ejpmr, 2017,4(11), 16-24

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

STUDY OF THE BIOLOGICAL EFFECTS OF AN AQUEOUS *MYRCIA RACEMOSA* EXTRACT ON BACTERIAL CULTURES IN THE PRESENCE AND ABSENCE OF STANNOUS CHLORIDE SOLUTION.

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Article Received on 22/08/2017 **Article Revised on 12/09/2017** **Article Accepted on 02/10/2017**

ABSTRACT

The species *Myrcia racemosa* (O.Berg) Kiaersk.is a phanerogamic plant belonging to the *Myrtaceae* family. Most drugs have numerous side effects that may restrict their use. An alternative to such medicinal products are medicinal plants, which offer an important perspective in the identification of bioactive compounds. The National Relation of Medicinal Plants of Interest to the SUS (Renisus) has the purpose of guiding studies and researches that may subsidize the elaboration of a list of medicinal and phytotherapeutic plants, to be made available for use by the population, with safety and efficacy for the treatment of disease. This study aimed to verify the action of an aqueous *Myrcia racemosa* extract in bacterial cultures in the presence and absence of the stannous chloride reducing agent. The extract was prepared from the infusion, filtered and subjected to refrigeration. To evaluate the cytotoxicity, we used the disc diffusion method in nutritive agar medium. After standardization of the turbidity of the saline solution (0.9% NaCl), to determine the CFU, it was compared with the 0.5 Mc Farland scale, after which the bacterial growth was evaluated in relation to the disks, which were impregnated with different volumes of $SnCl_2$ solution and / or with a volume of 12 µL of the vegetal extract. The results suggest that the aqueous extract of *Myrcia racemosa* has redoxi property in relation to the observed cytotoxic and antioxidant effects. This study reports, for the first time, the biological effects of this extract.

KEYWORDS: *Myrcia racemosa*, antioxidant, bactericidal, cytotoxic, stannous chloride.

1. INTRODUCTION

Since its emergence, Man makes use of several plants that have medicinal effects. The exploitation of natural plant resources has a very important impact for the economic development and also the valorization of the great Brazilian biodiversity. There are dozens of species that, when properly preserved, can be used for a variety of purposes, ensuring true effects that benefit human health. The knowledge gained in the field of natural product research is of great use for the sustainable exploitation and application of this raw material. Phytotherapy or plant therapy is one of the oldest therapeutic practices of mankind. It dates back to about 8500 a.c. and has origins in both popular (ethnobotanical) knowledge and scientific experience (ethnopharmacology). The plants contain active principles capable of curing various diseases and it was from the recognition of these therapeutic properties that the emergence of modern allopathic medicine occurred. The growing consumption of herbal medicines by the Brazilian population is notorious, which could be related to the advances in the scientific area, which allowed the development of recognized and safe herbal medicines, besides the growing tendency of the population to seek less aggressive therapies to primary health care (Yunes et al, 2001).

The Ministry of Health released in February 2009 the National List of Medicinal Plants of Interest to SUS (Renisus). This list includes medicinal plants that have

the potential to generate products of interest to SUS. The purpose of the relationship is to guide studies and research that may support the elaboration of a list of herbal and phytotherapeutic plants to be made available for use by the population, with safety and efficacy for the treatment of a particular disease. In addition, Renisus will subsidize the actions of the other ministries participating in the Program (Ministries of Civil Household, Agriculture, Livestock and Supply, Culture, Agrarian Development, Social Development and Hunger Control, Industry and Foreign Trade Development, Science, Technology and Innovation National Integration and Environment). Renisus should be reviewed and updated periodically, at the discretion of the Ministry of Health. The use of medicinal plants is an important practice both in folk medicine and in health. The availability of herbal and phytotherapeutic plants by SUS is enabling the use of scientifically based phytotherapy extracted from the set of plants used by successive generations of a population that had as only option for the treatment of their evils, the empirical use of medicinal plants easy access in each region of the country (Lorenzi and Matos, 2002, Brazil, 2009a).

