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Modulation Of Coagulase-Negative *Staphylococcus* Strains By *Lippia Origanoides* Essential Oil Associated With Beta-Lactam Antibiotics

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Abstract
Background*: Coagulase-Negative Staphylococcus (CoNS) can cause infection at the primary colonization site or spread to cause serious hospital infections such as bacteremia, septicemia and neonatal sepsis. In this context, the increase in bacterial resistance to conventional antibiotics has become a public health problem. Therefore, there is a need to intensify studies involving CoNS to implement more effective control measures. In this context, essential oils, which have antimicrobial activity, have emerged as alternatives for modulating the activity of antimicrobial-resistant bacterial strains. This study evaluated the modulatory effect of rosemary-pepper essential oil associated with antibiotics against CoNS strains.*
Materials and Methods*: Homogeneous populations of pepper rosemary were collected around CPCA/UFMG. Characterization was obtained by gas chromatography. The microorganisms were identified by MALDI - TOF MS and antibiotic resistance was determined by microdilution on plates. The STITCH bioinformatics platform was used to predict the potentiation of antibiotics through rosemary essential oil.*
Results*: Carvacrol (28.94%), o-Cymene (20.53%), γ-Terpinene (10.36%) and Thymol (2.91%) were obtained as the majority components of the oil. The association of pepper rosemary oil with the antibiotics amoxicillin and tetracycline reduced the concentration of antibiotic needed to inhibit 100% of the resistant strains of CoNS, indicating the synergistic effect of the oil with the antibiotics. In the networks and sub-networks created by the in silico analyses, it is possible to observe an interaction between the antibiotic amoxicillin and the bioactive compound in pepper rosemary oil, Camphor, through the histamine molecule. The antibiotic tetracycline was found to interact with the bioactive compounds in rosemary oil, linalool and limonene, via the nitric oxide synthase 3 (NOS_3) molecule. All the major components of the oil also acted in the chemical-protein interaction network, indicating possible synergisms between the antibiotics and the bioactive compounds in rosemary oil.*
Conclusion: *These results show that pepper rosemary oil has the potential to be used as an antimicrobial adjuvant in the treatment of infections caused by antibiotic-resistant CoNS bacteria.*
Key Word*: Synergism; Camphor; Histamine; Amoxicillin; Tetracycline, Limonene, Linalool.*

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I. Introduction

Among the main antibiotics used in dairy farming are the β-lactam group, which includes, among others, amoxicillin, penicillin, ampicillin, piperacillin and ceftazidime. These antimicrobials have a broad spectrum of action against gram-positive and gram-negative microorganisms [1]. However, some microorganisms can develop resistance mechanisms to β-lactams due to the presence of genes such as mecA [2]. The strain of *Staphylococcus* *aureus* that presents PBP2a, encoded by the mec complex, acquires resistance to all subclasses of beta-lactams, such as penicillins, carbapenems, monobactams and cephalosporins, except fifth-generation cephalosporins, also called anti MRSA [3].

CoNS have emerged as opportunistic bacteria, especially in hospitalized, immunocompromised, premature and implanted device patients [4]. They can cause infection at the primary site of colonization or spread to cause serious hospital infections such as bacteremia, septicemia and neonatal sepsis [5]. For this reason, interest in studying the susceptibility of CoNS to antimicrobials has increased [6]. In this context, the increase in bacterial resistance to conventional antibiotics has become a public health problem [7]. Therefore, it is necessary to intensify studies involving CoNS to implement more effective control measures, reducing severe cases and deaths, as well as minimizing costs compared to the usual treatment [8-9].

