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Original Research Article

Study of the Biological Effects of Aqueous Extracts of *Schinus terebinthifolius* Raddi, on the survival fraction of mutant and wild strains of *Escherichia coli*

[1]Silva E. J., [1]Nascimento C. C. H. C., [1,2]Nascimento S. F., [1,4]Vasconcelos, S. D. D. De, [5]Nogueira R. I., [3]Stephens P. R. S., [1]Barreto A. S. and [1,2*]Diré G. F.

[1]LaboratoryofChemicalandBiologicalAnalysis (LAQB), Foundation StateUniversity Center ofthe West Zone (UEZO), Avenue Manuel Caldeira de Alvarenga, Campo Grande, Rio de Janeiro, Brazil.

[2]Estácio de Sá University (UNESA), Rio de Janeiro, Brazil.

[3]Laboratory of Innovations in Therapies, Teaching and Bioproducts/LITEB, OswaldoCruz Institute, IOC/ FIOCRUZ), Rio de Janeiro, Brazil.

[4]Federal Center ofTechnologicalEducation Celso Suckow da Fonseca- CEFET-NI, Rio de Janeiro, RJ- Brazil

[5]Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Rio de Janeiro, Brazil.

***Corresponding Author's E-mail: Email:glauciodire@hotmail.com**

Abstract

The plants have been used by man for millennia without effective study that proves the exposure of the population to chemical substances and toxic agents present in these phytotherapeutic compounds. In this respect, biological tests allow the evaluation of these substances and their efficiency, in many cases, generally attributed to the various parts of the species used, as well as generating mechanisms that support and / or protect the population from exposure to the active principles that constitute such specimens. *Schinus terebinthifolius* Raddi belongs to the family *Anacardiaceae*, popularly known as "Aroeira", it is one of the 71 species of plants related in Renisus, being attributed to this species antimicrobial, anti-inflammatory, antifungal and insecticide actions. These activities are related to their chemical constitutions by having secondary metabolites, most of them represented by flavonoids, such as tannins, essential oils, saponins, alkaloids, terpenes and sesquiterpenes, substances which present in various parts of the plant, such as branches and leaves. As the majority of these substances are related to several popular uses. The objective of the present study was to evaluate the biological effects of aqueous extracts of *Schinus terebinthifolius* Raddi, on the survival fractions of mutant and wild strains of *Escherichia coli*. Branches and leaves were collected, selected, processed and extracted by infusion to obtain the extracts, the extracts were used in two strains of *E. coli* by the disc diffusion method. A 0.9% NaCl solution was used as the negative control. For antibacterial evaluation the positive control was attributed to antibiotics present in RENAME and in the antioxidant activity to stannous chloride and hydrogen peroxide. From the obtained results it can be concluded that the aqueous extracts contain phytochemical constituents that potentiated the effect of the antibiotics. In relation to the types of extracts studied, the dehydration presented the formation of inhibition halos for the mutant strain BW 9091, unlike the treatments with the *in natura* that there was no formation of inhibition halos, for both strains; which is related to the differences in concentrations of both extracts.

Keywords: *Schinus terebinthifolius* Raddi, *Escherichia coli*, Aqueous Extract, Phytotherapy, Rename, Renisus.

INTRODUCTION

The use of plants as food and treatment of diseases by the ancestors of modern man has always existed. Evidence that pre-agricultural communities already used them even before the field revolution shows a knowledge

of their nutritional and even medicinal properties (Honorio et al., 2016; Souza et al., 2016). With the development of societies, plants have become an important therapeutic resource for man, since they have numerous biologically active substances, which allow the synthesis of innumerable other synthetic substances for the treatment of the various diseases that affect humans (Guerra and Nodari, 2007; Ribeiro and Guimarães, 2013).

With the advancement of technology and the new notions of sustainability that have become a recurring practice since the twentieth century, the use of medicinal plants is increasing worldwide, especially in Brazil due to its immense vegetal biodiversity, which motivates new research in order to seek new drugs that meet the therapeutic needs and, at the same time, are safe for human health, as well as for all biological spheres (Bila and Dezotti, 2003; Guerra and Nodari, 2007).

Within the environmental sciences, the development of phytotherapeutic drugs stands out because it presents a safe and sustainable alternative, with molecules less aggressive environmentally and that meet the therapeutic needs, without the occurrence of bacterial resistance and persistent chemical residues. Therefore, new substances that act mainly as antimicrobials in therapeutic and laboratory practices have been researched (Calixto, 2000; Traesel et al., 2010).

Among these numerous substances are secondary metabolites such as essential oils, tannins, oil-resins and others such as terpenes, flavonoids, organic acids, and other chemical complexes (Lorenzi and Matos, 2002; Velázquez et al., 2003), are outstanding in the chemical apparatus that can be exploited for innumerable related researches, since they present diverse properties like anti-inflammatory, antioxidants, antimicrobial and numerous others that favor the investigation (Cowan, 1999).

In this aspect, the “aroeira”, *Schinusterebinthifolius* Raddi, a native species, belonging to the family *Anacardiaceae*, to which it presents many species with chemical peculiarities that make them necessary in popular medicine for presenting anti-inflammatory, cicatrizante, antidiarrheal and actions to contain gastric diseases , among others, but mainly due to the presence of some secondary metabolites such as tannins, oil-resins and others such as terpenes, flavonoids, organic acids, and other chemical complexes (Lorenzi and Matos, 2002; Velázquez et al., 2003).

This species is listed in the National List of Medicinal Plants of Interest to SUS (RENISUS); of a list with a list of medicinal plants that have the potential to generate products of interest to the Unified Health System and whose purpose is to direct studies and research that may subsidize the design of a list of medicinal and phytotherapeutic plants to be made available for use by the population, safely and effectively for the treatment of a particular disease (Brasil, 2014).

As previously mentioned, this species has many properties that make it a source of study for numerous researches, among them the microbiological aspects of bacterial assays with the use of strains of Escherichia coli, being outstanding the species of the genus Escherichia, the strains wild-type AB 1157 which are proficient in all DNA repair genes and BW 9091, mutant of the xthA gene, whose product, exonuclease III acts in the repair of oxidative lesions of DNA in exponential phase of growth (Silva et al., 2004), both used in this study.

The objective of this study was to evaluate the biological efficiency of two aqueous extracts of *Schinus terebinthifolius* Raddi on the survival fraction of mutant and wild Escherichia coli strains with associations of antibiotics broken down in the National List of Essential Medicines, RENAME (Brasil, 2017).

MATERIAL AND METHOD

Sampling and Obtaining Aqueous Extracts

The botanical material of *Schinus terebinthifolius* Raddi - aerial parts, in perfect phytopathological state, containing vigorous branches and leaves exposed to the sun - was sampled in the Santa Beatriz da Silva site, in the Guaratiba neighborhood, regional geographic subdivision Guaratiba Island, in the West Zone of the Municipality and State of Rio de Janeiro, 22°58'12.7"S and 43°33'05.8" W, at a topographic elevation of 87 meters on January 19, 2017, between 6 (six) and 7 (seven) hours, at 79% relative humidity and at room temperature of 29 °C.A voucher specimen was prepared and sent to the Herbarium of the Department of Botany of the Biology Institute of the Federal Rural University of Rio de Janeiro, UFRRJ, in the Municipality of Seropédica, RJ, registered in the *Index Herbariorum* with the abbreviation RBR, where it is conserved, under fall number RBR 39522. After sampling, the material was duly packed in low density polyethylene bags (PEBD) and taken to the Laboratory of Chemical and Biological Analysis, LAQB, of the Western State University Center Foundation - UEZO - , where the selection was made , sanitization, grinding for later extraction.