The Brazilian *Myrtaceae* belong to the tribe *Myrteae*, forming a phylogenetically cohesive group. *With approximately 1000 species and 23 genera occurring in Brazil*. *Myrtaceae* is one of the most representative families in floristic surveys, mainly in areas of Cerrado and Atlantic Forest. The species *Myrcia racemosa* (O.Berg) Kiaersk, belongs to the *Myrtaceae* family, are found in the Northeast (Alagoas, Bahia, Pernambuco, Sergipe), Southeast (Espírito Santo, Rio de Janeiro, São Paulo) and South (Paraná, Santa Catarina) of Brazil. It has a leaf type characterized as simple with the edge of the entire limb. They display opposite phylloxia and the way of life is that of a tree. The family was also identified in the southeast region of Brazil in the state of Rio de Janeiro, in the following areas: Restinga da Marambaia, Jacarepaguá, Maricá, Cabo Frio, Macaé and São João da Barra. In the Restinga da Marambaia region, there was an open non-flooded shrub, open flooded shrub, flooded forest and sandy cord forest. (Wilson et al., 2001).

Myrtaceae is one of the most diverse neotropical groups and therefore a good proxy of plant diversity in the region. *Myrcias.l.* is an informal group composed of three accepted genera (*Calyptranthes*, *Marlierea* and *Myrcia*) that make up the second largest Neotropical group of *Myrtaceae*, totaling about 700 species distributed in nine subgroups. Exclusively neotropical, the group occurs throughout the Neotropics with centers of diversity in the Caribbean, on the Guyana Plateau and in central-eastern Brazil. The Amazonian Forest has a relatively low diversity of the species *Myrcias.l.*, but seems to have been important in the early biogeographic history of ancient lineages. The lowland Atlantic Forest has a great variety of species, but the species-rich lineages did not originate in the area. The diversification of most subgroups of *Myrcias.l.* occurred throughout the Miocene, as reported for other neotropical taxa. During the Miocene, geological events may have influenced the evolution of Caribbean and Amazonian forest strains, but other regions were geologically stable and climate change was the most likely diversification factor. The evolution of many lineages in mountain areas suggests that *Myrcias.l.* can be particularly adapted to such environments (Santos et al, 2017).

Given their distribution, importance and richness, Myrtaceae species constitute a model system for studying the evolution of tropical plant diversity. In addition, chloroplast genome sequencing is an efficient tool for phylogenetic relationship studies (Machado et al, 2017).

Plants are rich in a wide variety of secondary metabolites with antimicrobial properties. Phytochemical studies in plant extracts in general and in essential oils (EOs) in particular are focused on the isolation and identification of components of complex mixtures in order to determine the structure - activity correlations (ie, physiological and / or ecological, Bases for the studies of pharmacognosy). Problems such as microbial resistance to existing antibiotics and the decline in the formulation of new antibiotics have generated a growing interest in herbal anti-infective drugs. Some plants are known to have EOs, especially superior plants, angiosperms and gymnosperms, belonging to approximately 50 families, with Myrtaceae being one of the families most frequently. The antimicrobial activity of EOs and their components has been demonstrated in a variety of microorganisms. Research is needed to develop strategies related to EOs in overlapping multidrug resistance and reducing the concentrations required to achieve a specific antimicrobial and / or antibiotic effect for human health and / or food safety (Saviuc et al, 2015).

Wubshet et al (2015) identified in the leaves of *Myrcia palustris* casuarinina, 3-O-β-d- (6 "-galoyl) galactopyranoside, kaempferol 3-Op-galactopyranoside, myricetin and quercetin as α-glucosidase inhibitors. four acetylated ellagic acid rhamnosides, i.e. 4-O- (2 ", 4" -O-diacetyl-α-1-rhamnopyranosyl) ellagic acid, 4-O- (2 ", 3" -O-diacetyl And 4-O- (2 ", 3", 4 "-O-Triacetyl-α-1-Rhamnopyranosyl) -4- O- (3", 4 "-O-diacetyl- ellagic.

Myrcia amazônica DC is a species predominantly found in northern Brazil and belongs to the *Myrtaceae* family. This family has several species used in folk medicine to treat gastrointestinal disorders, infectious diseases and hemorrhagic conditions and are known for their essential oil content. In this species, polyphenols and tannins were identified (Morais Rodrigues et al, 2016).

Mehriardestani et al (2017) report that *Myrtaceae* families contain a large number of plants with anti-trichomonas activity.

De Jesus et al. (2016) reported the novel antibacterial activity of essential oils (EOs) from nine plants *Myrcia ovata Cambessedes* against eight foodborne bacteria. *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Enterococcus faecalis* and *Pseudomonas aeruginosa* one in particular, *P. aeruginosa*, which is generally resistant to antimicrobial agents, was extremely sensitive to some EOs. The EOs of *Myrcia ovata Cambessedes* exhibit high inhibitory and bactericidal effects against foodborne bacteria, and may be an interesting alternative for future applications as natural antimicrobials in food systems.

