dependent DNA supercoiling reaction; inhibition of the relaxation of supercoiled DNA; and promotion of double-stranded DNA breakage. From these results, it is possible to speculate that these differences found in the mean inhibition zone in the *E. coli* AB 1157 and BW9091 strains in relation to Norfloxacin (Table 2) are in agreement with their enzymatic deficiencies in DNA repair. However, these differences were not significant for all associations with antibiotics including Norfloxacin, according to the experimental data analyzed by the Tukey test (Table 2). An increase in the highest mean inhibition was observed in the association of the aqueous extract of *E. astringens* Cambess. with 12mL of amoxicillin in both *E. coli* BW9091 and *E. coli* AB1157 (Table 1). However, all these differences were not significant according to the Tukey-Kramer test ($p > 0.05$) (Table 2). Although in both strains an average increase in halo inhibition can be seen in the association between amoxicillin and the extract (Table 2), it cannot be stated that the extract potentiates amoxicillin. Table 3 represents the calculation of the mean inhibition halos of the aqueous extract of *E. astringens* in association with oxidizing agents [stannous chloride ($SnCl_2$) and hydrogen peroxide, ($H_2O_2$)] in the strains *E. coli* AB1157 and BW9091. The tests with the antioxidant agents ($H_2O_2$ and $SnCl_2$) revealed, in the mutant strain, *E. coli* BW 9091, that the aqueous extract inhibited the oxidative capacity of stannous chloride (Table 3). However, it increased the oxidative capacity of hydrogen peroxide (Table 3). The association of the extract, stannous chloride and oxygen peroxide did not promote an increase in the mean when compared with either the isolated stannous chloride or the isolated hydrogen peroxide (Table 3). This difference in the mean becomes greater when the comparison is made with the isolated stannous chloride ($SnCl_2$) versus the association with extract and hydrogen peroxide, ($H_2O_2$) presenting a value of $p>0.001$ (Table 3).

**Table 2 -** Comparison and difference between the halos obtained in the *Escherichia coli* strains BW9091 and AB1157. Tukey-Kramer test and determination of significance.

| Comparison between: Solution - Solution | *E. coli* BW9091 | | *E. coli* AB1157 | |
|---|---|---|---|---|
| | Difference between the Halos (mm) | Tukey--Kramer (p) | Difference between the Halos (mm) | Tukey--Kramer (p) |
| 12mL of amoxicillin + 12mL of extract - 12mL of amoxicillin | 1,33 | p > 0,05 | 5,66 | p > 0,05 |
| Chloramphenicol + 12mL of extract - Chloramphenicol | 0,66 | p > 0,05 | 0,83 | p > 0,05 |
| Norfloxacin + 12mL of extract - Norfloxacin | 6,00 | p > 0,05 | 0,66 | p > 0,05 |
| Ampicillin + 12mL of extract - 12mL of Ampicillin | 2,66 | p > 0,05 | 2,33 | p > 0,05 |

**Table 3 -** Comparison and difference between the halos obtained obtained in the *Escherichia coli* strains BW9091 and AB1157 when subjected to oxidizing agents ($SnCl_2$ and $H_2O_2$). Tukey-Kramer test and determination of significance.

| Comparison between: Solution - Solution | *E. coli* BW9091 | | *E. coli* AB1157 | |
|---|---|---|---|---|
| | Difference between the halos (mm) | Tukey--Kramer (p) | Difference between the halos (mm) | Tukey--Kramer (p) |
| 12mL of $SnCl_2$+ 12mL of f extract - 12mL of $SnCl_2$ | 1,33 | p > 0,05 | 0,66 | p > 0,05 |
| 8mL of $H_2O_2$ + 8mL of $SnCl_2$ + 8mL of extract - 8mL of $SnCl_2$ | 14,66 | p <0,001 | 42,0 | p <0,001 |
| 8mL of $H_2O$ + 8mL of $SnCl_2$ + 8mL of f extract - 8mL of $H_2O_2$ | 2,00 | p > 0,05 | 4,70 | p > 0,05 |
| 12mL of $H_2O_2$ + 12mL of extract - 12mL of $H_2O_2$ | 6,00 | p > 0,05 | 6,67 | p > 0,05 |

