

## Evaluation preliminary phytochemistry and antimicrobial activity of extracts in *Eugenia uniflora* L. on *Escherichia coli* and *Staphylococcus aureus*

Avaliação fitoquímica preliminar e atividade antimicrobiana de extratos de *Eugenia uniflora* L. sobre *Escherichia coli* e *Staphylococcus aureus*

Evaluación preliminar de la fitoquímica y la actividad antimicrobiana de extractos en *Eugenia uniflora* L. sobre *Escherichia coli* y *Staphylococcus aureus*

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### Abstract

Due to the increased resistance of human and animal pathogens to current drugs, research for new antimicrobials has been encouraged, mainly through medicinal plants. *Eugenia uniflora* L., known as pitangueira, is popularly used with several therapeutic functions, justifying studies on this species. Thus, the objectives of this study were to evaluate the “in vitro” antibacterial activity of *Eugenia uniflora* L. leaf and bark extracts on *Escherichia coli* and *Staphylococcus aureus*. For the stages of phytochemical screening and microbiology, the usual methodologies in these areas were used to obtain the extracts and to evaluate the antibacterial activity with the use of positive and negative control. The results obtained showed that in the extract of the leaves, the presence of saponins, flavonoids, alkaloids and tannins was verified, while in the bark, only saponins and alkaloids were obtained. Leaf and bark extracts were able to inhibit the growth of *Staphylococcus aureus* only, promoting medium inhibition halos of 19.4 mm and 14.4 mm, respectively. The analyzes allowed to conclude that the extracts present interesting secondary metabolites and of great antimicrobial potential and that these extracts were able to inhibit only the strains of *S. aureus*, being resistant to the strains of *E. coli*.

**Keywords:** Bacterial activity; Natural products; Microorganisms.

### Resumo

Devido ao aumento da resistência de patógenos humanos e animais aos medicamentos atuais, pesquisas por novos antimicrobianos têm sido incentivadas, principalmente por meio de plantas medicinais. *Eugenia uniflora* L., conhecida como pitangueira, é popularmente utilizada com diversas funções terapêuticas, justificando estudos nesta espécie. Assim, os objetivos deste estudo foram avaliar a atividade antibacteriana “in vitro” de extratos de folhas e cascas de *Eugenia uniflora* L. sobre *Escherichia coli* e *Staphylococcus aureus*. Para as etapas de triagem fitoquímica e microbiologia, foram utilizadas as metodologias usuais nessas áreas para obtenção dos extratos e avaliação da atividade antibacteriana com uso de controle positivo e negativo. Os resultados obtidos mostraram que no extrato das folhas foi verificada a presença de saponinas, flavonóides, alcalóides e taninos, enquanto na casca foram obtidos apenas saponinas e alcalóides. Os extratos da folha e da casca foram capazes de inibir o crescimento de *Staphylococcus aureus* apenas, promovendo halos de inibição de meio de 19,4 mm e 14,4 mm, respectivamente. As análises permitiram concluir que os extratos apresentam metabólitos secundários interessantes e de grande potencial antimicrobiano e que esses extratos foram capazes de inibir apenas as cepas de *S. aureus*, sendo resistentes às cepas de *E. Coli*.

**Palavras-chave:** Atividade bacteriana; Produtos naturais; Microorganismos.

## Resumen

Debido a la mayor resistencia de los patógenos humanos y animales a los fármacos actuales, se ha fomentado la investigación de nuevos antimicrobianos, principalmente a través de plantas medicinales. *Eugenia uniflora* L., conocida como pitangueira, se utiliza popularmente con varias funciones terapéuticas, lo que justifica los estudios en esta especie. Así, los objetivos de este estudio fueron evaluar la actividad antibacteriana “in vitro” de extractos de hojas y corteza de *Eugenia uniflora* L. sobre *Escherichia coli* y *Staphylococcus aureus*. Para las etapas de cribado fitoquímico y microbiológico, se utilizaron las metodologías habituales en estas áreas para obtener extractos y evaluar la actividad antibacteriana utilizando controles positivos y negativos. Los resultados obtenidos mostraron que en el extracto de hoja se verificó la presencia de saponinas, flavonoides, alcaloides y taninos, mientras que en la corteza solo se obtuvieron saponinas y alcaloides. Los extractos de hojas y corteza fueron capaces de inhibir el crecimiento de *Staphylococcus aureus* únicamente, promoviendo halos de inhibición media de 19,4 mm y 14,4 mm, respectivamente. Los análisis permitieron concluir que los extractos presentan metabolitos secundarios interesantes y de gran potencial antimicrobiano y que estos extractos fueron capaces de inhibir solo las cepas de *S. aureus*, siendo resistentes a las cepas de *E. Coli*.

