

Chemical profile, bactericidal in vitro potential and toxicity against *Artemia salina* Leach of essential oils obtained from natural condiments

Perfil químico, potencial in vitro bactericida e toxicidade frente *Artemia salina* Leach de óleos essenciais obtidos de condimentos naturais

Perfil químico, potencial bactericida in vitro y toxicidad contra *Artemia salina* Leach de aceites esenciales obtenidos de condimentos naturales

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Abstract

Interest in essential oils and their food applications has grown due to the negative reaction of consumers to synthetic chemical additives intentionally added in industrialized products in order to increase their shelf life. In this context, the present study aimed to evaluate the chemical profile, toxicity to *Artemia salina* Leach and antibacterial activity in vitro of essential oils obtained from natural condiments on bacteria of clinical and food importance. Plant material was obtained in the municipality of São Luís-MA. The essential oils were extracted by hydrodistillation at 100 °C/3 h. Folin Ciocalteu methodology was performed for the determination of total phenolics. The toxicity assay was performed using the artemia salina Leach lethality bioassay. Antimicrobial activity followed the methodology described by the Clinical and Laboratory Standards Institute using the Broth Disc Diffusion and Dilution Method for the action of essential oils against the bacteria *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus* and *Bacillus cereus*. The presence of bioactive classes in the plant materials used in this research was identified and the essential oils obtained were classified as nontoxic in the toxicity assay, presenting low lethality to the micro crustacean *Artemia salina* Leach. In the bactericidal activity assay, the essential oils of *O. vulgare*, *T. vulgaris*, *C. zeylanicum* showed significant results, classified as efficient against the microorganisms tested. Finally, the use of essential oils classified as active and nontoxic is highlighted in this study as alternatives in the control and combat of pathogenic microorganisms presenting a proposal of natural product with low cost of obtaining and high market potential.

Keywords: Essential oils; Antimicrobial; Condiments.

Resumo

O interesse sobre óleos essenciais e suas aplicações em alimentos tem crescido devido à reação negativa dos consumidores em relação aos aditivos químicos sintéticos adicionados intencionalmente em produtos industrializados com a finalidade de aumentar a vida útil dos mesmos. Nesse contexto, o presente estudo teve por objetivo avaliar o perfil químico, a toxicidade frente a *Artemia salina* Leach e atividade antibacteriana in vitro de óleos essenciais de obtidos de condimentos naturais sobre bactérias de importância clínica e alimentar. O material vegetal foi obtido no município de São Luís-MA. Os óleos essenciais foram extraídos por hidrodestilação a 100 °C/3 h. Para a determinação dos fenólicos totais executou-se a metodologia de Folin Ciocalteu. O ensaio de toxicidade foi realizado através do bioensaio de letalidade frente *Artemia salina* Leach. A atividade antimicrobiana seguiu a metodologia descrita pelo Clinical and Laboratory Standards Institute utilizando o Método de Difusão de Disco e Diluição em Caldo para ação dos óleos essenciais frente às bactérias *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus* e *Bacillus cereus*. Identificou-se a presença de classes bioativas nos materiais vegetais utilizados nesta pesquisa e os óleos essenciais obtidos foram classificados como atóxicos no ensaio de toxicidade, apresentando baixa letalidade ao micro crustáceo *Artemia salina* Leach. No ensaio de atividade bactericida, os óleos essenciais de *O. vulgare*, *T. vulgaris*, *C. zeylanicum* apresentaram resultados significativos, classificados como eficientes frente aos microrganismos testados. Por fim, destaca-se o uso dos óleos essenciais classificados como ativos e atóxicos neste estudo como alternativas no controle e combate de microrganismos patogênicos apresentando uma proposta de produto natural com baixo custo de obtenção e alto potencial de mercado.

Palavras-chave: Óleos essenciais; Antimicrobiano; Condimentos.

Resumen

El interés por los aceites esenciales y sus aplicaciones alimentarias ha crecido debido a la reacción negativa de los consumidores a los aditivos químicos sintéticos añadidos intencionalmente en los productos industrializados con el fin de aumentar su vida útil. En este contexto, el presente estudio tenía como objetivo evaluar el perfil químico, la toxicidad para la *Artemia salina* Leach y la actividad antibacteriana in vitro de los aceites esenciales obtenidos a partir de condimentos naturales en bacterias de importancia clínica y alimentaria. Material vegetal: se obtuvo en el municipio de San Luis-MA. Los aceites esenciales fueron extraídos por hidrodestilación a 100 °C/3 h. Se realizó la metodología Folin Ciocalteu para la determinación de fenólicos totales. El ensayo de toxicidad se realizó utilizando el bioensayo de letalidad *Artemia salina* Leach. La actividad antimicrobiana siguió la metodología descrito por el Instituto de Normas Clínicas y de Laboratorio utilizando el Método de Difusión y Dilución de Discos de Caldo para la acción de los aceites esenciales contra la bacteria *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus* y *Bacillus cereus*. Se identificó la presencia de clases bioactivas en los materiales vegetales utilizados en esta investigación y los aceites esenciales obtenidos fueron clasificados como no tóxicos en el ensayo de toxicidad, presentando baja letalidad al micro crustáceo *Artemia salina* Leach. En el ensayo de actividad bactericida, los aceites esenciales de *O. vulgare*, *T. vulgaris*, *C. zeylanicum* mostraron resultados significativos, clasificados como eficientes contra los microorganismos probados. Por último, destacamos el uso de aceites esenciales clasificados como activos y no tóxicos en este estudio como alternativas en el control y combate de microorganismos patógenos que presentan una propuesta de producto natural con bajo coste de obtención y alto potencial de mercado.

Palabras clave: Aceites esenciales; Antimicrobiano; Condimentos.

1. Introduction

Among the ways of controlling microbial proliferation in foods, the use of chemical additives and the use of natural compounds as preservatives can be mentioned, with or without the aid of barrier technology. However, the suspicion about the toxicity of some chemical additives in products and the abuse of the use of such compounds have demanded more and more vigorous legislative measures in the world.

Natural extracts and essential oils are used to increase shelf-life and improve the sensory characteristics of food (Catellan, 2012). Essential oils are compounds derived from plant materials with the potential to be used as preservatives natural from foods. The growing consumer demand for the use of antimicrobial agents from natural sources justifies the inclusion of essential oils in food (Jiménez et al., 2018). Essential oils can have antimicrobial action in three ways: interference with the phospholipid double layer of the bacterial cell wall, by increasing the permeability and loss of cellular constituents, and by altering a variety of enzymatic systems such as those involved in the production of cellular energy and synthesis of structural components or destruction of genetic material (Sarto & Júnior, 2014).

The search for natural antimicrobial agents as an alternative to synthetic preservatives has been constant; in order to provide microbiological control and shelf life extension, thus excluding the disadvantages brought by the use of artificial additives, through the bactericidal action of essential oils. According to the legislation in force in Brazil, spices are defined as products made up of parts (roots, rhizomes, bulbs, barks, leaves, flowers, fruits, seeds, stems) of one or more plant species, traditionally used to add flavor or aroma to food. and drinks (Brasil, 2005). Among the plant species described in the referred legislation, we can highlight the *Thymus vulgaris*, *Origanum vulgare*, *Cinnamomum zeylanicum*, *Pimpinella anisum*, *Salvia rosmarinus* and *Piper nigrum*.

