

Evaluation of the Antimicrobial Potential of Essential Oil of the Leaf of *Passiflora Edulis*

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Abstract— The passion fruit of the genus *Passiflora edulis* generally presents three forms of use, that is, the in natural form, pharmaceutical applications, and industrial processes. Currently, research has reported the increasing use of oils extracted from passion fruit leaves for the purpose of bacterial inhibition. The objective of this work was to evaluate the antimicrobial action of *Passiflora edulis* leaves extract against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli* microorganisms using solvents with different polarities through disc diffusion and microdilution techniques. The oil extracted from the leaves of *Passiflora edulis* had satisfactory performance against the isolates of gram-positive bacteria *S. pyogenes*, in which it presented a good antimicrobial potential of inhibition compared to the other microorganisms tested. This action can be explained by the presence of constituents of the plant that cause this effect against the pathogen, such as: the presence of flavonols, proanthocyanidins, passion, polyphenols, tannins, aldehydes, among others.

Keywords— *Antimicrobial activity. Passionflower. Chemical extraction.*

I. INTRODUCTION

Feeding performs a very important role in maintaining health and promotion, currently consumers are looking for foods that may obtain these characteristics that is that provides directly benefits to health. Fruits are part of this complex healthy foods network, by being considered as an integral component of a balanced diet and with increasing of its functional properties, they reinforce even more the importance of its regular ingestion (Souza, 2016).

Passion fruit is a characteristic fruit of tropical areas that has been gaining prominence in industries mainly in the pharmaceutical industry because of the

various medicinal properties in extracts, leaves and flowers, as well as its antimicrobial and antioxidant potential presented in the pulp, leaves, seeds and bark. (Souza, 2016)

The genus *Passiflora edulis* is known as yellow passion fruit or passion fruit sour and is generally identified in herbaceous vines, subliming vines possessing a vigorous growth with five to ten meters, obtaining a radical systems, climbing stem, lobed and green leaves, floriferous yolk and also vegetative yolk in the axilla of the leaf, and the flowers are bisexual or unisexual. Moreover, its leaves present the presence of cyanogenic glycosides β -D-allopyranos benzyl compounds valuable for health. (Souza, 2016)

The passion fruit of the genus *Passiflora edulis* generally presents three forms of use, that is, in natural form, pharmaceutical applications and industrial processes. Currently research has reported the increasing use of oils extracted from passion fruit leaves for the purpose of bacterial inhibition due to a substance called Passicol that causes inhibition of the growth of bacteria, usually Gram negative and some studies have also observed the ability to inhibit *S. aureus*, that is a positive bacterium by presenting great resistance for several antimicrobial agents. (Galvão, et al; 2013)

From this it is clear that the passion fruit leaves of the genus *Passiflora edulis*, as well as all its fruit presents undeniable benefits to the health of the population, by demonstrating the possibility of the use of passion fruit leaves for the production of antibacterial phytotherapies by favoring both human health by use a natural drug as the sustainability of the Brazilian market. (Freitas, et al., 2017)

Desiring to define the antibacterial potential for consecutive use in the preparation of pharmaceutical formulations with the competence of making susceptible

resistant forms, this work has the objective to provide the evaluation of antimicrobial existence action of the extract of *Passiflora edulis* leaves against the microorganisms *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*, by using solvents with different polarities. Introduction is the initial part of the article, that outlines the delimitation of the subject that will be approached, the goals of the research and the other necessary factors to locate the article.

II. METHODOLOGY

Plant Material

The leaves were collected in a planting of *Passiflora edulis* in the city of Anagé - Bahia, from March to June 2018. The identification of the botanical material was performed in the herbarium of the State University of the Southwest of Bahia in the Campus of Vitória da Conquista - Ba, and an exsiccata of the species was made for identification of this one.

Place of Study

The extractive part was performed in the Institution of Higher Education Faculdade Independente do Nordeste - FAINOR, in the sector of health laboratories. The scope of the crude extract was based on the methodology of OLIVEIRA et al, 2016, in which modifications were made. The research was conducted in the State of Bahia, that presents a total of 417 municipalities and approximately 15,344,447 million estimated inhabitants (BRAZIL, 2017).

Method for extraction of oil

The methodology used was Clevenger, a complex that drags water vapor and / or steam distillation, by being the most used and economically possible way to extract vegetable oils. The water undergoes heating in a volumetric flask on a heating plate which then boils, the water vapors that are produced from this process are guided under pressure by giving continuity in another container, in the place where the plant material is. (PISTELLI, E. C., 2012).