Preparation of the extract *in natura*

The leaves and branches of *S. terebinthifolius* were sanitized with distilled water, crushed with a pair of scissors, so that the material presented an average standard of 1 cm², weighed in a semi analytical balance, in the amount of 250 grams and put in infusion in a polyethylene box with a volume capacity of 12 liters for 60 minutes in 1000 ml of distilled water heated to 80 °C. The final concentration of the *in natura* extract was 0.242 $g.mL^{-1}$ (24.2%); pH 4.52 at a temperature of 28.1 °C. The

extract was sieved with the aid of a polypropylene sieve and polyester cloth of dimensions 310x160x84 mm and added in an amber glass vial with a volume of 1000 ml for later use. Then it was cooled to a constant temperature of -20 °C in a freezer for plasma.

Preparation of Dehydrated Extract

A part of the *in natura* material was set to dry for five days, placed on the LAQB bench at room temperature, and after that period, the dehydrated leaves and branches of *S. terebinthifolius*, in perfect sanitary condition, were crushed in a domestic blender , so that the material had an average standard of 0.2 cm², weighed in a semi analytical balance, the amount of 250 grams and was infused in a glass container, with volumetric capacity of 3 liters, for 60 minutes in 1000 mL of distilled water heated to 80 °C. The final concentration of the dehydrated extract was 0.250 $g.mL^{-1}$ (25%); pH 4.65 at 25.8 °C.

The extract was sieved in order to remove the coarse particulate with the aid of a polypropylene sieve and polyester screen of dimensions 310 x 160 x 84 mm, filtered by gravity on qualitative filter paper, grade 1, 11 µm, Whatman GR 40, for removal of suspended particles, and added in an amber glass vial, with a volume of 1000 mL, for later use. 100 mL were fractionated in smaller vials with capacity of 10 mL, in order to facilitate the use in the experiments. Then it was cooled to a constant temperature of -20 °C in a freezer for plasma.

Preparation of the Culture Medium for the Antimicrobial Sensitivity Assay (Disco-Diffusion)

Weighed 15.2g of dehydrated medium of Muller Hinton Agar and added 0.4L of distilled water and homogenized for complete dissolution of the product. The culture medium solution was then autoclaved at 121 °C for 15 minutes at 101325 Pa (1atm) pressure. In a biological safety cabinet, 50 ml of the culture medium was poured into 150 mm glass Petri dishes with a volumetric pipette and cooled to room temperature until solidified. The plates were packed with flexible PVC transparent films and stored in a refrigerator at 5 °C until use in the experiments.

Reactivation of *Escherichia coli* strains

For the activation of the bacterial strains, with the aid of a 10 µL disposable bacteriological loop, samples of each bacterium were taken from the refrigerated stock (15% Glycerol in TSB Broth - Soy Tripitone), each placed in a tube containing 3 mL of trypticase soy broth. The tubes were sealed, homogenized and incubated in a bacteriological oven at 37 °C for 24 hours. After the incubation period, the activated material was seeded by depletion in a 90 mm Petri dish containing Müller Hinton agar medium with the aid of a 10 µL bacteriological loop and again incubated. After the 24 hour period within the biological safety booth, samples of colony forming units (UFC) were collected with the aid of a 10 µL disposable bacteriological loop and inserted into capped test tubes containing 5 ml of physiological solution (NaCl 0.9%) until reaching the McFarland scale turbidity pattern, whose 0.5 index corresponds to $1,5x10^{8}UFCmL^{-1}$, allowing the appropriate dilution level for sowing by exhaustion in Petri dishes of 150 mm with the aid of disposable swabs in the experiments.

Bacterial strains evaluated

In order to carry out the experiments, the bacterial strain of *Escherichia coli*: AB 1157, wild-type, was tested with all efficient genetic repair mechanisms of DNA and BW 9091, mutant of the xthA gene, whose product, exonuclease III acts in the repair of oxidative lesions of DNA in exponential phase of growth (Silva et al., 2004).

Antimicrobial Sensitivity Test (Disco-Diffusion)

In order to evaluate the sensitivity of bacterial strains to the action of the extracts, the antimicrobial susceptibility test was used, corresponding to the method most widely used in the microbiology laboratory, since it allows to analyze the susceptibility of the microorganisms against different antimicrobial agents, in a fast way is safe (Sejas et al., 2003).

In the conduction of the experiment, both the fresh and dehydrated aqueous extract, *Escherichia coli*, strains AB 1157 and BW 9091, previously activated as previously described, were used. In the plating, 150 mm Petri dishes containing 50 ml of agar medium were used. 18 plates were prepared, separated into 6 groups, all in triplicates; to each plate were added five discs, except those of the last groups, in which only 3 discs were positioned, making a total of 26 discs. (Peixoto et al., 2014).

Discs containing the antibiotics Chloramphenicol, Ticarcillin/Clavulanic Acid and Ampicillin Sulbactam were purchased from OxoidBrasil Ltda. and have pre-determined concentrations, in the order of: 30 $\mu g.mL^{-1}$ for discs impregnated with Chloramphenicol; 85 $\mu g.mL^{-1}$ for Ticarcillin/Clavulanic Acid; 20 $\mu g.mL^{-1}$ for disks containing Ampicillin Sulbactam.

The discs impregnated with Amoxicillin were previously prepared in the LAQB, from commercial tablets of the Neo-chemical laboratory in the concentration of 250 $mg.5mL^{-1}$. The solution was prepared from the dissolution of one tablet in 1 ml of distilled water.

After these procedures, the 150 mm Petri dishes were removed from the refrigerator, placed on the stand for 30 minutes until they reached room temperature and the excess moisture was absorbed, then they were marked with marking pen, arranged in each group, numbered the disks and dated. After this interval, in a biological safety booth, with the aid of sterile swabs, the previously activated bacterial strains were inoculated in the format of striations on the surface of the agar in three directions, thus allowing the complete depletion of the surface of the culture medium. After this step, the sterile paper disks were applied with a sterile forceps to avoid contamination. All discs were gently pressed allowing full contact with the surface of the agar. The distances of 30 mm between one disc and another and 15 mm of the edge of the plate were maintained, preventing the overlapping of inhibition halos (Sejas et al., 2003).
At the end of sowing, the plates were stored in a bacteriological oven for 24 hours at 35 °C. In reading the results we used a ruler to measure halos in antibiogram.

Statistical analysis

The results obtained were analyzed with the help of GraphPad (GraphPad Software, Inc., USA) statistical software from the Variance Analysis (ANOVA) tests. The arithmetic averages were compared in order to evaluate the proportionality between volume, treatments of the compounds associated with the aqueous extract of *S. terebinthifolius* and the diameter of the inhibition halo. And the Tukey-Kramer test compared the arithmetic means of the inhibition halos of the different treatments, establishing the significance for $p < 0.05$.