Tin chloride (II) or stannous chloride is a white crystalline solid of the chemical formula $SnCl_2$. It forms a stable dihydrate, but in aqueous solution it tends to undergo hydrolysis, especially if heated. $SnCl_2$ is used as a reducing agent in acid solutions, and in electrolytic baths for electroplating. $SnCl_2$ is widely used in everyday human life, for example, to conserve soft drinks, in the manufacture of foods and biocidal preparations. In nuclear medicine, stannous chloride is used as the reducing agent of technetium-99m, a radionuclide used for the radiolabelling of different cells and molecules. Despite this, stannous chloride is capable of generating reactive oxygen species (ROS) that can damage DNA (Mattos et al, 2000).

This salt is a powerful reducing agent and is also used in dental amalgams. Previous studies have shown that stannous chloride is capable of inactivating *Escherichia coli* cultures (Melo et al., 2001) and K562 cells (Dantas et al., 2002), as well as inducing single-strand breaks in plasmid DNA by generating radicals free in vitro (Dantas et al., 1999, Ferreira-Machado et al., 2004).

There are controversies about the cytotoxic, genotoxic and mutagenic effects of $SnCl_2$ in the literature. In bacterial inactivation experiments using strains of *Escherichia coli* AB1157 (wild type) and BW9091 (mutant xthA), data obtained showed that $SnCl_2$ showed a high toxicity. The SnCl2 -induced cell inactivation found in the XthA mutant strain suggests that the lack of the endonuclease IV enzyme may be responsible for the inability to repair DNA damage related to the $SnCl_2$ effect (Guedes et al, 2006).

Several authors have demonstrated the cytotoxic, genotoxic and mutagenic effects of $SnCl_2$. In relation to $SnCl_2$, it has been described that this salt is capable of producing ROS from a Fenton-like reaction, inducing lethality in *Escherichia coli* (*E. coli*) (Dantas et al, 1996), cytotoxicity and genotoxicity in eukaryotic cells, and genotoxicity in the DNA plasmid (Caldeira-de-Araujo et al, 1996, De Mattos et al, 2000).

Mutagenic properties were also reported for $SnCl_2$, with 8-hydroxyguanine (8-oxodGuo) being the predominant lesion responsible for G: C-T: A (Cabral et al., 1998) and mutagenicity in yeast and bacteria (Pungartnik et al, 2005).

In addition to many articles pointing to the production of ROS by $SnCl_2$, some researchers have shown that this agent is also capable of inducing a direct effect on DNA. De Mattos et al. (2005) suggested that stannous ions were able to bind to DNA, inducing the formation of ROS very close to that target.

Since it is known that natural products have been used by humanity since time immemorial and that the search for relief and cure of diseases by the ingestion of herbs and leaves may have been one of the first forms of use of natural products, it is of extreme relevance to investigate the biological effects related to the natural products that are consumed at the level of popular medicine, therefore, it becomes stimulating to verify the action of an aqueous extract of *Myrcia racemosa* in *Escherichia coli* and *Staphylococcus au*reus cultures in the presence and absence of the reducing agent stannous chloride.

MATERIAL AND METHODS

2.1 Preparation of Vegetable Extract

Plant samples (aerial parts, including leaves and stems) were collected at 6:30 am of a plant specie (*Myrcea racemosa*) belonging to the remaining resting vegetation located at the end of the Pedra de Itaúna street, in the Condomínio Pedra de Itaúna, Avenida das Américas-Pista Central, S / n, Barra da Tijuca, Rio de Janeiro - RJ - 23.011041, -43.423824. The samples were immediately transported to the Laboratory of Chemical and Biological Analysis (LAQB) of the Foundation State University Center of the West Zone (UEZO) where they underwent a selection and sanitization. 900g of the plant material (leaves), weighed in scale (Class II Bel Mark 2500), were quantified and then subjected to infusion for 60 minutes in water at 100°C. At the end, the infused extract was filtered to remove solid waste, packed in amber glass and subjected to refrigeration at a temperature of -20°C. After freezing, the samples were lyophilized (Lyophilizer, LIOTOP 220) and the final concentration adopted for the assays was 50 mg /mL.

2.2 Microbiological tests.

Microbiological tests were performed using the disc diffusion method. Gram-negative bacterial strains used were those of *Escherichia coli* of types AB1157, BW9091, ATCC25922 and the Gram-positive bacterial strain used was that of *Staphylococcus aureus* type ATCC 8096. The concentration of the bacteria was standardized using the range of 0.5 of the Mc Farland scale (Laborclin).