**Palabras clave:** Actividad bacteriana; Productos naturales; Microorganismos.

## 1. Introduction

The resistance of human and animal pathogens to drugs is one of the most documented occurrences of biological evolution, and a serious problem that affects both developed and developing countries. The use of more than a ton of antibiotics a day in some countries, has generated resistance in the colonies of bacteria, thereby causing a serious public health problem (Duarte (2006).

Considering this problem, a current plan in the treatment of diseases caused by bacteria has been seeking for possibilities that aim to reduce damage to health and obtain greater activity against infectious processes, since some conventional antibiotics used in treatments have lost their effectiveness (Vizzoto et.al.2010).

An alternative would be to look for new antibiotics from plants, many of which have antimicrobial properties. Numerous researches are being developed aimed at the discovery of new antimicrobial agents derived from plant extracts and other natural products, with the purpose of discovering compounds that have pharmacological activities compared to the traditionally used drugs, however these have greater efficacy against the resistance of pathogenic microorganisms, less toxicity and environmental impact (Brasil, 2018).

Antimicrobials are substances (natural, synthetic or semi-synthetic), which inhibit growth or destroy microorganisms, generating little or no damage to the host. They are classified as bacteriostatic (inhibit the growth of bacteria) and bactericidal (causing the death of the pathogen) (Brasil 2014), Tortora(2005).

Bacterial resistance is a phenomenon of natural evolution, where bacteria undergo spontaneous mutations or the acquisition of the gene by another microorganism through transformation and transfer of plasmids and this way they are able to interfere in a certain antibiotic mechanism of action. The survival and multiplication of bacteria with a new genotype can be benefited by natural and human-induced environments, which generate a great diversity of microorganisms Tomás-Menor et al. (2015).

Several factors contribute to this resistance to happen with the indiscriminate use of antibiotics, difficulties to differentiate clinically infections caused by viruses and bacteria, prescription without criteria, lack of control in the sale of these drugs, incomplete treatments, pressure from relatives for the prescription of antibiotics, false belief that the prophylactic use of these drugs could prevent the occurrence of complications, and lack of information about the possible adverse effects associated with the inappropriate use of antibiotics WHO. (2015)

The World Health Organization (WHO) evaluated six world regions where it was revealed that *Staphylococcus aureus* showed resistance to methicillin (beta-lactam antimicrobial belonging to the penicillin group), while the bacterium *Escherichia coli* demonstrated resistance to third generation cephalosporins and fluoroquinolones (antimicrobial used in the treatment of

urinary tract infections), 90% of cases in the American region, 60% in the European region, 80% of the cases in the African and Western Pacific regions, 50 % in the Eastern Mediterranean region, and 25% in Southeast Asia ( Fracarolli et al.. 2017).

This means that, standard treatments become ineffective and infections caused by resistant bacteria become more persistent, which increases the chances of spreading and causing the death of several people .(Oliveira, Paula ,Rocha (2015). In addition to causing an increase in the length of hospital stay of these patients, and consequently increase spending on more expensive and powerful antimicrobials, medical consultations, expenses with laboratory tests and costs with disinfections. Another negative point is the increased delay in seeing other patients, due to occupied beds (O'NEILL.2016).

It should also be noted that if nothing is done to decrease bacterial resistance by 2050, it will be the leading cause of death in the world, overcoming traffic accidents, cancers and myocardial infarctions. Currently, around 700,000 people die from resistant infections every year in the world, and if there is no control over the antibiotics use, according to the study's projection, around 10 million people will die from bacterial resistance throughout the year (Tavares 2018).

Thus, preventive measures and the rational use of medicines have been incorporated worldwide, in an attempt to reduce this resistance. In Brazil, the National Health Surveillance Agency (ANVISA), has been creating, since its creation, several actions related to antibiotic resistance, among which stand out: regulation of the antimicrobials sale control, prevention and control of infections and resistance in health services, establishment of limits for residues of veterinary medicines in food, verification of the presence of resistant microorganisms in specific foods and new resistance mechanisms and multi-resistant microorganisms in health services (Rozatto 2012).