The heat of the steam establishes that the cell walls untie. In view of this, the oil that is between the cells vaporizes along with the water vapors and the volatiles are guided towards the condenser by going to the cooling tube; then the collected oil is placed in a container. (PISTELLI, E. C., 2012)

Procedure

Unlike seeds and pulps, which are placed to dry in an oven at 60 ° C for 48 hours, the leaves were used green and then ground in a blender. Then, 100 g of sheets

were weighed using 500 ml of distilled water that were placed in the Clevenger apparatus for extraction of the oil at 50 ° C for two hours. Finally, in the extraction of the oils, they were submitted to the QUIMIS brand rotary evaporator under a temperature of 50°C, for total solvent separation of the water stored in an amber bottle in a refrigerator.

Biological test

In order to verify the antimicrobial action of the raw extracts of *Passiflora edulis*, standard microorganisms provided by the collection of FAINOR microorganisms were used to find some inhibitory activity for the development of the bacteria *Klebsiella* spp, *Streptococcus Pyogenes* and *Escherichia coli*. The bacteria were chosen randomly thereby aiming to determine between grampositive and gramnegative bacteria which had antimicrobial activity against the essential oil of the seeds of *Passiflora edulis*.

In the preparation of the inoculum, the young cultures of each bacteria were standardized in sterile saline solution (0.85% NaCl) until a turbidity compatible with the 0.5 scale agreement level of MacFarland (1x10⁸ CFU / ml) was obtained. The antimicrobial activity was done by following the in vitro paper disc diffusion method of Bauer and Kirby (1966).

Sterile filter paper discs measuring six mm of diameter were soaked with 10µL of the crude extract solution at 200,000µg / ml, so that each disk had a concentration of 1,000µg / ml. The strains were spiked in Muller-Hinton Agar medium and incubated at 37 ° C 24 hours prior to the test. Then the results were read, by measuring the space of the inhibition halo built around the disk, when occasionally present. The halo is measured in mm.

Broth macrodilution methodology for determination of Minimum Inhibitory Concentration (MIC)

The determination of MIC was performed by the CLSI test tube macrodilution method, based on the methodology of Santurio et al. (2007), with modifications. The technique was performed in tubes, by consisting initially of the preparation of serial dilutions of the oil of 1:10, 1: 100 and 1: 1000. To prepare the 1:10 dilution, 9 ml of absolute alcohol was taken together with 1 ml of essential oil. On the 1: 100 dilution the same amount of alcohol was used and 1ml of the 1:10 dilution was withdrawn, and the dilution was added 1: 100 and so on to give the 1: 1000 dilution.

Then, five tubes were prepared with serial dilutions, enumerated from 1 to 5. These tubes were composed of 8 ml of Muller-Hinton Agar liquid medium

together with 1 ml of the 1:10 dilution, 1 ml of tube 1 was transferred to tube 2, from the tube 2 to the 3 and successively to the last tube and finally 0.5 ml of the bacterial suspension that were added in all tubes, thus having a final volume of 8.5 ml. This process was performed in each tube by containing the dilutions.

The control tubes were prepared, where the same ones were divided into three and followed as the

following way: for the positive control, 4 ml of the bacterial suspension was added with 4 ml of Muller-Hinton Agar medium; for the negative control was added 4 ml of bacterial suspension with 4 ml of the antibiotic prepared; in the third tube only the pure Muller-Hinton Agar medium was added. For finalization of the technique, 10µl of the broth that was clear was seeded to know precisely the exact concentration of the oil.

III. RESULTS AND DISCUSSION

Table.1. Antibacterial activity of *Passiflora edulis* leaf essential oil

Microorganism	Halos formed by oil (mm)	Inhibition Halos (mm) Reference values *		
		**R	I	S
<i>Klebsiella</i> spp	5	≤ 14	15-17	≥ 18
<i>Escherichia coli</i>	0	≤ 15	16-20	≥ 21
<i>Streptococcus pyogenes</i>	13	≤ 11	12-23	≥ 24

* Reference values taken from the manual for Antibiogram by Kirby & Bauer disc diffusion technique. ** R - Resistant; S - Sensitive; I - Intermediate.

As described in Table 1 above, according to the studies performed, it was found that in the diffusion test tubes by using the plaque method, it was verified that the essential oil of the *Passiflora edulis* leaf was able to inhibit with intermediate halo the bacteria *Streptococcus pyogenes*, with the formation of the inhibition halo (13mm) around the disc that was impregnated with the essential oil. As compared with the other microorganisms tested, the results evidenced that the leaf essential oil had no antibacterial potential activity to inhibit the growth of *Klebsiella* spp and *Escherichia coli*, with halos of 5 mm and 0 mm respectively, by being considered resistant to the oil of *Passiflora edulis*.