Microbiological tests were performed in four stages

Step 1 (reactivation of strains): Retention of stock strains and reactivation in nutrient broth (TSB medium, Merck). Step 2 (Maintenance of colonies): After 24 h of incubation in bacteriological greenhouse (Ps -101, Scientific Solab), transfer of the strains to the nutrient agar medium (Merck).

After incubation (24h) in a bacteriological stove (Ps-101, Scientific Solab) at 35°C, samples of colony forming unit (CFU) and diluted in saline solution (NaCl 0.9%) and compared with the turbidity in relation to the 0.5 scale of MC Farland.

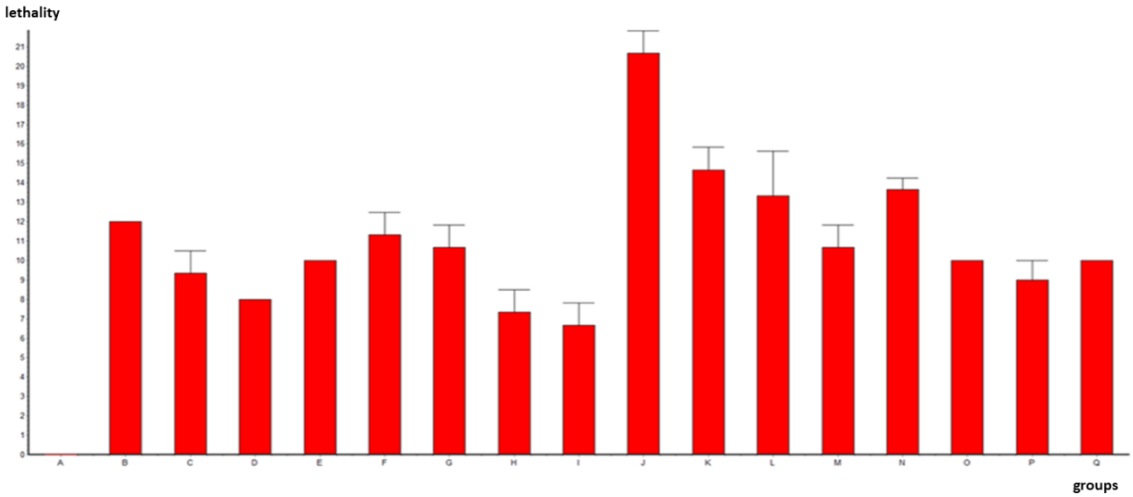
Step 4: Perform the disk diffusion test. The disc diffusion test was performed by depositing paper discs in sterile plates with different volumes (8, 12 and 24 µL) of an aqueous extract of *Myrcia racemosa* (50mg /mL) and / or with different volumes (8, 12 and 24 µL) of a stannous chloride solution (5mg /mL) ($SnCl_2$/Vetec). For insertion of the material into the plates, pipettes (Gopet II 0.5-10µL, 20-200µL and 100- 1000µL) were used.

2.3- Statistical analysis

The results were statistically analyzed using the Graph Pad In Stat3 software. The analyzes were performed using ANOVA with the Tukey-Kramer post-test.

3- RESULT

Graph 1- Effect of an aqueous extract of *Myrcea racemosa* on cultures of *Escherichia coli* and *Staphylococcus aureus* in the presence and absence of the stannous chloride reducing agent.



A-Sodium Chloride (NaCl 0.9%), B-Stannous Chloride (24 µL) and *Escherichia coli* AB1157, C-Stannous Chloride (12 µL) and Escherichia coli AB1157, D-Stannous Chloride (8 µL) and *Escherichia coli* AB1157, E- Chloride Stannous (12 µL) + Plant Extract (12 µL) and *Escherichia coli* AB1157, F-Stannous Chloride (24 µL) and *Escherichia coli* BW9091, G- Stannous Chloride (12 µL) and *Escherichia coli* BW9091; H-Stannous Chloride (8 µL) and *Escherichia coli* BW9091; I- Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* BW9091, J- Stannous Chloride (24 µL) and *Escherichia coli* ATCC25922; K- Stannous Chloride (12 µL) and *Escherichia coli* ATCC25922; L-Stannous Chloride (8 µL) and *Escherichia coli* ATCC25922, M-Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* ATCC25922; N-Stannous Chloride (24 µL) and *Staphylococcus aureus* ATCC 8096; O-Stannous Chloride (12 µL) and *Staphylococcus aureus* ATCC 8096; P- Stannous Chloride (8 µL) and *Staphylococcus aureus* ATCC 8096; Q- Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Staphylococcus aureus* ATCC 8096.