RESULTS AND DISCUSSION

Antibacterial activity

Aqueous extracts from branches and leaves of *Schinus terebinthifolius* were tested against 2 bacterial cultures by the agar diffusion method. Studies based on the literature show that several secondary metabolites identified in the species may be responsible for their antibacterial action, such as alkaloids (Ceruks et al., 2007) and terpenes (Malik et al., 1994), as well as the sesquiterpenes, substances present in greater quantity in the leaves (Barbosa et al., 2007). Recent investigations have demonstrated antimicrobial activity (Degáspari et al., 2005; Johann et al., 2008; Paiva et al., 2010; Machado et al., 2012; Gomes et al., 2013). In relation to the *E. coli* strain BW 9091 which was treated with the dehydrated extract, the formation of inhibition halos was observed and this result can be related to the considerations of Silva et al. (2004) that describe this bacterium as being mutant for the gene xthA, whose product, exonuclease III, acts in the repair of oxidative lesions of the DNA in exponential phase of growth, establishing a possible antibacterial action of the extract to present as constituents phytochemical alkaloids (Ceruks et al., 2007) and terpenes (Malik et al., 1994), as well as the polyphenolic compounds sesquiterpenos and flavonoids, substances present in greater quantity in the leaves (Barbosa et al., 2007). These results corroborate with those that were found in the Degáspari et al., (2005); Johann et al., (2008); Paiva et al., (2010); Machado et al., (2012) and Gomes et al., (2013) which in recent analogous investigations have demonstrated antimicrobial activity related to this plant species, associated to these metabolic compounds. In addition, phytochemical investigations carried out by Carvalho et al. (2008), testing different extracts, indicated that in relation to aqueous extract analysis, water is the most suitable solvent for the extraction of constituents in relation to the spectral range of 225 at 420 nm, which was analyzed. In this study, phenolic compounds as tannins, flavonoids, coumarins, cardiotonicheterosides, triterpenes, reducing sugars and steroids were found, which may be related to the antibacterial action of the aqueous extracts tested.

In combinations with antibiotics the aqueous extract of *S. terebinthifolius* was more effective when compared to the isolated antibiotics. In relation to the results obtained, related to the increase of halo, the synergic action, ie, a possible drug interaction between the phytocomplexes of the natural extract and the main functional groups of the antibiotics tested may be present. Some of the best sellers, derived from the secondary metabolism of *S. terebinthifolius*, interfere with the mechanism of action of antibiotics, resulting in potentiation of their pharmacological activities (Alexandre, Bagatini & Simões, 2008). Phytotherapeutic molecules can interact with these compounds, resulting in negative activities on the bacterial organism, which could justify the formation of the inhibition halos on the *E. coli* strain BW 9091 which was treated with the dehydrated extract, since herbal compounds are composed of complex mixtures of various chemical heterocytes that may be responsible for various actions such as antagonistic or synergistic effects with other medicinal products as a result of the interaction of several active chemical constituents (Williamson, 2005). However, in many cases, the chemical constituents responsible for the pharmacological activities of medicinal plants may increase the possibility of interactions when drugs are used concomitantly (Fugh-Berman & Ernst, 2001; Nicoletti et al., 2007). According to Nascimento et al., (2000) the by-products of medicinal plants may prevent, increase or even exert no effect on the medicinal effect of certain conventional compounds. In a review of the literature by Alexandre, Bagatini&Simões (2008), they verified that herbal medicines may interfere with the pharmacokinetics and/or pharmacodynamics of several drugs. Teles and Costa

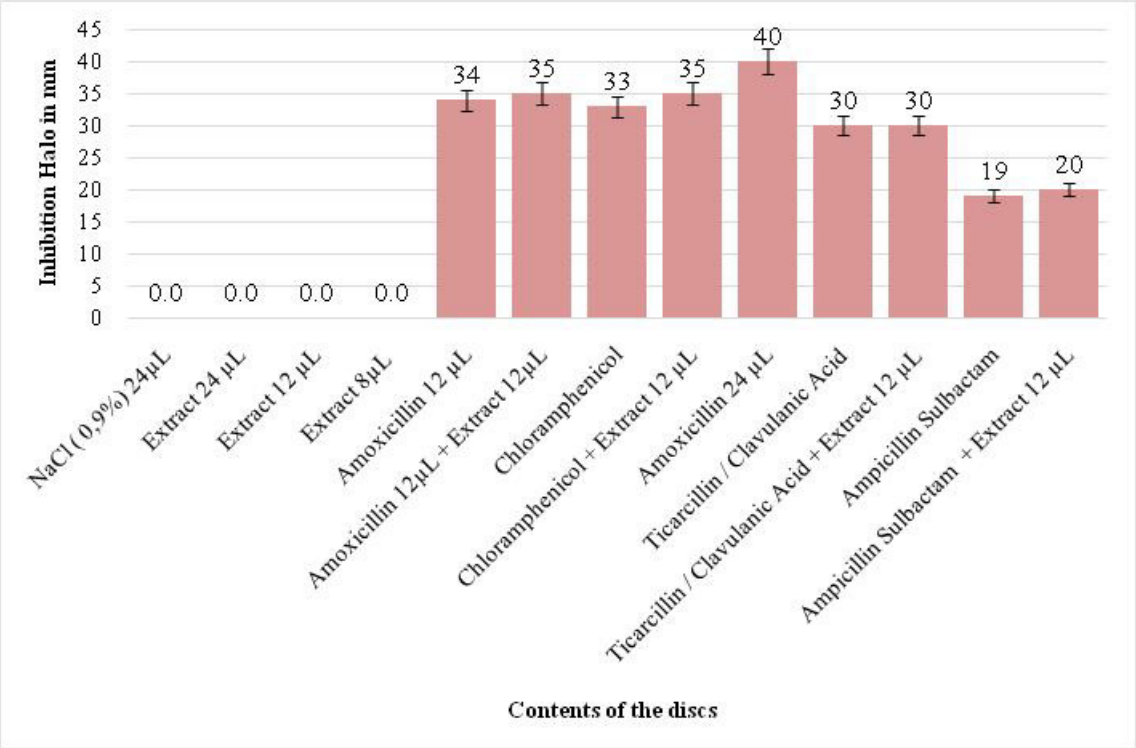

Figure 1. Correlation of the various treatments using different volumes associated or not to the *in natura* aqueous extract of *Schinus terebinthifolius* Raddi and the bacterial growth inhibition halos for strain AB 1157.