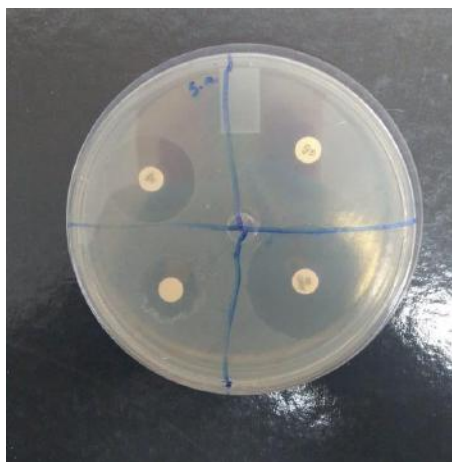


Fig.1. Verification of the inhibition halo of *Passiflora edulis* leaves by the disk diffusion technique of *Streptococcus pyogenes*.

This sensitivity of *Streptococcus pyogenes* was studied by Cushnie (2016), in which the presence of flavonols, secondary metabolite of the plant in question, is mentioned. This metabolite presents a great bactericidal activity due to the different mechanisms of action that lead to the aggregation effect in all the bacterial tissues, by being resolute against a gama of gram positive bacteria, such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Lactobacillus acidophilus*, among others .

Another metabolite present in the evaluated plant are the proanthocyanidins, derived from primary metabolites that inhibit the growth of several gram positive pathogens with characteristic of cocci and in pairs. (Caillet, 2014) Researches report the inhibition of gram positive bacteria before the leaves of *Passiflora edulis* due to the strong presence of a substance known as Passicol, however, it does not show any inhibition in relation to gram negative bacteria, as it is the case of resistance of *Klebsiella* spp and *Escherichia coli* (Petry 2011, Silva 2017, Silva 2014).

Phytochemical tests performed by Ferrari (2015) with hydroalcoholic extracts of the aerial parts, such as the leaves, established that the studied plant presents as secondary metabolites, condensed tannins, baphenes, flavones, flavonols, among others. It is hoped that with these phytochemicals, they will increase plant defense against pathogens and may therefore to result in biological activities (Johnson 2018; Kannan 2012).

The antimicrobial properties of certain classes of *Passiflora edulis* constituents, such as polyphenols, are related to their ability for suppressing microbial virulence

factors such as biofilm, adhesion and neutralization of bacterial toxins (Daglia, 2012).

However, some research has influenced that the lack of antimicrobial activity of *Passiflora edulis* essential oil with gram-negative bacteria (*Klebsiella* spp. And

Escherichia coli.) are due to the inability of forming complexes with soluble proteins present in the walls of membranes of these bacteria, that is, by being impossible to break the lipophilic membranes of the same ones. (Laupland 2012; Leitao 2016; Lugato 2014)

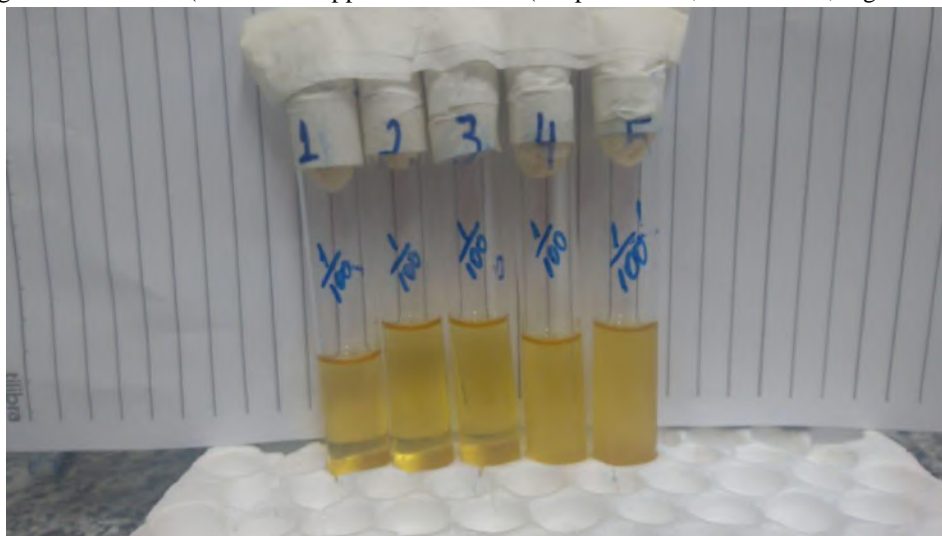


Fig.2: Verification of the CIM of *Passiflora edulis* leaves by the macrodilution technique on *Streptococcus pyogenes*.