From the analysis of variance (ANOVA), it was observed, in relation to the data analysis, that the value of p is <0.0001, considered extremely significant, that is, the variation between the columns is significantly higher than the expected. Analysis of the results from the Tukey-Kramer multiple comparison test allowed a value of p <0.001 when comparing the control with all groups.

When comparing the Stannous Chloride (24 µL) and *Escherichia coli* AB1157 groups with the Stannous Chloride (8 µL) and *Escherichia coli* AB1157 groups, a significant decrease (p <0.01) in $SnCl_2$ activity was observed as a function of the decrease in (p <0.01) was observed when comparing the Stannous Chloride (24 µL) and *Escherichia coli* BW9091 groups with the Stannous Chloride (8 µL) and *Escherichia coli* BW9091 groups. It can be observed that there was a significant decrease in the activity of $SnCl_2$ (p <0.05) when the Stannous Chloride (12 µL) and *Escherichia coli* BW9091 group were purchased with the Stannous Chloride group (8 µL) and *Escherichia coli* BW9091 of greater sensitivity of type BW9091 to $SnCl_2$ relative to type AB1157.

When comparing Stannous Chloride (24 µL) and *Escherichia coli* ATCC25922 with Stannous Chloride (12 µL) and *Escherichia coli* ATCC25922, a significant decrease (p <0.001) in $SnCl_2$ activity was observed due to the decrease in the volume of (p <0.001) and type *E. coli*BW9091 (p <0.001), a similar result (p <0.001) was observed in the sample. (24 µL) and *Escherichia coli* ATCC25922 with Stannous Chloride (8 µL) and *Escherichia coli* ATCC25922 were compared. When comparing Stannous Chloride (24 µL) and

Staphylococcus aureus ATCC 8096 with Stannous Chloride (12 µL) and Staphylococcus aureus ATCC 8096, a significant ($p < 0.01$) decrease in stannous chloride activity function of the low volume of the $SnCl_2$ solution. The results indicate that the strain of S. aureus ATCC8096 is less sensitive to the action of SnCl2 compared to the strain of E. coli ATCC25922.When comparing the stannous chloride group (24 µL) and *Staphylococcus aureus* ATCC 8096 with the group where stannous chloride (8 µL) and *Staphylococcus aureus* ATCC 8096 were associated, a very significant decrease of the action of $SnCl_2$ ($p < 0.001$), emphasizing the higher resistance of S. aureus strain ATCC8096 in relation to the effect of $SnCl_2$ when compared to the other bacterial strains.

Comparing the Stannous Chloride (12 µL) and *Escherichia coli* AB1157 groups with the Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* AB1157 group, it can be observed that the plant extract did not interfere with the action of $SnCl_2$. However, when we compared the Stannous Chloride (12 µL) and *Escherichia coli* BW9091 with Stannous Chloride (12 µL) + Vegetable Extract (12 µL) and *Escherichia coli* BW9091, we observed that the extract was able to ($p < 0.01$) the effect of $SnCl_2$ on the survival fraction of strain BW9091. Results similar to the one found with the BW9091 strain were found when comparing the Stannous Chloride (12 µL) and Escherichia coli ATCC25922 with Stannous Chloride (12 µL) + Plant Extract (12 µL) and Escherichia coli ATCC25922, observing that the extract was capable of decreasing ($p < 0.01$) the effect of SnCl2 on the survival fraction of the ATCC25922 strain.