Among the plant species highlighted, *Cinnamomum zeylanicum*, whose spice is the bark removed from the thin branches of the tree, which, when dried, curls, taking on the tubular shape of the so-called cinnamon stick. The residues and broken shells are ground to obtain powdered cinnamon (Stobart, 2018).

Salvia rosmarinus, belonging to the Lamiaceae Family, is a spice known since antiquity for its medicinal effects. Currently, several studies have pointed out this spice as an antioxidant and antimicrobial. The species *Rosmarinus officinalis* L., usually known as rosemary, comes from the Mediterranean Region and has a woody, erect and little branched subshrub with a height of up to 1.5 m. The very aromatic leaves are 1.5 to 4 cm long and 1 to 3 mm thick (Machado et al., 2013).

Origanum vulgare a spice originated from the Mediterranean, belonging to the Lamiaceae family, with characteristic flavor and aromas, with main application made in the food industry through flavorings for products called pizza type. The compound used for this purpose is the essential oil of oregano, the subject of evaluations regarding its biological properties, due to its potential antioxidants, antimicrobials (Porto, 2018). *Thymus vulgaris* L., popularly known as thyme, is a medicinal, aromatic and spice plant, belonging to the Lamiaceae family, originally from Europe and cultivated in the south and southeast of Brazil. This plant, widely used in folk medicine, has essential oil already reported as responsible for its antiseptic, expectorant, carminative and antispasmodic activities (Gonçalves et al, 2018).

Pimpinella anisum L., is a plant belonging to the Umbelliferae family, being one of the oldest medicinal plants. It is an annual herbaceous herb 30 - 50 cm tall, with white flowers and small green to yellow seeds, which grow in the eastern Mediterranean region, Western Asia, the Middle East, Mexico, Egypt and Spain. Its therapeutic properties include the ability to inhibit intestinal, carminative and antispasmodic fermentation (dos Santos & Martins, 2019). *Piper nigrum*, popularly known as black pepper, is a climbing plant, belonging to the Piperaceae family. This plant is composed of terpenes of great applicability and its different parts can be used as cosmetics, medicines, preservatives, insecticides and larvicides,

In the current context, the addition of spices remains a growing and widespread practice. Spices are used safely

around the world in order to increase shelf-life and improve the sensory characteristics of food (Catellan, 2012). Studies point out adverse reactions to synthetic additives, such as toxic reactions and the possible development of specific cancers. Several natural preservatives have been used to inactivate microorganisms, without adverse effects in relation to the nutritional values of food and human health.

The search for natural antimicrobial agents as an alternative to synthetic preservatives has been constant; in order to provide microbiological control and shelf life extension, thus excluding the disadvantages brought by the use of artificial additives, through the bactericidal action of essential oils. In this context, the present study aimed to evaluate the chemical profile, toxicity and the *in vitro* antibacterial activity of essential oils from natural spices against microorganisms of clinical and food importance.

2. Methodology

2.1 Plant material

Piper nigrum (black pepper) fruits, *Pimpinella anisum* (fennel) leaves, *Cinnamomum zeylanicum* sticks (cinnamon), *Origanum vulgare* leaves (oregano), *Salvia rosmarinus* leaves (rosemary) and *Thymus leaves* were obtained vulgaris (thyme) in dehydrated form and in the local market in the municipality of São Luís (MA), being transported to the Laboratory of Research and Application of Essential Oils of the Federal University of Maranhão (LOEPAV / UFMA).

Figure 1. Plant material (a) *Piper nigrum* (b) *Pimpinella anisum* (c) *Cinnamomum zeylanicum* (d) *Origanum vulgare* (e) *Salvia rosmarinus* (f) *Thymus vulgaris*.



Source: Authors (2021).

2.2 Phytochemical screening

A hydroalcoholic extract (Figure 2) was prepared from each of the plant materials obtained and these were subjected to chemical tests based on the methodology presented by Matos (2009). The tests performed to identify alkaloids, steroids,

phenolics, flavonoids, glycosides, cardiac glycosides, saponins and tannins are described below:

Figure 2. Hydroalcoholic extracts of plant materials.



Source: Authors (2021).

2.2.1 Steroids (Salkowsk test)

About 100 mg of dry extract was dissolved in 2 ml of chloroform. Sulfuric acid was carefully added to form a lower layer. A reddish-brown color at the interface indicated the presence of a steroid ring.

2.2.2 Alkaloids (Mayer's test)

1.36 mg of mercury chloride were dissolved in 60 ml and 5 mg of potassium iodide dissolved in 10 ml of distilled water, respectively. These two solvents were mixed and diluted to 100 ml using distilled water. To 1 ml of the aqueous acidic solution of the samples, a few drops of the reagent previously prepared were added. The formation of white or pale precipitation showed the presence of alkaloids.

2.2.3 Flavonoids

In a test tube containing 0.5 mL of alcoholic extract from the samples, 5 to 10 drops of diluted HCl were added and a small amount of Zn or Mg was added to the solution, which was then boiled for a few minutes. The appearance of a reddish pink or dark brown color indicated the presence of flavonoids.

2.2.4 Glycosides

A small amount of sample alcoholic extract was dissolved in 1 ml of water and then aqueous sodium hydroxide was added. The formation of a yellow color indicated the presence of glycosides.

2.2.5 Cardiac glycosides

About 100mg of extract was dissolved in 1ml of glacial acetic acid containing a drop of ferric chloride solution and 1ml of concentrated sulfuric acid was added. A brown ring obtained at the interface indicated the presence of an oxy sugar characteristic of cardenolides.

2.2.6 Saponins

A drop of sodium bicarbonate was added to a test tube containing about 50 mL of an aqueous sample extract. The mixture was shaken vigorously and held for 3 minutes. A honey comb like foam was formed and showed the presence of

saponins.

2.2.7 Phenols

For 1 mL of alcoholic sample solution, 2 mL of distilled water was added followed by a few drops of 10% aqueous ferric chloride solution. The formation of a blue or green color indicated the presence of phenols.

2.2.8 Tannins

In a test tube containing about 5 ml of an aqueous extract, a few drops of 1% lead acetate solution were added. The formation of a yellow or red precipitate indicated the presence of tannins.

2.3 Obtaining essential oils

For the extraction of essential oils, the hydrodistillation technique was used with a Clevenger glass extractor coupled to a round bottom flask wrapped in an electric blanket as a heat generating source. 70g of *P. nigrum* fruits, 30g of *O. vulgare* leaves, 80g of *P. anisum* leaves, 100g of *C. zeylanicum* sticks, 30g of *S. rosmarinus* leaves and 100g of *T. vulgaris* leaves were used, adding distilled water (1:10). Hydrodistillation was carried out at 100 ° C for 3 hours, collecting the extracted essential oil (Figure 3). Each essential oil was dried by percolation with anhydrous sodium sulfate (Na₂SO₄) and stored in a refrigerator until further analysis.

Figure 3. Essential oil obtained after 3 hours of extraction using the hydrodistillation method.



Source: Authors (2021).

The physicochemical parameters of essential oils were determined: refractive index, color and appearance according to the Brazilian Pharmacopeia (2019).

2.4 Quantification of total phenolics

The determination of the total phenolic compounds of the essential oil was carried out with adaptation of the method of Folin-Ciocalteu (Waterhouse, 2002). 5 mg of the essential oil diluted in 1 ml of ethanol was used. To this solution was added 3 mL of distilled water, 500 µL of the reagent Folin-Ciocalteu and 2.0 mL of 20% sodium carbonate. The formed

solution was taken to the water bath at 50 °C for 5 min, removed and left to cool; and then the reading was performed on a manual spectrophotometer, at a length of 760 nm. The standard curve was expressed in mg L⁻¹ of tannic acid.