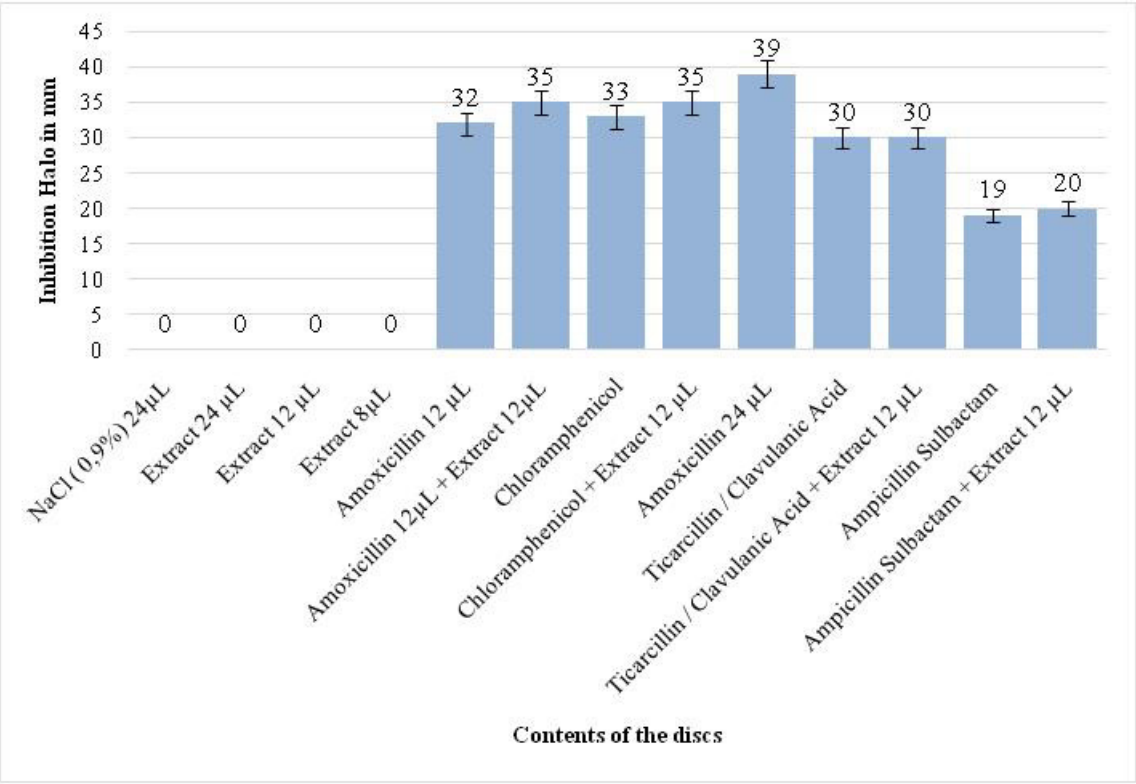

Figure 2. Correlation of the various treatments using different volumes, associated or not to the *in natura* aqueous extract of *Schinus terebinthifolius* Raddi and the bacterial growth inhibition halos for strain BW 9091.

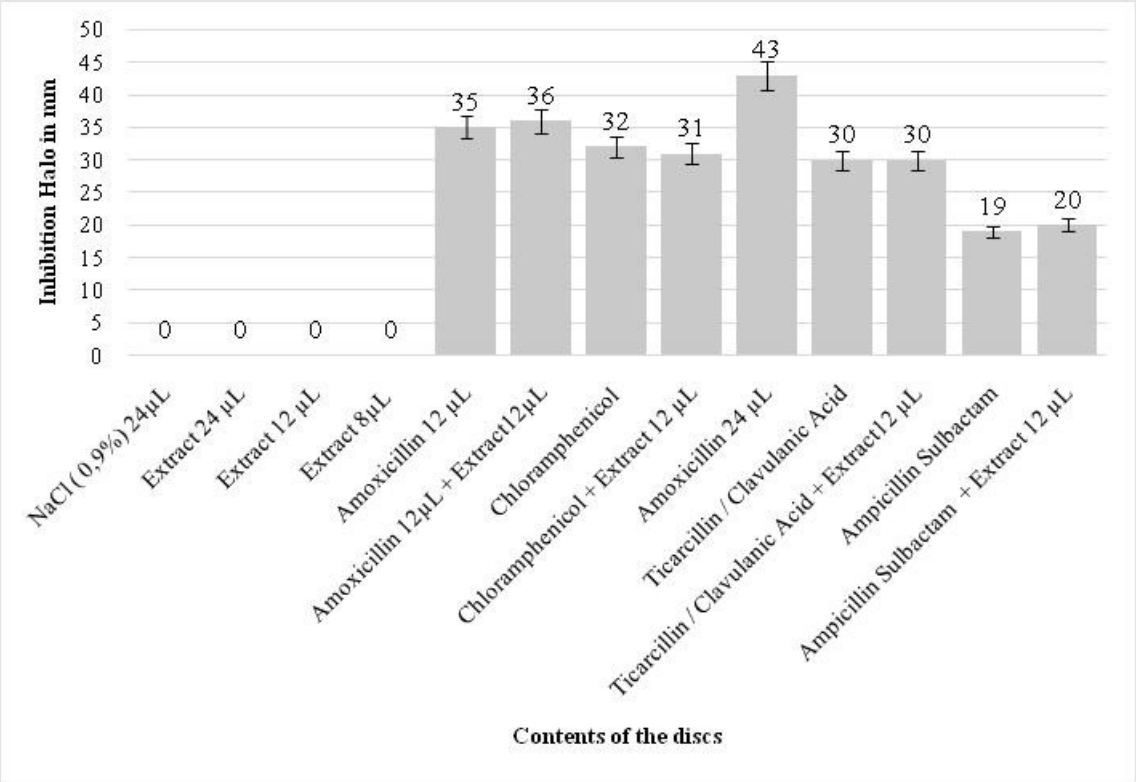

Figure 3. Correlation of the various treatments using different volumes, associated or not to the dehydrated aqueous extract of *Schinus terebinthifolius* Raddi and the bacterial growth inhibition halos for strain AB 1157.