Therefore, these differences are significant. The cytotoxic and genotoxic effects of the oxidizing agent $SnCl_2$ have been demonstrated in different experimental models and these appear to be mediated by free radicals (El-Demerdash; Yousef; Zoheir, 2005). Moreno *et al.* (2004) reported that a *Ginkgo biloba* L. extract was able to protect plasmid DNA from lesions induced by $SnCl_2$. According to the literature, stannous chloride causes lesions, mediated by the production of reactive oxygen species, both in vivo and in vitro; the damage induced by $SnCl_2$ causes a decrease in the transforming capacity of the plasmid pUC9.1. The number of lesions caused to the DNA is directly proportional to the incubation time with $SnCl_2$. In addition, the stannous ion is able to associate with the DNA molecule, inducing the generation of reactive oxygen species near the binding site, promoting modifications in the structure of the macromolecule; This association appears to lead to a preferential attack on nitrogenous bases, a fact that could be associated with the mutagenic potential of tin (El-Demerdash; Yousef; Zoheir, 2005). *E. coli* BW9091 does not produce exonuclease III, but it does produce endonuclease IV. Friedberg *et al.*, (1995) observed that endonuclease IV, a product of the nfo gene, is more important than exonuclease III, a product of the xthA gene, in the repair of AP sites (AP endonucleases - endonucleases that recognize apurinic or apyrimidine sites) generated during the repair of lesions induced by $SnCl_2$. This statement is supported by the fact that the *E. coli* BW9091 strain was not sensitive to the treatment performed, a fact that can be explained by the presence of the functional endonuclease IV protein. Hydrogen peroxide, in turn, is not considered an oxidizing agent; however, it easily diffuses through membranes and is capable of inducing damage

to DNA molecules through enzymatic reactions (Liochev, 2013) and the release of free radicals, such as OH. The hydroxyl radical ($OH^{\bullet}$) is the most reactive and non-selective in nature, thus it is a reactive product and, via the Haber Weis reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + HO^{-}$) or Fenton reaction ($O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + OH^{\cdot} + OH^{-}$), it can serve as an important precursor of $HO^{-}$. (Asghar, Raman, Daud, 2015). OH- has the ability to inactivate several proteins, including those in membranes, and is also related to the lipid peroxidation process (Ferreira; Matsubara, 1997). It is possible that the free radicals generated by hydrogen peroxide have decreased the concentration of flavonoids and tannins in the *E. astringens* extract through Haber Weis and Fenton reactions. Friedberg *et al.* (1995) found that xthA mutant bacteria are sensitive to oxidative lesions produced by agents such as UVA and UVB radiation and $H_2O_2$, suggesting that this enzyme participates in the repair of oxidative lesions. This evidence was corroborated by the demonstration that exonuclease III removes thymine fragmentation products, such as urea residues. The wild-type strain, *E. coli* AB1157, is proficient in all DNA repair mechanisms. Therefore, this strain is resistant to the oxidizing agents stannous chloride and hydrogen peroxide (Friedberg *et al.*, 1995). We observed that the oxidative capacity of *E. coli* was increased when associated with stannous chloride and oxygen peroxide (Table 1 an 3). Interestingly, when we compared the action of the extract with stannous chloride, we did not observe significant differences (Table 1 and 3). The comparison of stannous chloride associated with hydrogen peroxide and the extract showed significant differences. This leads us to believe that the oxidative capacity of the *E. astringens* extract was promoted by the change in the concentration and/ or chemical nature of the compounds present in the extracts, which consequently enhanced the loss of resistance of the strain to the action of these reagents (*E. coli* AB1157; Table 3).

## 4 CONCLUSION

The preliminary phytochemical analysis of the aqueous extract obtained from the leaves of *Eugenia astringens* obtained a positive result for the presence of polyphenolic compounds, among which tannins stand out, which have several pharmacological activities already described in the literature. The bioassay with *Artemia salina* revealed that the aqueous extract of the leaf of Eugenia astringens presents high toxicity with LD50 of 32.3 µg mL-*1* determined by linear regression analysis, with a correlation coefficient ($R^2$) equal to 0.9745 and equation of the line y = 0.0125x+77.5926. The aqueous extract of *Eugenia astringens* showed antioxidant activity, with a TEAC value of 3746.50 ± 210.71 µM trolox/g, determined by linear regression analysis with a correlation coefficient ($R^2$) equal to 0.9982, equation of the line y = 0.0002x + 0.0009 with a relative standard deviation of 5.62% between the measurements. The extract did not exhibit microbicidal action. The associations of the extract with antibiotics revealed an increase in the size of the halos and differences in these in the strains *E. coli* AB 1157 and BW9091. However, these differences were not significant according to

the experimental data analyzed by the Tukey test. The aqueous extract in *E. coli* BW 9091 inhibited the oxidative capacity of stannous chloride and increased the oxidative capacity of hydrogen peroxide. As for *E. coli* AB 11577, the extract promoted an increase in oxidative capacity when associated with stannous chloride and oxygen peroxide.

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