2.5 Lethality against *Artemia salina* Leach (toxicity against non-target organism)

Artificial saline solutions (60 g L⁻¹ of distilled water) were added in a rectangular container, with a divider containing approximately 0.02 cm thick spaced by 0.5 cm and evenly distributed (60 g L⁻¹ of distilled water) (60 g of sea salt / 1L of distilled water).

The container was placed inside an incubator illuminated by a fluorescent lamp, with aeration. On one side of this container, about 64 mg of *Artemia salina* cysts were added, since they did not cross the partition. The part of the system containing cysts was covered with aluminum foil, so that the organisms, at birth, were attracted by the light on the other side of the system, forcing them to pass through the partition. This procedure aims to homogenize the physical conditions of the test organisms. The incubation was performed for a period of 48 hours. Throughout the test the temperature was monitored.

To assess the lethality of *Artemia salina* Leach, a stock saline solution of each essential oil was prepared at a concentration of 10,000 mg L⁻¹ and 0.02 mg of Tween 80 (active surfactant). Aliquots of 5, 50 and 500 µL of this were transferred to test tubes and supplemented with saline solution previously prepared up to 5 mL, obtaining concentrations of 10, 100 and 1000 mg L⁻¹, respectively, at the end. All tests were performed in triplicates, where ten larvae in the nauplius phase were transferred to each of the test tubes. To control the blank, 5 mL of saline was used, for the positive control K₂Cr₂O₇ and for the negative control, 5 mL of a 4 mg L⁻¹ solution of Tween 80. After 24 hours of exposure, live larvae, those who did not move during the observation or with the slight agitation of the flask were considered dead. The criterion established by Dolabela was adopted.

2.6 Standardization of the microbial inoculum for sensitivity tests

Four strains of bacteria were used: *Escherichia coli* (ATCC 25922), *Salmonella* sp. (ATCC 700623), *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 11778). These were previously identified and confirmed by biochemical tests. Pure microbial cultures maintained on TSA agar were seeded into brain and heart infusion broth (BHI) and incubated at 35 ° C until they reached exponential growth phase (4-6 h). After this period, the cultures had their cell density adjusted in sterile 0.85% saline, in order to obtain a turbidity comparable to that of the McFarland 0.5 standard solution, which results in a microbial suspension containing approximately 1.5 x 10⁸ UFC mL⁻¹ according to the standards of the Clinical and Laboratory Standards Institute (CLSI, 2020).

2.7 Disc Diffusion Method (MDD)

The disk diffusion technique was performed according to the Clinical and Laboratory Standards Institute (CLSI, 2020), which standardizes disk diffusion sensitivity testing of antimicrobials. First, plates were prepared with the Mueller Hinton Agar (AMH) culture medium after its solidification was distributed to the microbial suspension on the agar surface and left to stand at room temperature for 30 min. Soon after, the disks containing 50 µL of essential oils and disks with defined concentrations of antibiotics are prepared. Using sterile forceps, the disks were distributed on the agar surface. The plates were incubated in a bacteriological oven at 35 ° C for 24 hours. The diameters of the inhibition halos were measured, including the diameter of the disk. These tests were done in triplicate. The values of the inhibition halos were the averages of the measurements of the three results. Tests performed in triplicate.

2.8 Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) assay was performed using the broth dilution technique, proposed by the Clinical and Laboratory Standards Institute (CLSI, 2020). First, essential oil solutions were prepared using 2% dimethylsulfoxide (DMSO), with serial dilutions being prepared in MH broth for the bacterial assay, resulting in concentrations of 10 to 1000 $\mu\text{g mL}^{-1}$. At each concentration, microbial suspension containing 1.5×10^8 CFU mL^{-1} of the *S. aureus*, *Bacillus*, *E. coli* and *Salmonella* strains were added. The tubes were incubated at 35° for 24h. Sterility and growth controls were performed for the test performed.

After the incubation period, MIC of essential oils and standardized HE was verified, being defined as the lowest concentration that visibly inhibited bacterial growth (absence of visible turbidity). Tests performed in triplicate. For the Minimum Bactericidal Concentration (CBM) assay, a 100 μL aliquot of the dilutions from the MH broth was used, which visibly inhibited microbial growth. The aliquots were inoculated in AMH and AMH (2% methylene blue) with subsequent incubation at 35 ° C for 24 hours. CBM was defined as the lowest concentration that visually in the MIC assay showed growth inhibition and that in cultures for bactericidal assays also did not show microbial growth.

3. Results and Discussion

3.1. Physico-chemical properties

The physical-chemical parameters are of great importance to determine the quality of a given product. According to Table 1, the parameters are presented physico-chemical properties of essential oils.

Table 1. Physico-chemical parameters of essential oils.

Essential oil	Refractive index (nD 25 °)	Color	Appearance
<i>Piper nigrum</i> (black pepper)	1,498	Colorless	
<i>Cinnamomum zeylanicum</i> (cinnamon)	1.605		
<i>Pimpinella anisum</i> (Fennel)	1,572		
<i>Origanum vulgare</i> (oregano)	1.505	Yellow	Clear
<i>Salvia rosmarinus</i> (Rosemary)	1,470		
<i>Thymus vulgaris</i> (thyme)	1.603		

Source: Authors (2021).

When observing the data presented in Table 1, comparisons were made with other studies that obtained the essential oils described in this research. Since, according to Vieira (2010), the refractive index of a substance is the relationship between the speed of light in the air and its speed in this substance.

Among the studies obtained, Melo et al. (2020) who reported that the refractive index of the essential oil of *P. nigrum* was 1.48 nD 25 °. Mendes et al (2012) observed in the essential oil obtained from the leaves of *C. zeylanicum* a refractive index of 1.533 nD 25 °. Hassan & Elhassan (2017) reported the refractive index of 1.55 nD 25 °for essential oil of *P. anisum*. Liston (2013) reported that the essential oil of *O. vulgare* presented itself with a refractive index of 1,495. Atti-Santos et al. (2010) reported the refractive index of 1.46nD 25 °for essential oil of *S. rosmarinus*. Imtara et al. (2021) reported the refractive index of 1.51 nD 25 ° for the essential oil of *T. vulgaris*. The essential oils studied showed compliance with the color and

appearance parameters of the studies presented in the literature.

The minimum variations observed are expected and according to Simões et al. (2007), this is based on the composition of the essential oil of a plant to be genetically determined, being, in most cases, specific for a certain organ and characteristic for its development, but with the variability of environmental conditions, they cause significant variations. Comparing the results for the essential oils studied with those in the literature, it can be observed that there was a similarity between them, with regard to the analyzed parameters. According to Costa et al. (2012) the differences in the values found can also be attributed to factors such as collection time, different types of soil, drying conditions and leaf storage time.

3.2. Phytochemical screening

Phytochemical analysis enabled the determination of secondary metabolites present in the analyzed plant materials, shown in Table 2.

Table 2. Phytochemical screening of plant materials used.