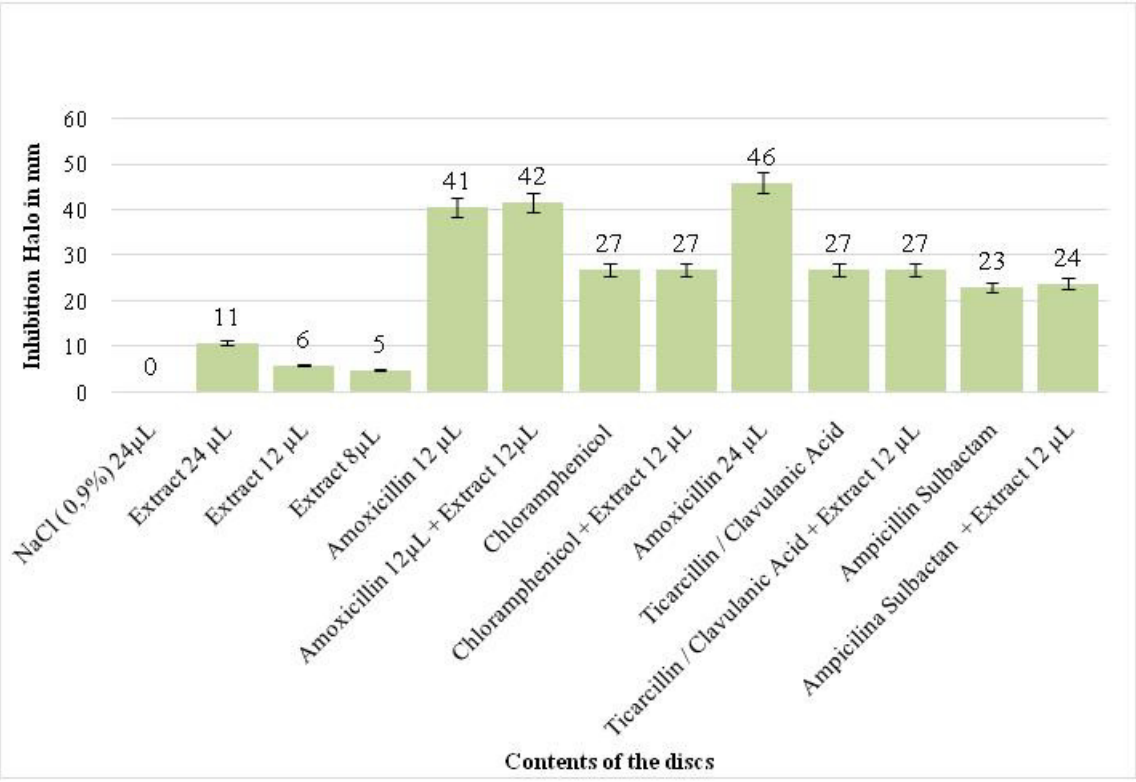

Figure 4. Correlation of the various treatments using different volumes, associated or not to the dehydrated aqueous extract of *Schinus terebinthifolius* Raddi and the bacterial growth inhibition halos for strain BW 9091.

(2014) to determine the existence of interactions and interferences in the combination of two aqueous extracts of *Punicagranatum* and *Plantago major*, on strains of *E. coli*, concluded that there is interaction between the extracts and the antibiotic tested, amoxicillin, which also may occur with other antibiotics associated with other extracts from other plants. These interactions may be related to the fact that antibiotics have many hydroxyl functional groups (-OH), a polar radical, which allows hydrocarbons, even nonpolar, to interact well with phytocomplexes, which in many molecular arrangements also present numerous polar radicals. Thus, these hydrocarbons form heterogeneous systems with water. In addition, in all cases, the polarity of the molecule will depend on various chemical factors, as well as the spatial arrangement of atoms and the presence of non-binding electrons. Many of these compounds with a -OH-functional group bonded to saturated carbon atoms are soluble in polar solvents because of the association of the hydrogen bonds. Thus, while many secondary plant metabolites are formed by phenolic compounds, the drug interaction may be more present since the phenols have the -OH group attached to a carbon atom of an aromatic ring thereby forming hydrogen bonding with water and increasing its solubility. Thus, the various organic functions give rise to a vast set of complex molecules whose solubility and interaction are directly related to their respective chemical structures. In this way, many biological processes are directly related to the solubility of the organic substances, and with the possibility of formation of the hydrogen bonds, which make them highly soluble in the aqueous phase (Martins, Lopes and Andrade, 2013).

When we searched several databases using the keywords: *Schinus terebinthifo*lius Raddi, antibiotic and the *E. coli* species tested, we found studies that evaluated the antibacterial property of this species, which demonstrated significant results regarding the bactericidal effect related to the aforementioned natural product (GOMES et al., 2013; SOUSA et al., 2013; Uliana et al., 2016; Ennigrou et al., 2017; Silva et al., 2017).

The type of aqueous extract used in the present study is in accordance with the considerations Santos et al. (2015), which affirm that there is no systematization that allows a correlation between the parts of the plant used and the type of extract tested with the antimicrobial property since the substances present in the various parts of the plant tissues, have a wide range of extraction, in the different types of extractors. In addition, the leaves appear to be the main part of the plant studied, with an antimicrobial property superior to the bark and the fruits, however, many studies use different parts of the plants in several types of extracts, such as the aqueous obtained from branches and leaves , as in the present study (Guerra et al., 2000; Freires et al., 2011; Alves el al., 2003; Degáspari et al., 2005). Chemical investigations have shown that polyphenolic compounds as flavonoids are the major constituents of leaf extracts of *S. terebinthifolius* (Farag, 2008; El-massry et al., 2009; Santana et al., 2012; Silva et al., 2017), which may be related to the formation of the inhibitory halos for the BW 9091 bacterial strain when tested against the fraction of the dehydrated aqueous extract, since studies by Verma et al. (2013) have shown that high concentrations of flavonoids induce to numerous biological alterations, among them alterations in the cellular membranes to significant damages the structure of the chromosomes (Porto et al., 2013; Silva et al., 2013; Giuliani et al., 2014). (Figure 1-4)

Antioxidant Correlation

In the present study, the antioxidant action of the aqueous extract of *S. terebinthifolius* leaves was determined by the agar diffusion method, where the positive controls for the action were tin chloride ($SnCl_2$) and hydrogen peroxide (H_2O_2). In the literature, we report antioxidant activity in experiments performed with aqueous extracts obtained from “aroeira” fruits (Degáspari et al., 2004); Gomes et al. (2010), using different extractors, determined antibacterial effect on *E. coli*. Such positive results were predicted, since studies have been reported in phenolic groups present in the studied species that have antioxidant activity, and can be affirmed due to the presence of flavonoids (Bobin et al., 1995; Degáspari et al., 2004; Souza et al., 2005; Ribas et al., 2006; Gomes et al., 2010) and other polyphenolic compounds (Lorenzi and Matos, 2002; Velázquez et al., 2003).

In relation to the experimental tests, it was observed that there was no antioxidant effect of the aqueous extract of the branches and leaves of *S. terebitnhifolius*, since there was no inhibition of the action of tin chloride on the strain BW 9091, whose characteristic is the sensitivity for this purpose (Silva et al., 2004) and AB 1157. It was also observed in relation to the two strains that the concomitant use of the extract plus stannous chloride and hydrogen peroxide did not significantly influence the action of the latter component when compared to the unit exposure. (Figure 5-8)