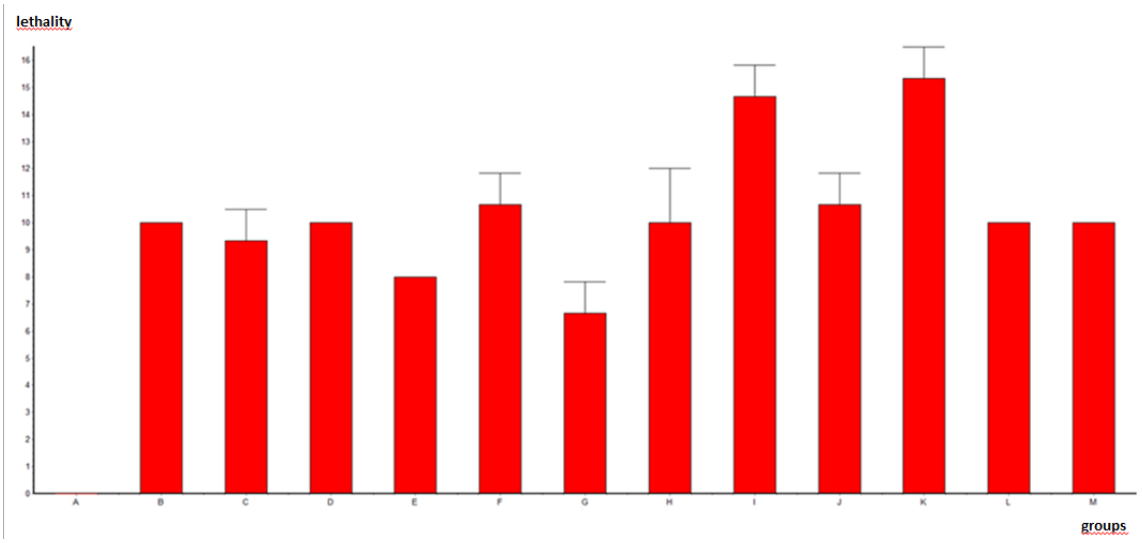
It is interesting to consider that when comparing the group Stannous Chloride (12 µL) and *Staphylococcus aur*eus ATCC 8096 with the Stannous Chloride (12 µL) + Plant Extract (12 µL) and Staphylococcus aureus ATCC 8096 group, there was no change in the effect of SnCl2 ($p > 0.05$), since the halo measure, related to the bacterial mortality fraction, was not modified.

When comparing the AB1157 strain with those of type BW9091, ATCC25922 and ATCC8096, it was observed that the extract exerted a significant effect ($p < 0.05$) on the action of $SnCl_2$ when comparing the Stannous chloride group (12 µL) + *Escherichia coli* AB1157, with the Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* BW9091, where for the BW9091 strain the extract exhibited an antioxidant effect.

Comparing the Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* BW9091 with the Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* ATCC25922 groups, the extract showed a (12 µL) + Plant Extract (12 µL) and *Escherichia coli* BW9091 with the group Stannous Chloride (12 µL) + Plant Extract ($p < 0.001$) in the ATCC25922 type strain, however, (12 µL) and *Staphylococcus aureus* ATCC 8096, the protective effect of the extract was found to be higher relative to the BW strain ($p < 0.05$) compared to the strain type ATCC8096.

When we compared the group Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* ATCC25922 with the Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Staphylococcus aureus* ATCC 8096 group, we observed that the extract did not interfere in ($p > 0.05$), allowing us to speculate that the extract exerts an expressive antioxidant effect in view of the results found with the type BW9091 strain which is mutant in relation to the repair mechanism due to oxidative stress because it is deficient expression of the enzyme exonuclease III. This protein is closely associated with the repair mechanism related to DNA damage, acting from the base excision repair system.

Graph 2 - Effect of an aqueous extract of *Myrcea racemosa* (12 µL) on cultures of *Escherichia coli* and *Staphylococcus aureus* in the presence and absence of stannous chloride reducing agent (12 µL).



A- Sodium Chloride (0.9% NaCl); B- Plant Extract (12 µL) and *Escherichia coli* AB1157; C- Stannous Chloride (12 µL) and *Escherichia coli* AB1157; D- Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* AB1157; E-Plant Extract (12 µL) and *Escherichia coli* BW9091; F- Stannous Chloride (12 µL) and *Escherichia coli* BW9091; G- Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* BW9091; H- Plant Extract (12 µL) and *Escherichia coli* AT25922; I- Stannous Chloride (12 µL) and *Escherichia coli* ATCC25922; J- Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* ATCC25922; K- Plant Extract (12 µL) and *Staphylococcus aureus* ATCC 8096; L-Stannous Chloride (12 µL) and *Staphylococcus aureus* ATCC 8096; M- Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Staphylococcus aureus* ATCC 8096.

From the analysis of variance (ANOVA), it was observed, in relation to the data analysis, that the value of p is <0.0001, considered extremely significant, that is, the variation between the columns is significantly higher than the expected. Analysis of the results from the Tukey-Kramer multiple comparison test allowed a value of p <0.001 when comparing the control with all groups.