Species	Class							
	1	2	3	4	5	6	7	8
<i>Piper nigrum</i> (black pepper)	+	-	+	-	-	+	+	+
<i>Cinnamomum zeylanicum</i> (cinnamon)	+	-	+	+	+	+	+	-
<i>Pimpinella anisum</i> (Fennel)	+	-	+	+	+	+	+	+
<i>Origanum vulgare</i> (oregano)	+	-	+	+	+	+	+	-
<i>Salvia rosmarinus</i> (Rosemary)	+	+	+	+	+	+	+	-
<i>Thymus vulgaris</i> (thyme)	+	-	+	+	-	+	+	+

Note: 1 - Steroids; 2 - Alkaloids; 3-Flavonoids; 4-Glycosides; 5-Cardiac glycosides; 6-Saponins; 7-Phenols; 8-Tannins. Source: Authors (2021).

Table 2 shows the presence of different classes in all analyzed plant materials. The presence of phytochemicals is largely influenced by several factors, including variety, genetic factors, maturation stage, climatic and edaphic conditions. According to Menezes Filho and Castro (2019) Temperature, water availability, ultraviolet radiation, addition of nutrients, environmental pollution and attack of pathogens are factors that can influence the amount of secondary metabolites present in plant extracts.

According to Alfaia et al. (2016), the natural function of many secondary metabolites has been investigated with greater tenacity, being recognized that these are essential for the existence of plants and in several biotechnological applications. Gomes et al. (2016), in his phytochemical study of the extract of *C. zeylanicum*, identified reducing sugars, phenols, tannins, steroids, triterpenoids, alkaloids, foamy saponins and flavonoids. In another study, Gomes et al. (2018), identified reducing sugars, phenols, tannins, steroids, triterpenoids, alkaloids, anthraquinones, foamy saponins and flavonoids.

Santos et al. (2020), when analyzing the phytochemical composition of the crude extract of *O. vulgare*, identified the presence of phenols, alkaloids, tannins, flavonoids and saponins. Chaves (2019), when analyzing the *O. vulgare* extract,

identified the presence of reducing sugars, tannins, phenols, depsides and depesdones, steroids and triterpenoids.

Gonçalves et al (2015) performed phytochemical screening in extracts of *O. vulgare*, *T. vulgare* and *S. rosmarinus*, identified the presence of reducing, phenolic, cardiac glycoside, saponin, tannins and terpenes for hydroalcoholic extracts of *S. rosmarinus*, *Origanum vulgare*, however in the extract of *T. vulgare*, they observed the presence of reducing sugars, phenolics, cardiac glycosides, saponins, tannins and found the absence of terpenes.

While Silva et al (2010) reported the presence of flavonoids, tannins, terpenes and phenols in the ethanolic extract of *T. vulgare*. Araujo (2005) when performing phytochemical screening in extracts of *P. anisum* identified the presence of alkaloids, anthraquinones, flavonoids. While in another study by Sousa et al. (2017), the authors identified the presence of phenols, tannins, flavanonols and xanthonenes in the extract of *P. anisum*. Almeida (2017) in his study on *P. nigrum*, reported the presence of alkaloids, propenylphenols, lignans, neolignans, terpenes, steroids, flavones.

3.3. Quantification of the total phenolic content (CFT)

The result of the total phenolic content of essential oils is shown in Table 3. The total phenolic content was expressed as tannic acid equivalents (mg EAT g⁻¹ plant material) the equation of the line obtained, where y represents the absorbance and equivalent concentration of tannic acid.

Table 3. Quantification of total phenolics (CFT) of essential oils.

Essential oil	CFT mg EAT g ⁻¹	Linear	R ²
<i>Piper nigrum</i> (black pepper)	13.99		
<i>Cinnamomum zeylanicum</i> (cinnamon)	519.96		
<i>Pimpinella anisum</i> (Fennel)	112.45	y = 0.0586 x + 0.06	(0.9980)
<i>Origanum vulgare</i> (oregano)	96.92		
<i>Salvia rosmarinus</i> (Rosemary)	74.03		
<i>Thymus vulgaris</i> (thyme)	368.77		

Note: CFT- Total Phenolic Content. Source: Authors (2021).

According to the results presented in Table 3, it was possible to confirm an important amount of phenolic compounds ranging from 13.99-519.96 mg EAT g⁻¹ in the essential oils obtained, with the greatest results obtained for *C. zeylanicum*, *P. anisum*, *T. vulgaris*, which is of great relevance since phenolics are often associated with several positive health effects, including antioxidant effects, decreased risk of cardiovascular diseases, anticancer mechanisms and anti-inflammatory properties (Singh et al., 2012). Phenolic compounds in plant foods have curative, preventive functions in physiological disorders in humans, making it interesting to determine the amount of total phenolic compounds in fresh and dehydrated condiment herbs (Soares, 2020).

The important amount of total phenolics observed in this study corroborates the data reported by Soares (2020) when analyzing the content of phenolic compounds in oregano, basil, mint and rosemary extracts in natura and dehydrated, reporting higher concentrations in oregano and rosemary, noting yet what dehydrated herbs have a greater extraction of phenolics, since dehydration increases solubility with a greater extraction of phenolics, reaffirming the significant amount observed in this study.

Still, Santos (2009), quantified the phenolic compounds of herbs belonging to the Lamiaceae family, noting that oregano presented the highest concentration of phenolic compounds of 5.350 equivalents of gallic acid (EAG) per milliliter of extract, while thyme presented 2,150 EAG mL⁻¹ and rosemary showed 2,440 EAG mL⁻¹. Santos (2014) reported in his study high levels of phenols for cinnamon 562 mg L⁻¹ gallic acid and emphasized that the ingestion of cinnamon can have a beneficial effect in controlling the variation in blood glucose levels. Similar results were also observed by Zielinski (2015) who reported in their study a good number of phenolic compounds of 100.45 EAG mg L⁻¹ in *Pimpinella anisum*.

Biazotto (2014) observed in his study low values of phenolic compounds in the grains of white pepper and reported that the peeling of grains of this type of pepper are the variables capable of reducing the levels of phenolics more significantly than the enzymatic browning applied to pepper black. In another study, Agbor et al. (2006) found low values of phenolic compounds in white pepper grains (*Piper nigrum*).

Therefore, as observed a significant number of phenolic compounds, it is emphasized that these are considered as considerable bioactive compounds, associated with several favorable health effects, among other functions, they are mainly related to the antioxidant activity in plants (Rocha et al., 2011). According to Achkar et al. (2013) the use of phenolic compounds, specifically phenolic acids, in food preservation, can increase the shelf life of the product between 15 to 200%. Thus, the significant results obtained in this stage are extremely important and encourage studies with biological activities

3.4. Lethality in front of *Artemia salina* Leach (toxicity bioassay)

Table 4 shows the Lethal Concentration 50% (LC₅₀) referring to the action of essential oil against *Artemia salina* L. and its subsequent classification according to the criterion of Dolabela (1997).

Table 4. LC₅₀ quantification for the action of essential oils against *Artemia salina* Leach and toxicity classification.

EO	LC ₅₀ (µg mL ⁻¹)	Linear	Standard error
<i>Piper nigrum</i> (black pepper)	261.5 (non-toxic)	y = 36.776x - 42.798	0.0823
<i>Cinnamomum zeylanicum</i> (cinnamon)	653.4 (non-toxic)	y = 16.135x - 8.1245	0.1485
<i>Pimpinella anisum</i> (Fennel)	293.8 (non-toxic)	y = 32.129x - 37.178	0.0913
<i>Origanum vulgare</i> (oregano)	> 1000(non-toxic)	-	-
<i>Salvia rosmarinus</i> (Rosemary)	> 1000(non-toxic)	y = 14,129x - 22,291	0.1891
<i>Thymus vulgaris</i> (thyme)	410.0 (non-toxic)	y = 23.706x - 26.678	0.1161

Source: Authors (2021).