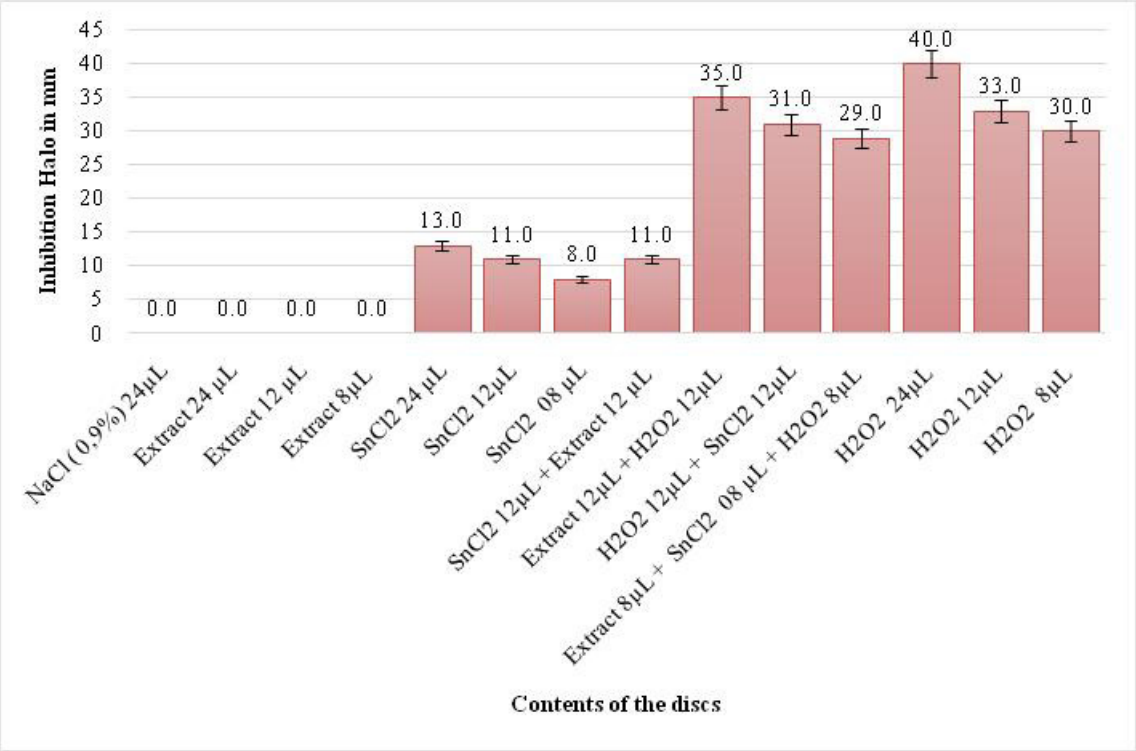

Figure 5. Correlation of the various treatments using different volumes, associated or not to the *in natura* aqueous extract of Schinus terebinthifolius Raddi and the bacterial growth inhibition halos for strain AB 1157.

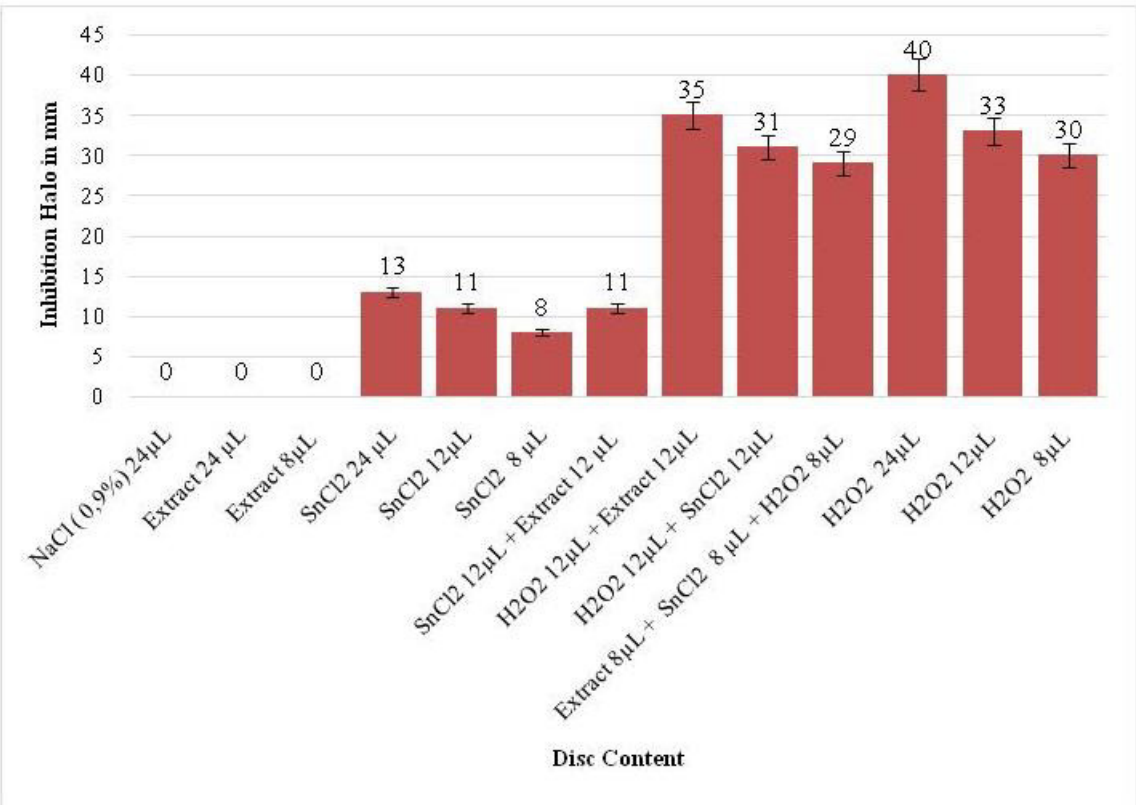

Figure 6. Correlation of the various treatments using different volumes, associated or not to the *in natura* aqueous extract of *Schinus terebinthifolius* Raddi and the bacterial growth inhibition halos for strain BW 9091.

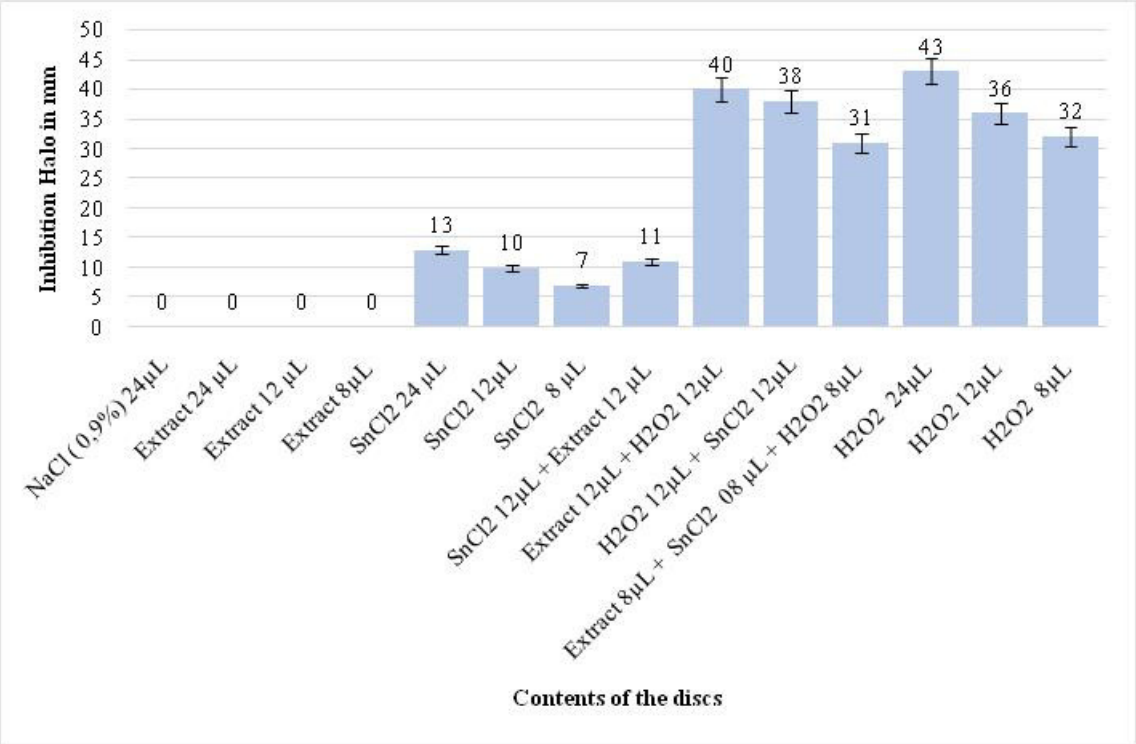

Figure 7. Correlation of the various treatments using different volumes, associated or not to the dehydrated aqueous extract of *Schinus terebinthifolius* Raddi and the bacterial growth inhibition halos for strain AB 1157.