In this context, essential oils, which have antimicrobial activity, have emerged as alternatives for modulating the activity of antimicrobial-resistant bacterial strains. Generally, the antimicrobial and modulating activity of essential oils is due to the presence of chemical compounds in the oils, such as quininones, phenols, alkaloids, flavonoids, terpenoids, peptides and other compounds from the secondary metabolism of each plant [10]. Among the medicinal plants used as essential oils with antimicrobial action is pepper rosemary (*Lippia origanoides*), native to the semi-arid northeast of Brazil. The essential oil has phenolic terpenoids, such as carvacrol ([2-methyl-5-(1-methylethyl) phenol]) and thymol (2-isopropyl-5- methylphenol), which confer the oil's antimicrobial activity [11]. These phenolic compounds are known for their biological properties, such as anti-inflammatory, antioxidant, antimicrobial and antitumor activities [12]. However, the synergistic effect of pepper rosemary oil with beta-lactam antibiotics is not yet fully understood.

Given the above, this study aimed to evaluate the modulatory effect of rosemary-pepper essential oil associated with antibiotics against *CoNS* strains.

II. Material And Methods

Obtaining and characterizing the essential oil

The essential oils of pepper rosemary (*Lippia origanoides*) were obtained by collecting them from homogeneous populations around the CPCA/UFMG. The harvesting period for pepper rosemary should be prioritized between 8 am and 10 am. As described by [13], the plant produces the highest essential oil content in the morning, with the highest production peak at 10 am due to the influence of the sun. To obtain the oil, the steam extraction technique was used in a pilot distiller (Linax®, model D20). After extraction, the oil was stored in an amber bottle and refrigerated (4-8°C) until it was characterized by chromatography.

The chromatographic analyses were carried out on a gas chromatograph, Agilent Technologies (GC 7890A), coupled to a mass spectrometer (MS 5975C), equipped with a DB-5 MS capillary column (Agilent Technologies, stationary phase 5% phenyl and 95% methylpolysiloxane, 30 m x 250 µm i.d. x 0.25 µm film thickness) and helium (99.9999% purity) as a carrier gas, (1mL min-1). The sample was injected (1 µL) in 1:5 split mode with the injector kept at 220 ºC. The heating ramp of the column started at 60 ºC with a rate of 3 ºC min-1 up to 240 ºC. Then, with a temperature gradient of 10°C min.-1 up to 270°C kept constant for 7 min. The interface with the mass spectrometer was maintained at 240°C and it was operated by electron impact (70 eV), in full scan mode, monitoring a mass range (40 to 550 m/z). The retention index of all the compounds was calculated from the retention time of a mixture of n-alkanes (C8- C32, Sigma USA) 20 ppm, split 1:100. The data generated was analyzed using the MSD Chemstation software together with the National Institute of Standards and Technology library [14].

Determination of MIC and MBC of essential oil

To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of pepper rosemary essential oil, a concentration of 120 µL of rosemary oil associated with the concentrations of the tested antibiotics (amoxicillin and tetracycline) was used. In the microtiter plates, 50 µL of the rosemary oil solution, 50 µL of the antibiotic solution, 100 µL of BHI broth + Twem 80 (0.1%), and 10 µL of the microorganism suspension were added and incubated for 24h at 37°C. After the incubation period, 10 µL of triphenyltetrazolium chloride (TTC 1%) was added and then incubated again for 2 hours. The wells that showed bacterial growth acquired a red coloration, while the wells without growth remained colorless. Therefore, the MIC was determined by the lowest concentration capable of inhibiting bacterial growth. To evaluate MBC, a portion of the wells that remained colorless was transferred to Petri dishes containing PCA medium. The plates were incubated at 37 °C for 24 hours and evaluated for the presence or absence of microbial growth. The plates whose oil concentration did not show colony growth were able to exert a bactericidal action on the bacteria.

Identification of microorganisms by MALDI - TOF MS

The CoNS strains were identified using the MALDI-TOF process, Matrix Associated Laser Desorption Ionization - Time of Flight. The identification process consisted of selecting a colony from each strain, placing it in a polymer matrix and inserting it into a device, where it was irradiated by a laser, which led to the ionization of the molecules through a vacuum tube until they reached the detector [15]. The detector has the function of detecting and comparing the peak of each molecule. To compare mass spectrometry peaks, the spectrophotometer has a computer with a database for interpreting the data.