According to the results presented in Table 4, it can be seen that essential oils were classified as non-toxic, therefore, their applications have their application potential encouraged. Knowledge of the toxicological potential of plants is an important factor in establishing the consumable limit. And in this toxicity test the essential oils of *O. vulgare*, *S. rosmarinus*, *C. zeylanicum* and *T. vulgaris*, presented the LC₅₀ well above the criterion of 250 mg L⁻¹ to be classified as non-toxic.

Similar results are justified by Pereira et al. (2015) when analyzing the toxicological potential of condiment plants, found a low toxicological potential for rosemary and fennel and associated their atoxicities with beneficial non-toxic compounds in their compositions, when consumed within limits. The results described by Ramos et al. (2011) also corroborate

the data reported in this study, when analyzing the cytotoxicity of thyme essential oil, reporting that it presents 25% lysis at the minimum tested concentration (1.25 mg mL^{-1}), showing low toxicity. As the concentration of essential oil increased by 25 and 50 times, the percentage of lysis increased visually to 50%. In another study, Reis (2012) reported that the essential oil of cinnamon leaves has 162.1 mg L^{-1} ,

For oregano essential oil, Santin (2013) evaluated acute skin and eye irritation and skin sensitization with in vivo tests and observed that 3% essential oil causes mild and reversible acute skin and eye irritation for seven days. Cleff et al. (2008) studied the toxicity of 3% oregano essential oil administered daily for 30 days in rats by oral and intra vaginal route and observed that there was no macroscopic alteration in the reproductive and digestive tract, liver, spleen and kidneys and also did not contact changes in clinical, hematological evaluations.

Cardoso et al. (2005) evaluated the toxic effect of piperine isolated from black pepper on liver, kidney and blood cells of mice and observed that there was no significant modulation for the hematological parameters analyzed.

According to Bakkali et al. (2006), the cytotoxic activity of essential oils is probably related to the lipophilicity of their chemical constituents, which, due to their interaction with the cytoplasmic membrane, crosses the cell wall, breaking the structures of the different layers of polysaccharides, fatty acids and phospholipids, reaching several targets to same time, thus leading to cell death.

Everton et al. (2020) emphasizes that many studies in the literature do not yet disclose toxicity of the plants under study in a specific test such as the bioassay against *Saline artemia*. The study of toxicity is extremely important, as it provides information about the quality of the environment or part of it, thus making it possible to measure the lethal concentration of a given product (Botelho et al., 2010).

According to Reis (2012), the toxicity bioassay with *Saline artemia* provides an estimate of the concentration of a substance by measuring a biological response, in which there is only one parameter involved: life or death. Since this assay is specific, it allows the assessment of acute toxicity and, therefore, is considered essential as a preliminary bioassay in the study of compounds with potential biological activity (Reis, 2012). Thus, this study brought the toxicity test with very significant results for condiments widely used in daily life, but without in-depth studies on their toxicity.

3.5. In vitro bactericidal activity of essential oils

The results for the disk-diffusion assays for determining the inhibition halos for antimicrobial activity are shown in Table 5.

Table 5. Diameter of the inhibition halos (mm) for the action of essential oils against the pathogenic microorganisms tested.

EO	DHI - Diameter of inhibition halos (mm)			
	<i>B. cereus</i>	<i>E. coli</i>	<i>Salmonella sp.</i>	<i>S. aureus</i>
<i>Piper nigrum</i> (black pepper)	NI *	NI *	<9R	NI *
<i>Cinnamomum zeylanicum</i> (cinnamon)	32S	30S	31S	36S
<i>Pimpinella anisum</i> (Fennel)	<9R	<9R	<9R	<9R
<i>Origanum vulgare</i> (oregano)	12S	15 S	19 S	13 S
<i>Salvia rosmarinus</i> (Rosemary)	<9 R	NI *	<9R	<9R
<i>Thymus vulgaris</i> (thyme)	30 S	27 S	24 S	32 S

Note: NI *, there was no inhibition of the microorganism by the action of essential oil; S-Sensitive; R-Resistant. Source: Authors (2021).

As seen in Table 5, the strains were classified as sensitive and resistant according to Moreira et al. (2005), being defined according to the diameter of the inhibition halo formed, being classified as resistant when the inhibition halo is less than 8 mm and sensitive to 9 to 14 mm halos. Thus, the essential oils of cinnamon, oregano and thyme are described as active in the control and combat of pathogenic microorganisms *B. cereus*, *E. coli*, *Salmonella sp.* and *S. aureus*.

Similar results have been reported by Jiménez et al. (2018) when evaluating the antimicrobial action of essential oils of *C. zeylanicum* and *P. nigrum* against *S. aureus*, *Salmonella sp.* and *E. coli*, the authors reported minor zones of inhibition for essential oil *C. zeylanicum*, When compared to this study, where they obtained the 8.20 mm halo for *S. aureus*, 10.15 mm for *Salmonella sp.* and 12.30 mm for *E.coli*. While the results for *P. nigrum* were similar to those found in this study, having no effects for these microorganisms.

In another study by Salviano et al. (2017) using the bark of *C. zeylanicum* the authors reported activity for the peel essential oil, since the authors' essential oil was more efficient compared to *E. coli* with 36 mm, while in this study it observed the best efficiency for *S. aureus*, for *Salmonella*, they observed a 23 mm inhibition zone while the one found in this study was 31 mm and the results for *B. cereus* were similar to this study. In a study by Binatti et al. (2016) when analyzing the hydroalcoholic extract of fennel obtained a 6 mm inhibition halo for *S. aureus*, 8 mm for *Salmonella* and did not obtain an inhibition halo formed for *B. cereus*.

In addition, Reis et al. (2020) when analyzing the essential oils of oregano, thyme and rosemary and black pepper against *S. aureus*, *Salmonella sp.* and *E. coli*, reported the greater effectiveness of bacterial action to the essential oil of *O. vulgare* where they observed the 31 mm inhibition zone for *S. aureus*, 13 mm for *Salmonella* and 19 mm for *E. coli*, however for *T. vulgare* observed the formation of 11.7 mm inhibition halo for *Salmonella*, 16 mm to *E. coli* and did not obtain inhibition halo for *S. aureus*, which reinforces the satisfactory results obtained in that study, where for *S. aureus* we obtained the best efficiencies of the essential oil of *T. vulgare* with the 32 mm inhibition halo, the results of rosemary and *P. nigrum* corroborate with that of this study, the authors report inactivity of the two essential oils.

The biological action observed is explained by Carvalho (2012) that the plant's potential is due to the presence of flavonoids, which have the ability to complex proteins and bacterial cell wall, causing their lysis. Viuda-Martos et al., (2011) point out that the susceptibility of a bacterium to an essential oil is the result of differences in the structure of the cell membrane.

The results for the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays for determining antimicrobial activity are shown in Table 6.

Table 6. Minimum Inhibitory Concentration and Minimum Bactericide ($\mu\text{g mL}^{-1}$) of essential oils against microorganisms.