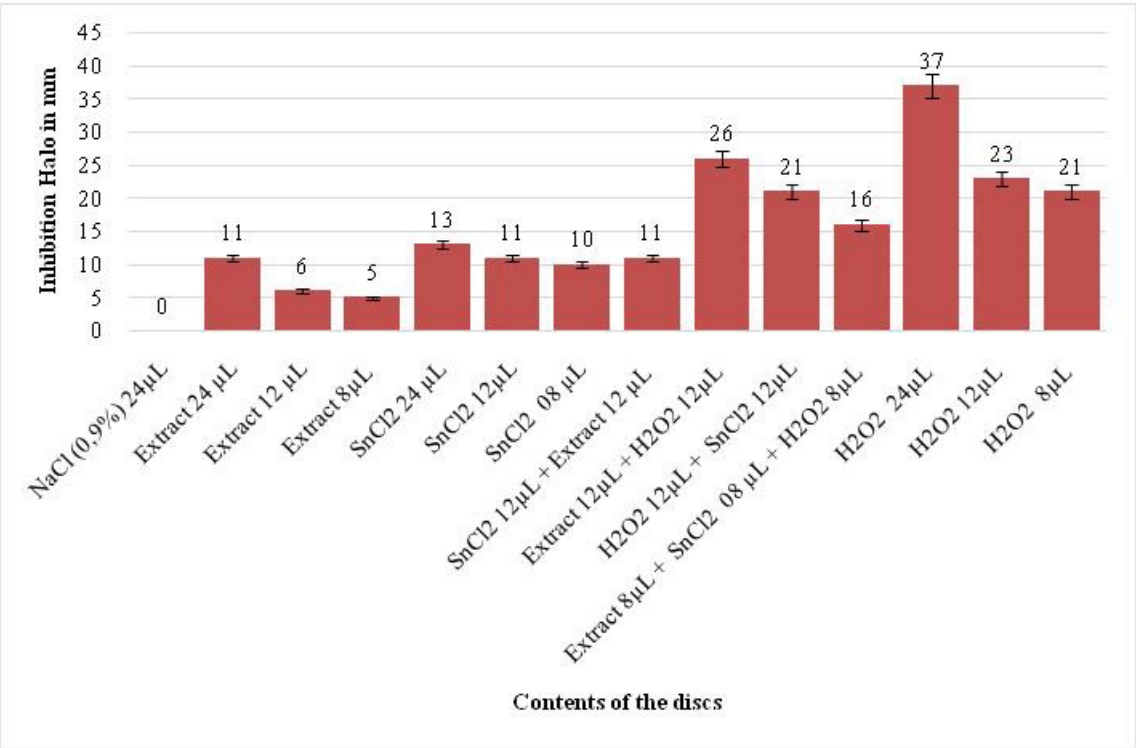

Figure 8. Correlation of the various treatments using different volumes, associated or not to the dehydrated aqueous extract of *Schinus terebinthifolius* Raddi and the bacterial growth inhibition halos for strain BW 9091.

CONCLUSION

From the obtained results it can be suggested that possibly the aqueous extracts studied present phytochemical constituents that in association with the antibiotics, present certain drug synergism which potentiated the effect of the antibiotics. In relation to the types of extracts studied, the dehydration presented the formation of inhibition halos for the mutant strain BW 9091, unlike the treatments with the *in natura* that there was no formation of inhibition halos, for both strains; which is related to the differences in concentrations of both extracts.

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REFERENCES

ALEXANDRE RF, BAGATINI F, SIMÕES CMO (2008). Interações entre fármacos e medicamentos fitoterápicos à base de *ginkgo* ou *ginseng*. Revista Brasileira de Farmacognosia, [s.l.], v. 18, n. 1, p.117-126, mar.

BARBOSA, D. F.; LEMOS, P. C. P. A medicina na Grécia antiga. Revista de Medicina, [s.l.], v. 86, n. 2, p. 117 - 119, 20 jun. 2007.

BILA DM, DEZOTTI M (2003). Fármacos no meio ambiente. Quím. Nova, São Paulo, v. 26, n. 4, p. 523-530, Aug

BOBIN MF, RAYMOND M, MARTINI MC (1995). Propriedades de absorção UVA/UVB de produtos naturais. CosmetToil (edição em português) 7: 44-50

BRASIL. Consulta pública n° 37, de 5 de setembro de 2017. Secretaria de Ciência, Tecnologia e Insumos Estratégicos; Diário Oficial da União. Brasília, DF, Nº 173, p 710, 05 set. 2017.

BRASIL; Ministério da Saúde; Portal da Saúde. Plantas de Interesse ao SUS. 2014. Disponível em: <http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/leia-mais-o-ministerio/465-sctie-raiz/daf-raiz/ceaf-sctie/fitoterapicos-cgafb/l1-fitoterapicos/12552-plantas-de-interesse-ao-sus >. Acessoem: 03 nov. 2017.

CALIXTO JB (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian J. Med. Biol. Res. [s.l.], v. 33, n. 2, p.179-189, fev..

CARVALHO CA, et al. (2008). Estudo espectrométrico de diferentes estágios fenológicos da *Brassicaoleracea* var. *capitata*. Revista Brasileira de Farmacognosia, [s.l.], v. 18, n. 2, p.249-257, jun.

CERUKS, M. et al. (2007). Constituintes fenólicos polares de *Schinus terebinthifolius* Raddi (*Anacardiaceae*).. Química Nova, [s.l.], v. 30, n. 3, p. 597-599.

COWAN MM (1999). Plant Products as Antimicrobial Agents. Clin.Microbiol.Rev., [s.l], v. 12, n. 4, p.564-582, out.

DEGÁSPARI C. H, et al. (2004). Atividade Antioxidante de Extrato de Fruto de Aroeira (*Schinus terebinthifolius* Raddi). Visão Acadêmica, Curitiba, v. 5, n. 2, p.83-90, dez..

EL-MASSRY KF, et al. (2009). Chemical compositions and antioxidant/antimicrobial activities of various samples prepared from *Schinus terebinthifolius* leaves cultivated in Egypt. J Agric Food Chem, v. 57, p. 5265–5270.

ENNIGROU, A. et al. (2017). Maturation-related changes in phytochemicals and biological activities of the Brazilian pepper tree (*Schinus terebinthifolius* Raddi) fruits. South Afr. J. Botany, [s.l.], v. 108, p.407-415, jan.

FARAG SF (2008). Polyphenolic compounds from the leaves of *Schinus terebinthifolius* Raddi. Bull. Pharm. Sci, [s.l.], v. 31, p. 319-329.

FREIRES IA, et al. (2011). Atividade antifúngica de *Schinus terebinthifolius* (Aroeira) sobre cepas do gênero *Candida*. Revista Odontológica do Brasil Central, [s.l.], v. 20, n. 52.