Plants are considered to be a relevant source of biologically active natural products, in which many medicines have been originated and marketed worldwide. Reports by researchers in the field of natural products reveal a wide variety of structural forms, biological activities and physicochemical properties of these plants. Despite this, available data show that only 15% to 17% of vegetables have been analyzed for their therapeutic potential (Bell 2014), Silva, N.C.C. (2010).

The active compounds present in medicinal plants are produced during the secondary metabolism, and have several pharmacological properties, highlighting here the antimicrobial action, which has been the focus of numerous studies in the world, because of bacterial resistance to antibiotics. Therefore, researches are justified in order to obtain a better knowledge about the properties of plants, even those that have already had their antimicrobial action proven (Madigan 2016) .

From the last two decades, the phytotherapics consumption (medicine obtained from active vegetable raw material, with proof of its safety and effectiveness based on clinical evidence and showing regularity in its quality) has increased a lot worldwide, and, with this, investments increased in research in the search for new herbal medicines, giving greater credibility to the phytotherapy use (Santos 2016), (Rozato2012).

Finally, the proper use of this practice in the treatment of diseases, based on active compounds that are extracted from different parts of the plant, such as leaves, flowers, fruits, roots, stems and barks, are fundamental to restore the physiological functions of the organism (Saad, 2016).

The plants have developed defense mechanisms throughout their natural evolution process. Several authors point out that, one of the ways they deal with stress conditions is through substances that provide, in some way, to overcome the challenges. Among these compounds are the secondary metabolites (Monteiro et.al.2005)

Although these secondary metabolites are not fundamental for the development and growth of plants, they guarantee certain advantages for the survival and adaptation of plants in the environment, such as, for example, protect against ultraviolet (UV) radiation, herbivores attacks, bacteria, fungi, and help to attract pollinators and other animals that are seed dispersers, thus allowing the perpetuation of their species (Auricchio 2005).

Several secondary metabolites have diverse biological activities which are striking. They arouse much interest, not only for their biological actions generated by plants in response to stimuli to the environment, but also for their great pharmacological activity (Oliveira et.al.2008).

Among medicinal plants, the species *Eugenia uniflora L.*, popularly known as pitangueira belonging to the Myrtaceae family, is a plant that has edible fruits and is recognized and appreciated throughout Brazil. Its leaf tea is used in folk medicine as stoma, hypoglycemic, hypotensive and anti gout disease (Rodrigues et al.2018). Furthermore, in the literature, there is a large number of studies that have proven the antimicrobial action of pitanga on various pathogenic microorganisms (Leite et.al.2014).

Thus, this work presents a study on the antimicrobial potential and the preliminary phytochemical profile of the extracts of the leaves and barks of the species of *Eugenia uniflora L*

## **2. Methodology**

### **2.1 Collection of botanical material**

The leaves and bark of pitangueira (*Eugenia uniflora L.*) were collected in October, 2019, on a rural property in Cataguases, State of Minas Gerais. The samples were taken to the laboratory of UNIFAMINAS University Center, in Muriaé campus, and submitted to identification by the specialist, Dr. Braz Antônio Pereira Cosenza and the testimony material was made.

### **2.2 Preparation of extracts**

The extracts were prepared based on the usual methodology in phytochemical screening with modifications (CLISI 2012). The leaves and bark of pitangueira were dried in an oven at a 23° C temperature for 7 days, and later the dry material was grinded separately (leaf and peel) in a blender and standardized with the aid of meshes to obtain a fine powder, being stored in a beaker for later use.

### **2.3 Microbiological analysis**

Microbiological tests were prepared based on the methodology de Castro (2004), Cavalcante (2019) with modifications. The antimicrobial activity of the extracts from the peels and leaves of pitangueira were carried out using the agar diffusion method by the paper disc technique. The standard strains selected for the study were *Staphylococcus aureus* (ATCC BAA-97) and *Escherichia coli* (ATCC 35218).

In a separate plate, antibiotic disks of 10µg of Norfloxacin, 10 µg of Ciprofloxacino and 10 µg of Amoxicillin as a positive control, and on another plate it was made the negative control, where a disk impregnated with 20 µL sterile distilled water was put, then the plates were incubated at 37° C for 24 hours and later they were analyzed. The tests were performed in triplicate for each selected strain.

The measurements of the halos promoted by the extracts were performed with the aid of a caliper and the final result was determined by the arithmetic mean of the size of the halos (mm) found.