Among the fifteen strains identified were five species of *Staphylococcus*: *Staphylococcus auricularis*, *Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus warneri*.

Microdilution plates to determine antibiotic resistance

The microdilution test [14] began by standardizing the inoculum on the McFarland scale at 0.5 (1.5x108 CFU/ml), observing turbidity in saline solution. After standardizing the inoculum, it was transferred to nutrient broth in a test tube, Brain Heart Infusion - BHI, adjusting the final volume in the broth so that when it was transferred to the pools on the Elisa plates, the final concentration in the pools was approximately 5 x 105 CFU/ml.

Amoxicillin was used at concentrations of 32, 16 and 8µg.ml-1. The concentrations of Tetracline were 16, 8 and 4 µg.ml-1. 20 µL of microorganism inoculum were transferred to titration plates and added to 160 µL of antibiotic solution at the concentrations previously prepared and incubated for 24 hours. After the incubation period, the sensitivity of the microorganism to the antibiotic was measured using the 1% Triphenyl tetrazolium chloride (TTC) reagent, adding 20 µL to each well.

For the antibiotic and essential oil association test, I followed the standard methodology described by [14], using the antibiotic and the oil in a 1:1 ratio.

Predicting the potentiation of antibiotics using pepper rosemary essential oil

To contribute to the understanding of the action of antibiotics on non-aureus *Staphylococcus* in the presence of rosemary essential oil, in silico analyses were carried out using the online platform STITCH database (version 5.0; http://stitch.embl.de/), as described by [16]. The configuration of the platform was based on a confidence score of 0.4 (medium confidence), the species homo sapiens and the expansion of the networks until antibiotics appeared connected to the networks. The data sources included Textmining, Experiments, Databases, Co-expression, Neighborhood, Gene fusion, Co-occurrence, Predictions. Firstly, a chemical-protein interaction network was drawn up using all the chemical compounds contained in the composition of rosemary essential oil (Table 1), including one of the antibiotics (amoxicillin or tetracycline). In the second network, in addition to one of the antibiotics, we used the chemical compounds mostly present in pepper rosemary oil (Carvacrol, o-Cymene, γ-Terpinene and Thymol). The third network was built using the Cytoscape platform, version 3.10.1. The input was sub-networks obtained from the STITCH platform, which had compounds connected to both the antibiotics and the chemical compounds in the essential oil of rosemary.

Data analysis

Descriptive analyses were carried out using the absolute and relative frequencies of bacterial strains sensitive to antibiotics and pepper rosemary oil combined with antibiotics.

The interpretation of the chemical-protein interaction networks was based on data obtained from scientific literature, presenting pertinent explanations for the potentiation of antibiotics in the presence of rosemary essential oil against strains *of* non-aureus *Staphylococcus*.

III. Results And Discussion

The MBC of the oils under study was 120ul.ml^{-1}, and this concentration was used for the following tests. This concentration corroborates others described in the literature when using rosemary oil extracted from plants collected in the same region [17-18].

From the chromatographic analysis of the essential oil of rosemary (Table 1), Carvacrol (28.94%), o-Cymene (20.53%), γ-Terpinene (10.36%) and Thymol (2.91%) were obtained as the majority components (Figure 1), similar to the results of [17-18]. Among the main compounds that confer antimicrobial activity to the oil are carvacrol and thymol. These compounds can permeate the cell membrane causing disturbances in the plasma membrane of the microorganism [19-21], by different mechanisms such as changes in cell pH, and these chemical characteristics are desirable for inhibiting multidrug-resistant strains. However, other compounds have also been reported with therapeutic potential in infectious and inflammatory processes, such as camphor [22-23], linalool [24-25] and limonene [26].