Essential oil	MIC / MBC $\mu\text{g mL}^{-1}$	<i>B. cereus</i>	<i>E. coli</i>	<i>Salmonella sp.</i>	<i>S. aureus</i>
<i>P. nigrum</i> (black pepper)	CIM	NI *	NI *	1000	NI *
	CBM	NI *	NI *	> 1000	NI *
<i>C. zeylanicum</i> (cinnamon)	CIM	200	200	200	200
	CBM	400	400	400	400
<i>P. anisum</i> (Fennel)	CIM	1000	1000	1000	1000
	CBM	> 1200	> 1200	> 1200	> 1200
<i>O. vulgare</i> (oregano)	CIM	400	200	200	400
	CBM	600	400	400	600
<i>S. rosmarinus</i> (Rosemary)	CIM	1000	1000	1000	1000
	CBM	> 1200	> 1200	> 1200	> 1200
<i>T. vulgaris</i> (thyme)	CIM	200	200	200	200
	CBM	400	400	400	400

Note: MIC, Minimum Inhibitory Concentration; MBC, Minimum Bactericidal Concentration; NI * there was no inhibition of the microorganism by the action of essential oil. Source: Authors (2021).

According to Aligianis et al. (2001), the classification of antimicrobial activity for plant specimens, according to the MIC results, is considered to be strongly inhibited: MIC up to $500\mu\text{g mL}^{-1}$; moderate inhibition: MIC between 600 and $1000\mu\text{g mL}^{-1}$; and weak inhibition: MIC above $1000\mu\text{g mL}^{-1}$. Thus, according to Table 6, essential oils from *O. vulgare*, *T. vulgaris*, *C. zeylanicum* showed bactericidal activity and strong inhibition, while the essential oils of *P. anisum* and *S. rosmarinus* were moderately inhibitory and *P. nigrum* showed no activity.

Studies have already reported the inhibitory action of the essential oils of *O. vulgare*, *T. vulgaris*, *C. zeylanicum*. In the study by Jiménez (2018) the essential oil of *C. zeylanicum zeylanicum* inhibited the growth of *S. aureus*, *E. coli* and *Salmonella*. Andrade et al., (2012) also observed the bacterial inhibition of the essential oil of *C. zeylanicum* against *S. aureus* and *E. coli* and found the presence of 14 constituents in essential oil, the majority being: (E) - cinnamaldehyde (77.72%), (E) - cinamyl acetate (5.99%) and the monoterpene 1,8-cineole (4.66%), this result in the inhibition of bacterial growth may be related to the presence of the major component cinnamic aldehyde in high concentration. According to the literature, the mechanism of action of aldehydes causes damage to lipids and proteins, where it is believed that the carbonyl group is capable of binding to proteins, preventing the action of amino acid decarboxylases, for example in *Enterobacter aerogenes* which are gram-negative bacilli.

Cattelan (2012) in his study, when analyzing bacterial activity essential oil of *O. vulgare*, *T. vulgaris* and *S. rosmarinus* against *E. coli*, *S. aureus*, *S. typhi*, *P. aeruginosa*, *B. subtilis* and *B. cereus*, reported that the essential oil of *S. rosmarinus* had a good moderate bacterial action, being consistent with our study, observed that the essential oil of *O. vulgare* was effective in bacterial inhibition and unlike this study, there was no activity of essential oil in relation to *B. cereus*, which reinforces the satisfactory results obtained in this study, where for *B. cereus* we obtained a MIC of 400 $\mu\text{g mL}^{-1}$, Casttelan also reported for *T. vulgaris* essential oil efficiency in bacterial inhibition only for *E. coli*, whereas in the present study, we obtained the MIC of 200 $\mu\text{g mL}^{-1}$, for all microorganisms tested.

The compounds responsible for the antibacterial action of the essential oil of *T. vulgaris* are believed to be the phenolics, present in high levels. According to the literature, the essential oil of *T. vulgaris* has thymol as a major compound (46.6%). Studies report that phenolic compounds are able to alter the permeability of the plasma membrane and penetrate bacterial cells, where they interact in metabolic mechanisms and this interaction affects the membrane permeability and thus, the loss of its resistance potential occurs (Hyldgaard, 2012). Costa Júnior et al., (2019) when analyzing the essential oil of *O. vulgare* observed the inhibitory action of the essential oil obtaining the MIC of 800 $\mu\text{g mL}^{-1}$. Castilho et al., (2012) observed the inhibitory action of essential oil of *O. vulgare* front to *S. aureus* and *E. coli* and also pointed out that its broad spectrum of antimicrobial activity is due to the presence of phenolic derivatives, such as carvacrol and thymol.

Cutrim et al. (2019) reported weak inhibition of rosemary essential oil ahead the bacteria *S. aureus* and *E. coli*, obtained the 1700 MIC $\mu\text{g mL}^{-1}$ for *E. coli* and 1500 $\mu\text{g mL}^{-1}$ for *S. aureus*, thus confirming the results obtained in the present study. Da Silva et al. (2019) reported in his study the inactivity of the antibacterial action of the extract of *P. anisum*, However, Gessinger (2013) reported that this essential oil is effective against the bacterium *Micrococcus luteus*, thus presenting great divergence in the literature showing bactericidal activity and the absence of this activity. Studies recommend modifying the methodology used to verify the active principles of plants before and after the extraction process.

Reis et al. (2020) in their study reported that the essential oil of *P. nigrum* did not inhibit the microorganisms tested at any concentration. In another study, Indui et al. (2006) evaluated the extract of *P. nigrum* against *E. coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila* and observed that the extract did not show antimicrobial activity.

Hyldgaard (2012) reports that Gram-negative bacteria are generally less susceptible than Gram-positive bacteria, as the outer membrane of Gram-negative bacteria contains hydrophilic lipopolysaccharides (LPS), which create a barrier for macromolecules and hydrophobic compounds, providing bacteria Gram-negatives with greater tolerance for hydrophobic antimicrobial compounds like those found in essential oils. The literature shows that most constituents of essential oil have multiple targets, so it is difficult to predict how susceptible a microorganism is and why the susceptibility varies from strain to strain.

4. Final Considerations

Finally, the potential of all essential oils in this study is highlighted again, since they presented an important quantity of phenolic compounds, showing themselves as promising for application and use in food. In addition, all essential oils studied they were non-toxic, since oils with high toxicity are not recommended for biological applications. The essential oils of *O. vulgare*, *T. vulgaris*, *C. zeylanicum*, present themselves as promising for application in the conservation of foods, both species showed better antibacterial activity considered extremely efficient in the control of pathogenic microorganisms, represented by *E. coli* and *Salmonella* sp. as Gram-negative, *S. aureus* and *B. cereus* as Gram-positive.

References

Achkar, M. T., Novaes, G. M., Silva, M. J. D., & Vilegas, W. (2013). Antioxidant property of phenolic compounds: importance in the diet and food preservation. *Journal of the Vale do Rio Verde University*, 11 (2), 398-406.