## 2.4 Phytochemical Screening

Phytochemical tests were performed according to the methodology of Tomita (2014) with some adaptations. The extracts of leaves and bark of *Eugenia uniflora* L. were subjected to analysis for the detection of the main classes of secondary metabolites based on specific chemical reactions which generate colorings or precipitate that indicate their presence.

These tests aim to qualitatively characterize the presence of saponins, flavonoids, alkaloids, tannins, coumarins and polysaccharides.

## 3. Results

The characterization of the main secondary metabolites of the vegetable was carried out through color reactions and characteristic precipitation. The preliminary study of the chemical constituents of extracts of leaves and bark of pitanga (*Eugenia uniflora* L.) allowed the identification of the following secondary metabolites, shown in Table 1.

**Table 1** - Result of the phytochemical screening of *Eugenia uniflora* L. leaf and bark extracts

Class of secondary metabolites	leaf	Bark
Saponins	(+)	(+)
Alkaloids	(+)	(+)
Flavonoids	(+)	(-)
Tannins	(+)	(-)
Coumarinas	(-)	(-)
Polysaccharide	(-)	(-)

Legend: (-) Absent (+) Present Source: Authors.

In determining the phytochemical profile of *Eugenia uniflora*, the tests demonstrate the presence of saponins in both extracts. The formation of a persistent foam layer in (Tube A) and (Tube A<sub>1</sub>) in Figures 1 and 2 respectively, suggests the presence of this component. The foaming effect occurs due to the emulsifying properties of this compound, which has an amphiphilic character, that is, it presents a hydrophobic part (sapogenin or aglycone) and a hydrophilic part (sugars).

The presence of alkaloids was also identified in the two plant extracts (Tube C) and (Tube C<sub>3</sub>) due to the appearance of a reddish orange precipitate (Figures 1 and 2).

While in the flavonoid detection test (Tube B), only the extracts of the pitanga leaves demonstrated this component, being evidenced by the pink coloration as shown in Figure 1.

Tannins were also identified only in the leaves of the pitanga, through the formation of a greenish black color (Tube D), as shown in figure 1. This reaction occurs by the addition of ferric chloride, which causes a color change due to the ability to oxidize with the hydroxyl groups present in the ring aromatic of the tannins, causing the solution to darken, leaving the color strongly greenish

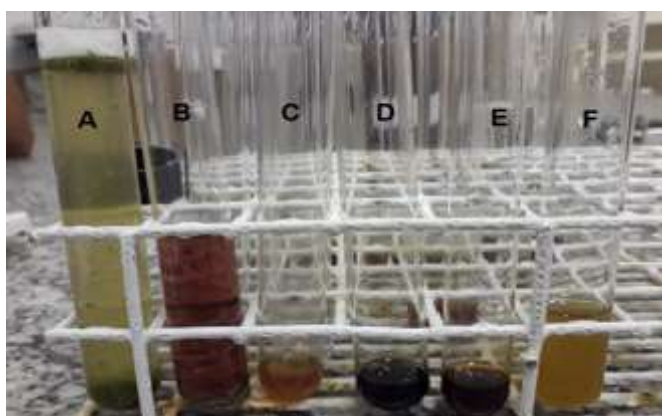
The test was negative for coumarin and polysaccharide in both extracts, since in the first there was no yellow color (Tube E) and (Tube E<sub>5</sub>) and in the second it did not show blue color (Tube F) and (Tube F<sub>6</sub>), as shown in figures 1 and 2 respectively.

**Figure 1** - Result of the phytochemical prospecting of the leaf *Eugenia uniflora* L. Positive test for (A) Saponin, (B) Flavonoid, (C) Alkaloid, (D) Tannins and negatives for (E) Coumarin and (F) Polysaccharide.



Source: Authors.

**Figure 2** - Result of phytochemical prospecting of bark *Eugenia uniflora* L.- Result of phytochemical prospecting of bark *Eugenia uniflora* L. Positive test for (A1) Saponins and (C3) Alkaloids and negatives for (B2) Flavonoids, (D4) Tannins, (E5) Coumarins and (F6) Polysaccharides.



Source: Authors.