When comparing the Vegetable Extract (12 µL) and *Escherichia coli* AB1157, Vegetable Extract (12 µL) and *Escherichia coli* BW9091, Vegetable Extract (12 µL) and *Escherichia coli* AT25922 and Vegetable Extract (12 µL) and *Staphylococcus aureus* ATCC 8096 with (0.9% NaCl), it was observed that there was an extremely significant difference (p <0.001) between the groups with the control group [Sodium Chloride (NaCl 0.9%)], expressing the microbicidal effect of the extract.

When comparing the Plant Extract (12 µL) and *Escherichia coli* AB1157 group with the Stannous Chloride (12 µL) and *Escherichia coli* AB1157 group, it was observed that there was no difference between the extract and stannous chloride (p> 0, 05), a similar result (p> 0.05) is found when comparing the Stannous Chloride (12 µL) and *Escherichia coli* AB1157 groups with the Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* AB1157 group.

Comparing the plant extract group (12 µL) and *Escherichia coli* BW9091 with the group Stannous chloride (12 µL) and *Escherichia coli* BW9091 it was observed that there was no difference between the action of the extract with that of stannous chloride (p> 0.05 However, when comparing the Stannous Chloride (12 µL) and *Escherichia coli* BW9091 group with the Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* BW9091 group, it was observed that the extract was able to (p <0.01) the effect of lethality of SnCl2 on strains of *E. coli* type BW9091.

The *Escherichia coli* ATCC25922 and *Escherichia coli* ATCC25922 groups showed a very significant difference (p <0.001) in the group Extract Vegetable (12 µL) and *Escherichia coli* ATCC25922, in relation to the higher lethality effect of (12 µL) and *Escherichia coli* ATCC25922 with the group Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Escherichia coli* ATCC25922, observed It was found that the plant extract was able to significantly decrease (p <0.01) the lethality of $SnCl_2$ on strains of *E. coli* ATCC25922.

When comparing the Plant Extract (12 µL) and *Staphylococcus aureus* ATCC 8096 groups with the Stannous Chloride (12 µL) and *Staphylococcus aureus* ATCC 8096 group, an extremely significant difference (p <0.001) was observed in relation to the lethality effect of the extract on *S. aureus* strain ATCC 8096 in comparison to the oxidative effect of $SnCl_2$. However, when comparing the Stannous Chloride (12 µL) and *Staphylococcus aureus* ATCC 8096 groups with the Stannous Chloride (12 µL) + Plant Extract (12 µL) and *Staphylococcus aureus* ATCC 8096 group, it can be observed that there is no significant difference between the (p> 0.05), indicating a possible interaction of the extract with the tin chloride which would reduce the effect of lethality of the extract on the strain of *S. aureus* ATCC 8096, in a way, maintaining unchanged the effect of the stannous chloride.

DISCUSSION

The use of natural products as coadjunters in the treatment of different pathologies has resurfaced, with emphasis, in recent times. A medicinal plant is one that contains one or more active principle that gives therapeutic activities, however, the active principle is a substance, or group of substances, responsible for certain reactions in the body. In this study where the biological effects of an aqueous extract of *Myrcia racemosa* on the fraction of lethality, from the measurement of the halos, of bacterial cultures in the presence and absence of the stannous chloride reducing agent, was evaluated, in principle, expressive bactericidal effect of said aqueous extract.

The cytotoxic and genotoxic effects of $SnCl_2$ have been demonstrated in different experimental models and these seem to be mediated by free radicals (El-Demerdash et al., 2005; Almeida et al., 2007). Moreno et al. (2004) reported that a Ginkgo biloba extract was able to protect the plasmid DNA from the $SnCl_2$-induced lesions. 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 $SnCl_2$, 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 DNA macromolecule; this association seems to lead to a preferential attack on the nitrogenous

bases, a fact that could be associated with a mutagenic tin potential (El-Demerdash et al., 2005).

Our results with an aqueous extract of *Myrcia racemosa* indicated that this extract exhibited microbicidal action in all bacterial strains studied, expressing a greater lethality effect on the *S. aureus* Gram-Positive strain ATCC 8096 (Figure 2), however it was possible to observe that the extract had a lower lethality effect on the ATCC25922 type strain and a higher lethality effect on the ATCC8096 type strain (Figure 2). These findings allow us to speculate that the greater action of the extract on the cell wall of Gram-positive bacteria could be related to a greater oxidation on the thick layer of peptidoglycans, thus denaturing the wall more intensely in comparison to the effect to the cell wall of the Gram-negative bacterium.