- Alfaia, D. P. S. (2016). Phytochemical evaluation, cytotoxic and antimicrobial analysis of the crude ethanolic extract of the leaves of *Annona muricata* L. (Annonaceae). *Amazon Biota* (Biote Amazonie, Biota Amazonia, Amazonian Biota), 6 (1), 26-30.
- Aliagiannis, N., Kalpoutzakis, E., Mitaku, S., & Chinou, I. B. (2001). Composition and antimicrobial activity of the essential oils of two *Origanum* species. *Journal of agricultural and food chemistry*, 49 (9), 4168-4170.
- Almeida, L. A. D. (2017). Phytochemical characterization of *Piper nigrum* L.
- Andrade, M. A., Cardoso, M. D. G., Batista, L. R., Mallet, A. C. T., & Machado, S. M. F. (2012). Essential oils of *Cymbopogon nardus*, *Cinnamomum zeylanicum* and *Zingiber officinale*: composition, antioxidant and antibacterial activities. *Revista Ciência Agronômica*, 43 (2), 399-408.
- Araujo, P. K. A. quality control of samples of *pimpinella anisum* l. *Commercialized in the city of Palmas*.
- Atti-Santos, A. C., Rossato, M., Pauletti, G. F., Rota, L. D., Rech, J. C., Pansera, M. R., & Moyna, P. (2005). Physicochemical evaluation of essential oils of *Rosmarinus officinalis* L. *Brazilian Archives of Biology and Technology*, 48 (6), 1035-1039.
- Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – a review. *Food and chemical toxicology*, 46 (2), 446-475.
- Biazotto, F. D. O. (2014). Antioxidant, anticholinesterase activity and metabolic profile of different types of peppers: Implications for Alzheimer's disease (Doctoral dissertation, University of São Paulo).
- Binatti, T. T., Geromel, M. R., & Fazio, M. L. S. (2016). Antimicrobial action of spices on bacterial development. *Food Hygiene*, 30 (260/261).
- Botelho, R. G., Inafuku, M. M., Maranhão, L. A., Neto, L. M. Olinda, R. A., Dias, C. T., & Tornisielo, V. L. (2010). Acute and chronic toxicity of neem extract (*Azadirachta indica*) for *Ceriodaphnia dubia*. *Pesticides: ecotoxicology and environment magazine*, 20.
- Brazil. Ministry of Health. RDC ANVISA No. 276, of September 22, 2005. Technical regulations for spices, seasonings and sauces. Official Gazette [of the Federative Republic of Brazil], Brasília, DF, 2005. Section 1.
- Cardoso, J. F. R., Evangelista, D. W., Viana, E. B., Lima, M. E. F., Soares, B. A., Barreto Junior, C. B., & Danelli, M. G. M. (2005). Evaluation of the toxic effect of Piperine isolated from black pepper (*Piper nigrum* L) in mice. *Rural University Magazine*, 25 (1), 85-91.
- Carvalho, C. A. D. (2012). Botanical, phytochemical characterization and evaluation of the biological activity of extracts of *Piptadenia gonoacantha* (Mart.) JF Macbr (FABACEAE).
- Castilho, P. C., Savluchinske-Feio, S., Weinhold, T. S., & Gouveia, S. C. (2012). Evaluation of the antimicrobial and antioxidant activities of essential oils, extracts and their main components from oregano from Madeira Island, *Portugal. Food Control*, 23 (2), 552-558.
- Chaves, R. D. S. B. (2019). Evaluation of the larvicidal activity of the crude ethanolic extract and essential oils of the leaves of *origanum majorana* L. and *origanum vulgare* L. against *Aedes aegypti* (Linnaeus, 1762).
- Costa, L. M., Resende, O., Gonçalves, D. N., & Sousa, K. A. (2012). Quality of crambe fruits during storage. *Revista Brasileira de Sementes*, 34 (2), 293-301.
- Costa Júnior, P. S. P., Ferreira, M. A., Bishop, A. S. D. R., Vieira, B. B., & Evangelista-Barreto, N. S. (2019). Antimicrobial activity of essential oils of different spices. *Hig. Food*, 2697-2701.
- Costa, I. C. D. (2020). Polymeric nanoparticles filled with *Piper nigrum* essential oil: chemical and morphological characterization.
- Cutrim, E. S. M., Teles, A. M., Mouchrek, A. N., Mouchrek Filho, V. E., & Everton, G. O. (2019). Evaluation of the antimicrobial and antioxidant activity of essential oils and hydroalcoholic extracts of *Zingiber officinale* (Ginger) and *Rosmarinus officinalis* (Rosemary). *Revista Virtual de Química*, 11 (1), 60-81.
- Everton, G. O., Rosa, P. V. S., Neves, S. C., Pereira, A. P. M., Lima, E. C. S., Mendonça, I. P., ... & Mouchrek Filho, V. E. (2020). Chemical characterization, antimicrobial activity and toxicity of the essential oils of *Pimenta dioica* L. and *Citrus sinensis* L. Osbeck. *Research, Society and Development*, 9 (7), e803974842-e803974842.
- Gessinger, M. M. (2013). Mathematical modeling of the supercritical extraction process of *pimpinella anisum* l. And study of its antimicrobial activity. *Graduation Magazine*, 6 (2).
- Gomes, E. M. C., Pena, R. D. C. M., & Silva, S. S. S. M. (2016). Phytochemical composition and fungicidal action of crude extracts of *Cinnamomum zeylanicum* on *Quambalaria eucalypti*. *Amazon Biota* (Biote Amazonie, Biota Amazonia, Amazonian Biota), 6 (4), 54-58.
- Gomes, E. M. C., Firmino, A. V., Pena, R. D. C. M., & Almeida, S. S. M. D. S. D. (2018). In vitro inhibitory effect of *Cinnamomum zeylanicum* Blume extracts on the control of *Cylindrocladium candelabrum*. *Forest Science*, 28 (4), 1559-1567.
- Gonçalves, J. H. T., Santos, A. S., & Morais, H. A. (2015). Antioxidant activity, total phenolic compounds and phytochemical screening of dehydrated condiment herbs. *Magazine of the Vale do Rio Verde University*, 13 (1), 486-497.
- Gonçalves, B. T., Lages, L. Z., Alves, Pic, & Gandra, And the Antibacterial Activity Of *Thymus* Essential Oil (*Thymus Vulgaris* L.) In Front Of Food Pathogens.
- Hassan, O. M., & Elhassan, I. A. (2017). Characterization of essential oils from fruits of Umbelliferous crop cultivated in Sudan I. *Pimpinella anisum* L. (Anise) and *Anethum graveolens* L. (Dill). *Journal of Pharmacognosy and Phytochemistry*, 6 (1), 109-112.
- Hyldgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential oils in food preservation: mode of action, synergies and interactions with components of the food matrix. *Frontiers in microbiology*, 3, 12.