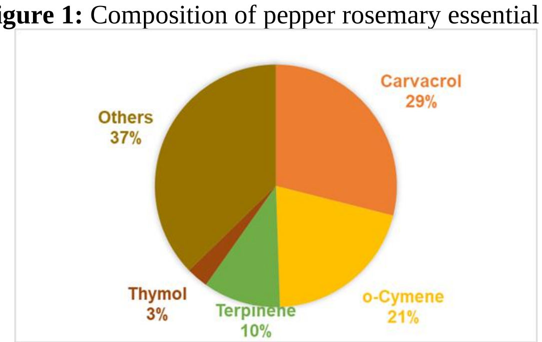
Table 1: Majority composition of the essential oil of Rosemary (*Lippia origanoides*)

N°	Retention time (min)	Compound	Retention rate	Absolute Area	Relative area (%)
1	5.39	β-Thujene	-	638076	0.93
2	5.59	α-Thujene	924	271081	0.39

3	6.04	Camphene	946	378649	0.55
4	7.09	β-Pinene	974	1152399	1.67
5	8.02	α-Terpinene	1014	1292519	1.88
6	8.30	o-Cymene	1022	14151249	20.53
7	8.43	Limonene	1024	533222	0.77
8	9.46	γ-Terpinene	1054	7139775	10.36
9	11.02	β-Linalool	1095	360338	0.52
10	12.95	Camphor	1141	192822	0.28
11	14.33	4-Terpineol	1174	153274	0.22
12	16.38	Thymol methyl ether	1232	6819516	9.89
13	18.71	Bornyl acetate	1284	1116267	1.62
14	19.03	Thymol	1289	2006529	2.91
15	19.42	Carvacrol	1298	19948820	28.94
16	22.52	α-Cubebene	1345	243463	0.35
17	23.11	unknown		364263	0.53
18	24.34	Caryophyllene	1408	5813346	8.43
19	24.92	α-Bergamotene	1411	370801	0.54
20	24.98	α-Guaiene	1437	194834	0.28
21	25.78	α-Humulene	1436	673240	0.98
22	26.83	Germacrene D	1484	568357	0.82
23	26.94	δ-Selinene	1492	220912	0.32
24	27.16	unknown		1371992	1.99
25	27.42	Chamigrene	1503	1093382	1.59
26	27.56	unknown		347076	0.50
27	27.98	β-Bisabolene	1505	818514	1.19
28	28.34	δ-Cadinene	1513	436043	0.63
29	29.26	unknown		131872	0.19
30	30.11	unknown		129213	0.19
				68931844	100.00

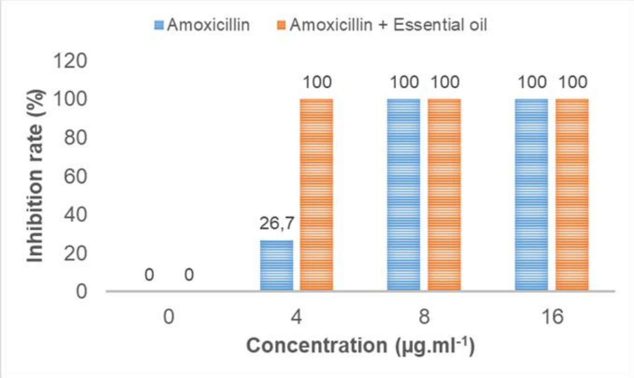
The chemical composition of *L. origanoides* oil can show phytochemical variation according to the main constituents of its essential oils. Genetic variation can cause quantitative and qualitative variation in the plant's secondary metabolism. And, because it represents a chemical interface between plants and the surrounding environment, the synthesis of secondary metabolites is often affected by environmental conditions [27] such as seasonality, temperature, water availability, ultraviolet radiation, nutrient availability, altitude, atmospheric pollution and mechanical stimuli or pathogen attack [28]. Due to the chemical composition of essential oils, they can interfere in different ways with the symbiosis between bacteria, mitigating the virulence factor, replication and antibiotic production, as well as affecting the formation of biofilms, motility and sporulation processes [29-30].