It can be seen in Table 2 above and in Figure 3 (A and B) that the averages of the halos of the leaf extracts obtained zones of inhibition greater than the halos of the extracts of the bark. *Eugenia uniflora* L. on *Staphylococcus bacteria aureus*, having extract of the leaves of pitanga, halos with an average diameter of 19.4 mm, already for the bark, obtained halos of 14.4 mm. A probable justification for this has occurred due to the fact that more compounds of antimicrobial activity (saponins, flavonoids, alkaloids and tannins) were found in the leaves than in the bark (saponins and alkaloids) in the phytochemical screening of this study. *Eugenia uniflora* L.

**Table 2** - Result of the phytochemical screening of *Eugenia uniflora* L. leaf and bark extracts.

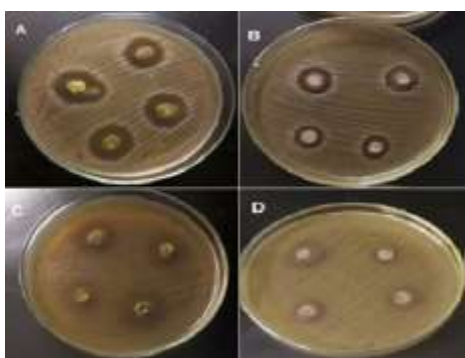
Class of secondary metabolites	leaf	Bark
Saponins	(+)	(+)
Alkaloids	(+)	(+)
Flavonoids	(+)	(-)
Tannins	(+)	(-)
Coumarinas	(-)	(-)
Polysaccharide	(-)	(-)

Legend: (-) Absent (+) Present Source: Authors.

When analyzing the response of that same extract of leaves and bark of pitanga against strains of *Escherichia coli*, as shown in Figure 3 (C and D), it was found that this strain was resistant to the two extracts, with no inhibition halos.

Therefore, it can be seen that the studied extracts only obtained antimicrobial activity on *Staphylococcus aureus*, being resistant to strains *Escherichia coli* as shown in Figure 3.

**Figure 3** - Halo inhibition of extracts of leaves and bark of *Eugenia uniflora* L. about *Staphylococcus aureus* and *Escherichia coli*. Extract of leaves (A) and bark (B) on *Staphylococcus aureus* and extracts of leaves (C) and bark (D) on *Escherichia coli*.



Source: Authors.

When analyzing the inhibitory results found in this study with the literature, it can be seen that the analyzed plant has different responses in relation to its antimicrobial action.

*Staphylococcus aureus* and *Escherichia coli* analyzed have sensitivity to the action of the antibiotics of Norfloxacin and Ciprofloxacin references.

Table 3 shows the diameter of the antibiotic inhibition halos compared to that of the extracts, as well as the use of the negative control to confirm the bactericidal activity in a comparison of the extract activity against the commercialized antibiotics.

#### 4. Discussion

The qualitative presence of secondary metabolites can be seen according to the use of preliminary phytochemical analysis. Saponins, identified own in high water solubility, high molecular weight and complexing capacity, being an important pharmacological agent to be used as anti-inflammatory, antifungal, antimicrobial, expectorant and diuretics Martinez (2005).

Analyzing the presence of flavonoids, in their this Bouchardat analytical test occurs the formation of an insoluble (precipitated) complex due to the presence of metals (Lameira et.al, 2003). Although its mechanism is not clearly elucidated, states that this is due to a reduction caused by magnesium in contact with acid, which changes the color of the flavonoid compound. (Fiuza et.al, 2008)

For tannins, and this reaction occurs by the addition of ferric chloride, which causes a color change due to the ability to oxidize with the hydroxyl groups present in the aromatic ring of the tannins, causing the solution to darken, leaving the color strongly greenish (Oliveira et al, 2008).

The result of phytochemical tests for leaf extracts *Eugenia uniflora L.* in this study, they corroborate the researches of that report the presence of saponins, flavonoids, alkaloids and tannins, as well as the absence of polysaccharides, presenting the same results found in our research.(Scur et al.2008)

The presence of saponins, flavonoids and tannins in the phytochemical characterization of pitanga leaf extracts, was reported in research by [31, 32] but did not find the presence of alkaloids and coumarin, unlike this research that found alkaloids.(Abrantes, 2017, Lorenzoni, 2013) It is also reported] the presence of tannins and saponins, however they did not detect flavonoids and alkaloids in their research, while in this study the presence of these two chemical constituents was found ( Bouzada, 2009).

While researching analyzing secondary metabolites present in the extract of pitanga leaves, they identified the presence of flavonoids and tannins (Castro et al. 2019, Silva & Lima, 2016).