- Imtara, H., Al-Waili, N., Aboulghazi, A., Abdellaoui, A., Al-Waili, T., & Lyoussi, B. (2021). Chemical composition and antioxidant content of honey *Thymus vulgaris* and essential oil of *Origanum vulgare*; its effect on carbon tetrachloride-induced toxicity. *Veterinary World*, 14 (1), 292.
- Indu, M. N., Hatha, A. A. M., Abirosh, C., Harsha, U., & Vivekanandan, G. (2006). Antimicrobial activity of southern Indian condiments on *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila*. *Brazilian Journal of Microbiology*, 37 (2), 153-158.
- Jiménez, M., Domínguez, J. A., Pascual-Pineda, L. A., Azuara, E., & Beristain, C. I. (2018). Elaboration and characterization of O / W cinnamon (*Cinnamomum zeylanicum*) and black pepper (*Piper nigrum*) emulsions. *Food Hydrocolloids*, 77, 902-910.
- Liston, M. D. S. (2013). Study of the composition and application of the essential oil of *Origanum vulgare* L. As an antimicrobial agent in *sururu* (*mytella falcata*).
- Machado, B. A. S., Ribeiro, D. S., & Druzian, J. I. (2013). Prospective study on the antimicrobial activity of some aromatic plants. *Prospecting Notebooks*, 6 (1), 97.
- Menezes Filho, A. C. P., & Souza Castro, C. F. (2019). Phytochemical classes of secondary metabolites in foliar ethanolic extracts of species of the Brazilian cerrado. *Revista Saúde & Ciência Online*, 8 (1), 45-61.
- Melo, C. F. M., Furlan Junior, J., & Huhn, S. (1997). Black pepper: essential oil and oleoresin. In Embrapa Amazônia Oriental-Article in congress proceedings (ALICE). In: international seminar on pepper-do-reino and cupuaçu, 1996, Belém, PA. Anais ... Belém, PA: EMBRAPA-CPATU: JICA, 1997.
- Mendes, L. S. D. S. (2012). Chemical Study and Larvicidal Activity in Front Of *Aedes Aegypti* Of The Essential Oil Of The Leaves Of *Cinnamomum Zeylanicum* Breyn (Cinnamon).
- Moreira, M. R., Ponce, A. G., Del Valle, C. E., & Roura, S. I. (2005). Inhibitory parameters of essential oils to reduce a foodborne pathogen. *LWT-Food Science and Technology*, 38 (5), 565-570.
- National Health Surveillance Agency (2019). *Brazilian Pharmacopoeia*. (6a ed.), Editora Fio Cruz, 1. 1-546 p.
- Porto, L. L., & Rosa, L. R. V. D. (2018). Evaluation of the antimicrobial potential of essential oils of coriander (*Coriandrum sativum* L.) And oregano (*Origanum vulgare* L.) (Bachelor's thesis, Universidade Tecnológica Federal do Paraná).
- Pereira, E. M., Leite Filho, M. T., by Assis Mendes, F., Martins, Ana, & Rocha, A. P. T. (2015). Toxicological potential against *Artemia Salina* in condiment plants sold in the city of Campina Grande, PB. *Green Journal of Agroecology and Sustainable Development*, 10 (1), 52-56.
- Ramos, A. V. G., Eninger, M. C., Tiunan, T. S., & Adamczuck, R. S. S. Antibacterial and cytotoxic activity of essential oil of thyme (*Thymus vulgaris* L.) And evaluation of synergistic effect with synthetic food preservatives. *Food*, 38.
- Reis, J. B., from Figueiredo, L. A., Castorani, G. M., & Veiga, Smom (2020). Evaluation of the antimicrobial activity of essential oils against food pathogens. *Brazilian Journal of Health Review*, 3 (1), 342-363.
- Reis, J. B. (2012). Analytical study, evaluation of toxicity and molluscicidal activity of the essential oil *Cinnamomum zeylanicum* Blume (cinnamon) against the snail *Biomphalaria glabrata* (Say, 1818).
- Rocha, W. S., Lopes, R. M., Silva, D. B. D., Vieira, R. F., Silva, J. P. D., & Agostini-Costa, T. D. S. (2011). Total phenolic compounds and condensed tannins in native fruits of the cerrado. *Revista Brasileira de Fruticultura*, 33 (4), 1215-1221.
- Salviano, L. F., Geromel, M. R., & Fazio, M. L. S. (2017). Antibacterial activity of essential oils of green and roasted coffee (*Coffea arabica*), Cocoa (*Theobroma cacao*), Ceylon cinnamon bark and leaf (*Cinnamomum zeylanicum*). *Hig. Food*, 107-111.
- Santin, R. (2013). Antifungal potential and toxicity of essential oils of the Lamiaceae family.
- Santos, R. D. (2009). Phenolic compounds of lamiaceae herbs in the oxidative stability of butter and evaluation of the toxicity of rosemary extract (*Rosemarinus officinalis* L.).
- Santos, E. J. D. (2014). Effect of ingestion of cinnamon tea *C. burmannii* on postprandial glycemia in non-diabetic adult individuals (Doctoral dissertation).
- Silva, C. A. L., Amaral, C. A., Santos, C. M., Leite, D. S., & Santos Brasil, J. (2019). Antimicrobial activity of plants of popular use in Brazil: chamomile (*Matricaria chamomilla*), fennel (*Pimpinella anisum*) and jucá (*Caesalpinia ferrea*). Antimicrobial activity of plants of popular use in Brazil: chamomile (*Matricaria chamomilla*), fennel (*Pimpinella anisum*) and jucá (*Caesalpinia ferrea*), 1-388.
- Santos, J. R. N., Teles, A. M., Ferreira, C. G., & Mouchrek, A. N. (2020). Evaluation of the bactericidal and antioxidant activity of the essential oil and the hydroalcoholic extract of oregano (*Origanum vulgare*). *Research, Society and Development*, 9 (10), e7829108410-e7829108410.
- Santos, R. A. M., & Martins, K. M. (2019). Quality control of vegetable drugs *Matricaria recutita* L., *Peumus boldus* M. and *Pimpinella anisum* L., sold in pharmacies in Maringá-PR. *Journal of Biology & Pharmacy And Agricultural Management*, 15 (4).
- Souza, C. A. S., Almeida, L. N., Santos Cruz, E., Silva, C. M. L., Júnior, J. A. C. N., Silva, F. A., & Serafini, M. R. (2017). Physico-chemical quality control and phytochemical characterization of the main medicinal plants sold at the Lagarto-SE fair. *Scientia Plena*, 13 (9).
- Sarto, M. P. M., & Junior, G. Z. (2014). Antimicrobial activity of essential oils. *UNINGÁ Review Magazine*, 20 (1).
- Schumack, K. D. S. P., Almeida Barbosa, A. C., Santos, L., Santos, T. G., & Lima, C. P. (2017). Antimicrobial activity of the essential oils of *Thymus vulgaris* L., *Eucalyptus citriodora* Hook and *Illicium verum* Hook f. *Proceedings of EVINCI-UniBrasil*, 3 (1), 244-244.

Simões, C. M. O., Schenkel, E. P., Gosmann, G., Mello, J. C. P., Mentz, L. A., & Petrovick, P. R. (2007). *Pharmacognosy: from plant to medicine*. 1002 pg. Porto Alegre / Florianópolis: Editora da UFSC / Editora da UFRGS.

Silva, T. B., & Rangel, E. T. (2010). Evaluation of the antimicrobial activity of the ethanol extract of thyme (*Thymus vulgaris* L.) In vitro. *Electronic Pharmacy Magazine*, 7 (2), 11-11.

Singh H. P., Kaur S., Negi K., Kumari S., Saini V., Batish, D. R. & Kohli R. K. (2012). Assessment of in vitro antioxidant activity of essential oil of *Eucalyptus citriodora* (lemon-scented Eucalypt; Myrtaceae) and its major constituents. *LWT-Food science and Technology*, 48 (2), 237-241.

Soares, V. G. (2020). Content of phenolic compounds and physico-chemical analyzes in different fresh and dehydrated condiments of rosemary, mint, basil and oregano.

Stobart, T. (2018). *Herbs, spices and condiments: from A to Z*. Editora Schwarcz-Companhia das Letras.

Vieira, R. (2010). Photosensitivity Study of Essential Oil of *Rosmarinus officinalis* Accompanied by UV-VIS Spectroscopy. 2010. 45 f. Course Conclusion Paper - Federal Technological University of Paraná (UTFPR), Toledo.

Viuda-Martos, M., Mohamady, M. A., Fernández-López, J., Abd ElRazik, K. A., Omer, E. A., Pérez-Alvarez, J. A., & Sendra, E. (2011). In vitro antioxidant and antibacterial activities of essential oils obtained from Egyptian aromatic plants. *Food Control*, 22 (11), 1715-1722.

Zielinski, A. A. F. Evaluation of phenolic compounds and in vitro antioxidant activity of teas: classification, modeling and optimization by chemometric techniques.