Figure 1: Composition of pepper rosemary essential oil



Among the fifteen strains of CoNS identified by MALDI-TOF MS, only 26.67% of the microorganisms were inhibited by the lowest concentration of Amoxicillin (4 µg.ml-1), while the concentrations of 8 and 16 µg.ml-1 showed an inhibition profile in 100% of the strains. When the antibiotic and essential oil were combined, the lowest dose of the antibiotic showed a sensitivity profile in 100% of the strains, demonstrating the potentiation effect of the antibiotic by the essential oil of pepper rosemary, inhibiting the CoNS strains even at the lowest concentration of the antibiotic (Figure 2). Therefore, this association between the essential oil of rosemary and pepper could be promising in the fight against bacterial strains resistant to amoxicillin. The high prevalence of multidrug-resistant isolates reinforces the current concern about the spread of resistant bacteria in the food production chain [31]. The synergistic effect of the antibiotic with essential oils can efficiently prevent the growth of strains at lower concentrations than those required for the individual antimicrobial in vitro, i.e. it makes it possible to reduce the dose of the antibiotic to be administered [32-34]. Other studies indicate that essential oils contain compounds that modulate resistance to antimicrobials, which can act as adjuvants in the antibiotic therapy of bacterial infections that are resistant to the synthetic antibiotics available on the market [35].

Figure 2: Inhibitory effect of pepper rosemary essential oil (*Lippia origanoides*) associated with the antibiotic Amoxicillin on coagulase-negative *Staphylococcus* strains



The in silico analyses point to possible molecular mechanisms that may be involved in the potentiation of the antibiotic amoxicillin by association with the essential oil of pepper rosemary. In the network and sub-networks shown in Figure 2, it is possible to observe an interaction between the antibiotic amoxicillin and the bioactive compound in pepper rosemary oil, Camphor, via the histamine molecule.

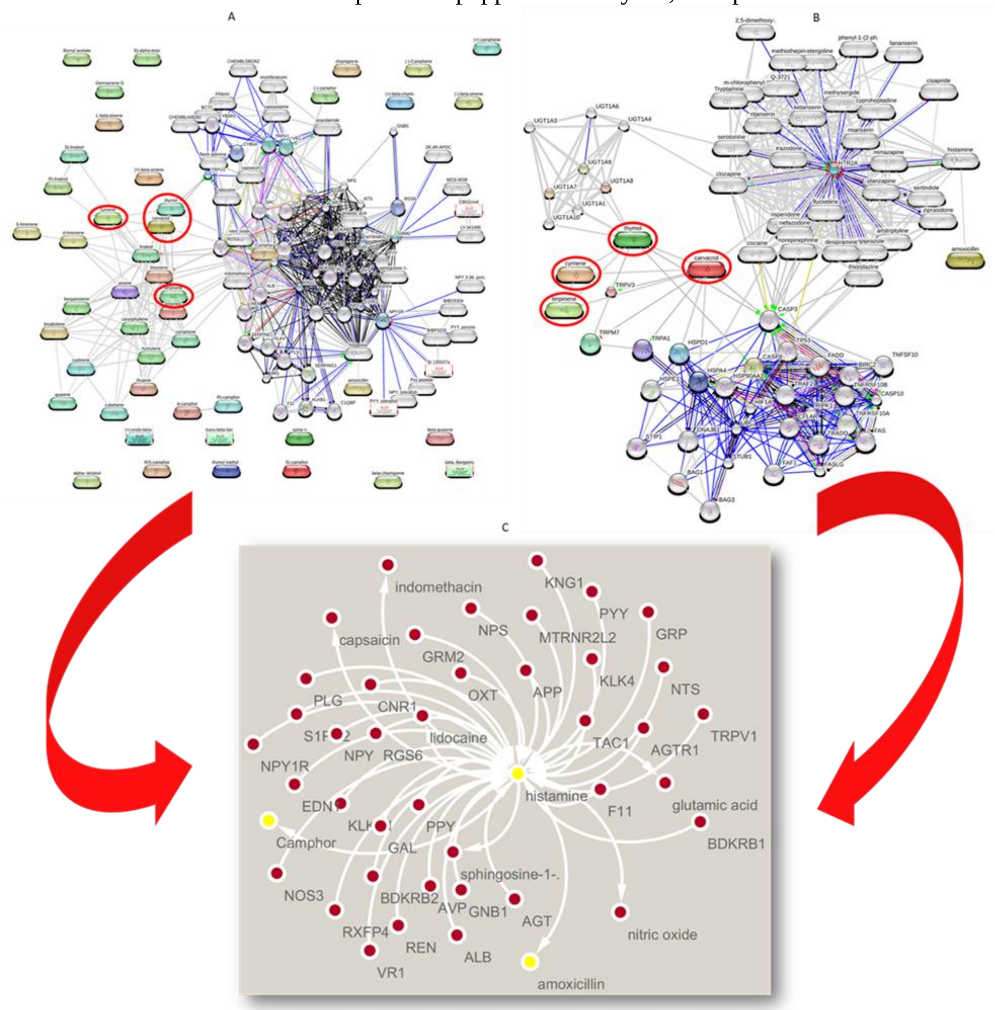
Histamine is a chemical messenger generated mainly in mast cells. Through various receptors, it mediates cellular responses, including allergic and inflammatory reactions, gastric acid secretion and neurotransmission in some regions of the brain [36]. Histamine is synthesized from the amino acid histidine, which is decarboxylated by histidine decarboxylase to form the amine histamine [37]. Histamine is one of the chemical mediators released in the tissues in response to stimuli such as cell destruction (as a result of cold, organism toxins, trauma, insect and spider venoms), allergies and anaphylaxis [38]. Histamine helps inflammation by releasing nitric oxide through the vascular endothelium, causing vasodilation of small blood vessels; increased secretion of pro-inflammatory cytokines in various types of cells and local tissues; increased permeability of capillaries [39-40].