Macrodilution analyzes confirmed that the essential oil of *Passiflora edulis* seed presented an effective response against clinical isolates of *Streptococcus pyogenes*. In the 1:10 dilution there was no growth in any of the 5 tubes, in the 1: 100 dilution there was no growth only in the first one. From the second tube to the fifth, there was growth. As it had growth in the 1: 100 dilution, consequently in the 1: 1000 would also be seen growth.

The concentration measure (or concentration average) is studied for the ability to cause some inhibition in the growth of the test microorganism. That is, in the 1:10 dilution is the lowest concentration of the oil of *Passiflora edulis* leaf capable of causing the bacteria *Streptococcus pyogenes* death as it is exposed to that concentration. Thus, it is possible to emphasize the positive power of the oil extracted from the leaves of *Passiflora edulis* concerning to the bacterial sensitivity when contacting with the oil. In all experiments positive and negative control of medium and microbial growth with the inoculum was adequate.

According to Nkhata (2012) the essential oil of *Passiflora edulis* has antibacterial activity due to its chemical constitution, with the presence of tannins, aldehydes, saponins and glycosides that are associated with its potential. According to Nascimento (2010), the antimicrobial activity also results from the composition and concentration of the species or essential oil in question, of the type, of the microorganism in question,

the composition of the substrate, the processing and the storage condition, among others.

The essential oil of *Passiflora edulis* Sims is described as a volatile, lipophilic substance, usually odoriferous and liquid, by belonging to the secondary metabolism plants. It may have activity attributed to antibacterial and antifungal, as well as in the sectors of perfumery, personal hygiene and cosmetics. (Simões & Spitzer, 2004, Costa, 2008)

The combination of natural products with antibiotics enriches the disintegration of bacterial membranes through the formation of a complex by agents associated with this structure (Pimentel 2015; Serpa 2012). The natural products associated with antibiotics, exerts an activity against many bacterial species, either increasing the activity of the antibiotic itself or decreasing the natural resistance of the bacteria. Thus, due to this specificity, these compounds are classified as modifiers of antibiotic activity (Siebra, 2016; Shahidi, 2013).

Passiflora edulis has large medicinal properties, such as Arrais (2012) mentions in its studies, anti-inflammatory, sedative, healing, antimicrobial and antidiarrheal (KRIEF, 2014) Medicinal plants are more and more being used in the popular medium, by fact of people are more and more seeking the use of herbal medicine as auxiliary therapy, or even, by taking the place of advances in medicine. Currently, in Brazil, there is phytotherapy in the Unified Health System (UHS), incorporated by the Ministry of Health, that has the goal of

increasing the applicability of medicinal plants in the day by day of the population. (2006)

Klebsiella spp and *Escherichia coli* are opportunistic bacteria that cause serious infections by leading to a high degree of morbidity and mortality mainly in immunocompromised patients. Because they have the ability to arrest themselves to local cells, they are responsible for the greater gamma of urinary tract infections. (RONALD, 2013) *Streptococcus pyogenes*, with high virulence and morbidity and mortality, is a species of grampositive cocci, that can be found in the upper airways and cause infections (Walker 2013).

In this respect, the phytochemicals that are found in plants and may make some clinical office against these pathogens, they have importance in the arsenal of antimicrobial agents, since the durability of any antibiotic is limited and, in turn, the public is more and more clarified with the problems of superprescription and the indiscriminate use of antibiotics (Alves, 2013).

Paterson (2015) affirms that there is nowadays a large scale of natural compounds that are available with the exemption of medical prescription, in drugstores, herbal and general food stores. Thus, the research of plant extracts with antimicrobial action presents an exit for combating pathogenic microorganisms, thus leading to the search for new chemical molecules derived from plant species as a promising source of new antimicrobial agents (Nunes 2013; Oliveira 2016)

IV. CONCLUSION

Before this study, it was observed that the oil extracted from the leaves of *Passiflora edulis* had satisfactory performance against the isolates of gram-positive *S. pyogenes* bacteria, in which it presented a good antimicrobial potential of inhibition compared to the other microorganisms tested. This action can be explained by the presence of plant constituents that cause this effect against the pathogen, such as: presence of flavonols, proanthocyanidins, passicol, polyphenols, tannins, aldehydes, saponins and glycosides, among others, which generate high bactericidal activity, suppression of factors of virulence and complexation with the structures of the bacteria. Thus, the leaf oil from *Passiflora edulis* can be used as a source for the treatment of infections caused by *S. pyogenes*, obviously, by requiring more embracing clinical studies to certify its antimicrobial potential.

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