FUGH-BERMAN A, ERNST E (2001). Herb-drug interactions: Review and assessment of report reliability. British J. Clin. Pharmacol. [s.l.], v. 52, n. 5, p.587-595, 20 dez.

GIULIANI, C. et al. The flavonoid quercetin inhibits thyroid-restricted genes expression and thyroid function. Food and Chemical Toxicology, [s.l.], v. 66, p.23-29, abr. 2014.

GOMES FS. et al. (2010). Isolation and Antimicrobial Activity of Lectin from Schinus terebinthifolius Leaves. J. Biotechnol. [s.l.], v. 150, p.453-453, nov.

GOMES FS. et al. (2013). Antimicrobial lectin from *Schinus terebinthifolius* leaf.Journal of Applied Microbiology.[s.l.], v. 114, n. 3, p. 672-679.

GUERRA MP, NODARI RO (2007). Biodiversidade aspectos biológicos, geográficos, legais e éticos. In: Simões, C. M. O., Schenkel, E. P., Gosmann, G., Mello, J. C. P., Mentz, L. A., Petrovick, P. R. (Ed.). Farmacognosia: da planta ao medicamento. 6. ed. Porto Alegre: Editora da UFRGS, p.14-28

HONORIO ICG, BERTONI BW, PEREIRA AMS (2016). *Uncaria tomentosa* and *Uncariaguianensis* anagronomic history to be written. Cienc. Rural, Santa Maria, v. 46, n. 8, p. 1401-1410, aug.

JOHANN S. et al. (2008). Inhibitory effect of extracts from Brazilian medicinal plants on the adhesion of *Candida albicans* to buccal epithelial cells.World J. Microbiol. Biotechnol. [s.l.], v. 24, n. 11, p. 2459-2464.

LORENZI H, MATOS FJA (2002). Plantas medicinais no Brasil: nativas e exóticas. 2. ed. São Paulo: Instituto Plantarum;.

MACHADO, J.A. et al. (2012). In vitro Evaluation of the antimicrobial potential association of *Schinus terebinthifolius*Raddi and *Syzygiumaromaticum* L. J. Pharm. [s.l.], v.2, n. 3, p. 438-443.

MALIK, M.S. et al. (1994). Studies on the essential oil of *Schinus terebinthifolius*. Science International. [s.l.], v. 6, n. 4, p. 351-352

MARTINS CR, LOPES WA, ANDRADE JB (2013). de. Solubilidade das substâncias orgânicas. Química Nova, [s.l.], v. 36, n. 8, p.1248-1255.

NASCIMENTO GGF, et al. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian J. Microbiol. [s.l.], v. 31, n. 4, p.247-256, out.

NICOLETTI, Maria Aparecida et al. (2007). Principais interações no uso de medicamentos fitoterápicos. Infarma, [s.l.], v. 19, n. 1/2, p. 32-40.

PAIVA PMG.; et al. (2010). Antimicrobial activity of secondary metabolites and lectins from plants. In: MENDEZ-VILLAS, A. Current research, technology and education topics in applied microbiology and microbial biotechnology. FormatexResearch Center: Espanha, p. 396-406.

PEIXOTO AG. et al. (2014). Chemical Analysis of Biological Effects of a Hydroalcoholic Extract PunicaGranatum (Pomegranate). Innovative J. Med. Health Sci, v. 4, n. 6, p. 172-179,

PORTO, Luiz Carlos Santos et al. (2013). Evaluation of acute and subacute toxicity and mutagenic activity of the aqueous extract of pecan shells [*Caryaillinoinensis* (Wangenh.) K. Koch]. Food and Chemical Toxicology, [s.l.], v. 59, p.579-585, set.

RIBAS, Marina de Oliveira et al. Efeito da *Schinus terebinthifolius* Raddi sobre o processo de reparo tecidual das lesões ulceradas induzidas na mucosa bucal do rato. Revista Odonto Ciência: Fac. Odonto/PUCRS, [s.l], v. 21, n. 53, p.245-252, jul. 2006.

RIBEIRO KS, GUIMARÃES ALA (2013). O uso de medicamentos à base de plantas medicinais por médicos do SUS no município de Teresópolis/RJ. Revista Agrogeoambiental, Pouso Alegre, Edição Especial n. 1, p. 61-65, ago.

SANTOS, M. R. G. et al. (2015). Brief review on the medicinal uses and antimicrobial activity of different parts of *Schinusterebinthifolius*raddi. Int. J. Pharm. Pharm. Sci. [s.l.], v. 7, n. 12, p.1-7, out.

SEJAS, Lilian M. et al. (2003). Avaliação da qualidade dos discos com antimicrobianos para testes de disco-difusão disponíveis comercialmente no Brasil. Jornal Brasileiro de Patologia e Medicina Laboratorial, [s.l.], v. 39, n. 1, p.27-35

SILVA, C. R., et al. (2004). Absence of mutagenic and citotoxic potentiality of *senna* (*Cassia angustifolia*Vahl.) evaluated by microbiological tests. Revista Brasileira de Farmacognosia, [s.l.], v. 14, p. 1-2

SILVA, M. M. da et al. (2017). *Schinus terebinthifolius*: phenolic constituents and in vitro antioxidant, antiproliferative and *in vivo* anti-inflammatory activities. Revista Brasileira de Farmacognosia, [s.l.], v. 27, n. 4, p.445-452, jul.

SOUZA TM. et al. (2005). Avaliação da atividade fotoprotetora de *Achilleamillefolium* L. (*Asteraceae*). Revista Brasileira de Farmacognosia, [s.l.], v. 15, n. 1, p.1-5, mar.

SOUZA, L.F. et al .Plantas medicinais referenciadas por raizeiros no município de Jataí, estado de Goiás. Rev. bras. plantas med., Botucatu, v. 18, n. 2, p. 451-461, Junho 2016.

TELES DG, COSTA MM. (2014). Estudo da ação antimicrobiana conjunta de extratos aquosos de Tansagem (*Plantago major* L., *Plantaginaceae*) e Romã (*Punica granatum* L., *Punicaceae*) e interferência dos mesmos na ação da amoxicilina *in vitro*. Revista Brasileira de Plantas Medicinais, [s.l.], v. 16, n. 21, p.323-328

TRAESEL, Carolina Kist et al. (2010). Serum biochemical profile and performance of broiler chickens fed diets containing essential oils and pepper. Comparative Clinical Pathology, [s.l.], v. 20, n. 5, p.453-460, 30 maio

ULIANA, M. P. et al. Composition and biological activity of Brazilian rose pepper (Schinus terebinthifolius Raddi) leaves. Industrial Crops And Products, [s.l.], v. 83, p.235-240, mai. 2016.

VELÁZQUEZ, et al. (2003). Antioxidant activity of Paraguayan plant extracts. Fitoterapia, [s.l.], v. 74, n. 1-2, p.91-97, fev..

VERMA M., et al. (2013). Pharmacological evaluation of hyperin for antihyperglycemic activity and effect on lipid profile in diabetic rats. Indian J Exp Biol. 51(1): 65-72.

WILLIAMSON, E. M (2005). Interactions between herbal and conventional medicines. Expert Opinion on Drug Safety, [s.l.], v. 4, n. 2, p.355-378, mar.