In its phytochemical screening [36], it detected the presence of saponin, flavonoids and tannins, different from the studies of which did not detect saponins and flavonoids, only tannins( Michelin et al. 2005, Trugilo et al.2003).

The peridermis is a very important part of the plant, as it has the function of protecting the trunk of the plant against the invasion of pests, being considered a physical barrier. The bark has chemical constituents inside it that act as bactericides, insecticides and fungicides, preventing microorganisms or pests from proliferating in the changes of plants (Ferreira, 2014).

The bark has greater amounts of tannins, as it is the place where the greatest problems with injuries and invasions of xylophagous agents occur in the wood, this metabolic being used as protection. However, in the phytochemical screening of this research, this secondary metabolite was not found (Alvarenda et al. 2017).

The negative result does not rule out the possibility of obtaining the presence of this secondary metabolite, as many times the methodology used was not able to detect the chemical substances or the quantity of these compounds was reduced in the extract (Leite et al.2014).

Research by highlights having found in the bark of *Eugenia calycina*, same gender and family as *Eugenia unifloraL.* the presence of saponins, flavonoids and tannins, and absence of coumarins and alkaloids. These results differ from this research in that no flavonoids and tannins were found, with only the presence of alkaloids and saponins being detected.(Gobbo-Neto & Lopes, 2007).

In a phytochemical analysis of the stem bark of *Psidium cattleianum*, belonging to the family Myrtaceae, same family of *Eugenia uniflora* it was found the presence of saponins, flavonoids and tannins, but no alkaloids were detected in their shells, unlike this research where the presence of alkaloids was found. The distinction of these bark components found in these researches, may be related to the difference between species, since each plant produces secondary metabolites according to the environment (Silveira et al., 2009).

The differences between the metabolic groups found in the analyzes and those mentioned in the literature can be related to several factors, one of which is seasonality, since the quantity or even the nature of the chemical constituents is not regular during the year, which could explain the difference between the results obtained in this work and those described in the



literature. Other factors that can influence are temperature, ultraviolet radiation, exposure to pathogens, altitude, plant age, water and nutritional availability (Ayres et al, 2008).

The phytochemical analysis of the extracts is extremely important to identify the different classes of secondary metabolic, and later to test their action against the various existing pathogenic microorganisms.

Microbiological studies of plant extracts are widely used as a preliminary step in the search for bioactive or compounds that have antimicrobial action, and this step is fundamental for the discovery of plants that have pharmacological action (Lima, Silva, 2016).

Antimicrobial agents tend to be more efficient in gram-positive bacteria, while in gram-negative bacteria they are generally more resistant. The cause of this resistance of gram-negative bacteria (*Escherichia coli*) is related to your cell wall. They have in their cell an external membrane of lipopolysaccharides, which hinders the action of antimicrobial (bioactive) substances present in plant extracts, this may justify the lack of sensitivity of these bacteria to the extracts analyzed in the present study, making it more resistant to action of extracts. Gram-positive bacteria, on the other hand, have a less complex cell wall with less lipids than gram-negative bacteria ( Migliato et al.2011, Albuquerque, Soares, Oliveira, 2017, Bezerra et al. 2016).

Very similar results were obtained Santos(2008), which verified the action of the leaves extract only for *Staphylococcus aureus*, this extract being resistant to strains of *Escherichia coli*, results that were also verified in this research.

Rodrigues et al(2008) evaluated the antimicrobial action of five samples of the dried leaves of *Eugenia uniflora L.* on *Staphylococcus aureus*, commercialized in the city of Curitiba and Metropolitan Region, and identified an average of inhibition halos with a range from 19.5 to 20.5, values very close to those found in this research.

In studies of Bouzada et al (2008), Brandelli et al (2009), satisfactory results were also found on the strains of *Staphylococcus aureus*, with inhibition halos of 12 mm and 11 mm, respectively.

In contrast, (Rodrigues et al. 2009) analyzed 41 medicinal plants traditionally used in Brazil, seeking to find new antimicrobial drugs, among the analyzed leaves, *Eugenia uniflora L.*, was one of those that had no inhibitory antimicrobial action on *Staphylococcus aureus* and *Escherichia coli*.