Calyptranthes tricona is a species (*Myrtaceae*) native to southern Brazil. The plants belonging to this family are folkloric used for analgesia, inflammation and infectious diseases. However, little is known about the toxic potential of many species belonging to this family. Kich et al. (2017) observed that the phenol content and the antioxidant activity only present in the ethanol extract produced from the plant also highlighted that phytochemical screening showed steroids, triterpenoids, condensed tannins and flavones as the main compounds. However, they emphasized that both the alcohol and leaf hexane extract were able to induce DNA damage dependent on human lymphocytes. In treating the cells with the extracts, both inhibited cell death in response to oxidative stress induced by H_2O_2. These results may be somewhat related to those we found, since the plant used in our study is from the family *Myrtaceae* and native to Brazil, possibly sharing phytochemical characteristics compared to *C. tricona*. as can be observed in figure 1, where we show that the extract when incubated together with the stannous chloride solution exerted a greater protective effect with respect to the action of $SnCl_2$ on the strain of type BW9091, emphasizing that despite the microbicide effect the extract also expresses an antioxidant potential, possibly depending on the oxidative stress variation of the medium, thus responding to compounds with cytotoxic and antioxidant activity.

Da Cunha et al. (2016) pointed out that an ethanolic extract from the leaves of *Eugenia uniflora L.* (Myrtaceae) presented some polyphenolic compounds with high content, such as quercetin, quercetin, isoquercitrin, luteolin and ellagic acid, which may be at least partially responsible for their beneficial effects in relation to treatment for intestinal disorders and hypertension. We highlight that quercetin and ellagic acid, as an example, are constituents common to the genus *Myrcia*, which denotes the antioxidant potential, also attributed to this genus, according to the results found in this study.

Our results would be in agreement with those observed by Fu et al (2016), where tropical fruits such as persimmon, guava (belonging to the family *Myrtaceae*) and conquered fruit had a very high antioxidant activity and also contained high levels of total phenolics. They indicated that the three tropical fruits possessed expressive antioxidant and antibacterial activity, which supported the possibility of introducing the fruits into a new category - functional foods, as well as new natural antimicrobial agents and food preservatives. In addition, phenolic compounds detected in fruits could be used as a potential natural antibacterial and antioxidant agent.

The results obtained in this work are similar to those reported by Papoutsi et al (2008), where they reported that the nut extracts exhibited concentration-dependent antioxidant capacity in an evaluation of the antimicrobial capacity against gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*); gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*) and fungi (*Candida albicans*, *Cryptococcus neoformans*).

Regarding the results found with the strain Staphylococcus aureus ATCC 8096, our results are similar to those described by Peixoto et al. (2006) suggested that, in relation to the strain of Staphylococcus aureus, the alcoholic extract of *Punica granatum* possibly exhibits cytotoxic, genotoxic, oxidizing and inhibitory action of the protein synthesis, effects that could allow the incorporation of the pomegranate extract as alternative to the treatment of diseases caused by this type of bacteria.

Another important consideration of our work, which is possible to suggest, is an important protective effect of *Myrcia racemosa* extract against stannous chloride (antioxidant) dependent on the level of oxidative stress, besides its microbicidal (cytotoxic) effect, which have not yet were described by other authors.

The different results found in this work, regarding the cytotoxic, microbicidal and antioxidant effects can be explained by the presence of different substances present in the aqueous extract of *Myrcia racemosa*, that depending on the concentration these compounds may be able to induce lesions in the cell wall of the bacteria, in bacterial DNA and / or even protect the same cell against chemical agents such as stannous chloride. Thus, some compounds such as terpenoids, present in the extract of *Myrcia racemosa* can demonstrate a cytotoxic effect mainly by apoptosis, as described by Fernandes et al. (2003), which could also be related to its bactericidal effect, according to which the results found in this work, besides being associated with the antioxidant effect.

CONCLUSION

According to the experimental data obtained and analyzed, we can speculate that in the aqueous extract of *Myrcia racemosa* it has substances with redox properties,

exhibiting a bactericidal effect as observed in the induction of lethality in the studied bacteria, besides the antioxidant effect dependent on the level of oxidative stress, according to the action of minimizing the effect of $SnCl_2$ on the mutant strain of E. coli BW9091. Other studies will be carried out to try to elucidate the mechanisms of action involved in the effects of the *Myrcia racemosa* extract on its different levels of toxicity.

Thanks

The authors are grateful for the important contribution they made to this work to the Foundation for Research Support of the State of Rio de Janeiro (FAPERJ) for the financial support.

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