Analyzing the extracts of the leaves and bark of *E. uniflora L* (Lorenzoni et al. 2013) obtained effective inhibitory halos only for *S. aureus*, with halo diameters of 21.1 mm, obtaining no results compared to *E. coli*. These results were superior to those found in this work and also in the literature already mentioned. A probable justification is that two parts of the plant were used to prepare the extract, thus increasing the chances of having more secondary metabolites with antimicrobial action.

Castro et al (2019) analyzed the activity of an extract prepared with the leaves and bark of the *Eugenicis uniflora L.* bacteria present in the water of a fish pond and obtained average halos of 8.33mm, 9.52mm and 10.05mm in each plate tested.

In the research by (Lopes, 2008) it was observed that the extract of the pitanga bark was able to inhibit the *Giardia lamblia*, a parasite of the mammalian intestine at minimum inhibitory concentrations of 0.313 mg mL<sup>-1</sup>. It is noticed that the extract of the bark of the pitanga, in addition to inhibiting bacterial growth, also has an action against protozoa.

The antimicrobial inhibition is related to the chemical compounds present in the plant, such as tannins, flavonoids, alkaloids, saponins, which interact synergistically, thus inhibiting the growth of microorganisms, low concentrations of these constituents can interfere with their antimicrobial potential (Martins, 2012).

There are several factors that can influence its content such as the season, the maturity stage of the plant, the form and time of collection, temperature, altitude, solar radiation, nutritional status, water availability and atmospheric composition(Pereira, 2007). Stressful situations such as floods and fires can also influence the concentrations of these

compounds (Dias et al. 2010). Which can justify the difference in the halos of inhibition found in the different literature and also in this study.

When compared to the action of antibiotics tested with extracts of leaves and bark of pitanga about bacteria *E. coli* and *S.aureus*, shown in the table 3, it can be seen that the antibiotics Norfloxacin, Ciprofloxacin and Amoxicillin demonstrated a higher degree of sensitivity than the extracts of leaves and barks of *Eugenia uniflora* L. on *Staphylococcus aureus*, being the antibiotic Norfloxacin with the largest inhibition zone of 30.9 mm.

For *Escherichia coli*, only antibiotics Norfloxacin and Ciprofloxacin were sensitive to this bacterium, since amoxicillin and leaf and bark extracts were resistant. According to Pereira (2007) *Escherichia coli* are producers of  $\beta$ -lactamases an enzyme that has the ability to break  $\beta$ -lactam rings, consequently inactivating penicillins, this may justify the lack of sensitivity of these bacteria to Amoxicillin in this experiment.

The tests carried out in this study are of great importance, since the extracts analyzed demonstrated antimicrobial actions, which may contribute to new investigations of substances that have pharmacological actions in the treatment of bacterial infections.

Therefore, the phytochemical tests performed in this research were only qualitative, that is, they only verify the presence or not of a constituent. The presence of secondary metabolites found in the extracts of *Eugenia uniflora* L. suggests that they can act synergistically in activity against bacteria. From a qualitative analysis of these extracts can contribute to greater better information accuracy on the most prevalent concentration of metabolites and relate them to the antimicrobial activity and the studied plant organ.

## 5. Final Considerations

It is concluded that the extracts of leaves and bark of *Eugenia uniflora* L. presented inhibitory effects to strains of *Staphylococcus aureus*, the leaves having a more satisfactory effect than the peels in relation to their inhibition.

These same extracts, however, were resistant to strains of *Escherichia coli*, not occurring to the formation of halos.

The phytochemical tests performed on the leaves and bark extracts of *Eugenia uniflora* L., allowed to verify the presence of secondary metabolites that may be related to antibacterial activity The *Staphylococcus aureus*.

In the leaf extracts, chemical constituents were found, such as saponin, flavonoids, tannins and alkaloids, in contrast, in the bark extract they found only saponins and alkaloids.

The results allowed to conclude that in a comparison, antibiotics (Norfloxacin, Ciprofloxacin and Amoxicillin) obtained biggest action that extracts from the leaves and bark of *Eugenia uniflora* about the strains *Staphylococcus aureus*.

For *Escherichia coli*, the antibiotic Norfloxacin, Ciprofloxacin were active, while Amoxicillin and plant extracts did not show inhibition activity against bacterial strains. Even in these conditions, the extracts have good potential for the development of natural products.

We highlight that further studies are needed to complement this research and to prove The inhibition potential presented by the analyzed plant, from a quali-quantitative analysis of the extracts, aiming at their chemical elucidation, as well as the quantification of the presence of these metabolites.

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