Some beta-lactams such as penicillins, when in contact with previously sensitized cells, induce mast cell degranulation and the release of soluble inflammatory mediators such as histamine, leading to immediate clinical manifestations and even anaphylaxis [41]. On the other hand, in infectious processes, the main function of the immune response to infection is to phagocytose the bacteria, in addition to releasing granules and mediators, such as histamine, which amplify the inflammatory response by acting on microbicidal activity to eliminate the bacteria [42]. Thus, in the presence of microorganisms, inflammatory chemical mediators can raise body temperature, which has an inhibitory effect on different pathogens. In addition, they promote the activation of macrophages and induce the synthesis of nitric oxide, thus exerting an antimicrobial effect [43].

The literature reports a probable anti-histamine effect of essential oils with camphor as one of the major components. [44] found that these oils block histamine receptors on a histamine-induced contractile response. In addition, [45] demonstrated that the topical action of the oils inhibits the release of histamine from peritoneal mast cells. Although studies show that camphor has anti-inflammatory properties, being able to inhibit neutrophil migration in vitro, decrease cell infiltration and show topical anti-edematogenic activity [46], appearing to have an antagonistic action to the antibiotic amoxicillin, other studies show the synergistic effect of this compound to beta-lactam antimicrobials. Several hydrophobic compounds, such as camphor, present in the composition of essential oils [47-49], can interact with the cell membrane increasing its permeability to antibiotics, as well as inhibiting efflux pumps. Similar results were obtained with the essential oil of *Lippia origanoides* [50], which contains camphor. Other authors have also demonstrated the antibacterial activity of compounds such as camphor [51-54]. *Staphylococcus* aureus showed susceptibility to the action of oil, which has Camphor as a molecule described as responsible for antibacterial activity [55]. The data obtained in this study, in line with reports in the literature, suggest that the synergistic action of pepper rosemary oil with antibiotics occurs as a result of the combined action of different compounds and not just one isolated compound, as well as being an action carried out by different molecular mechanisms.

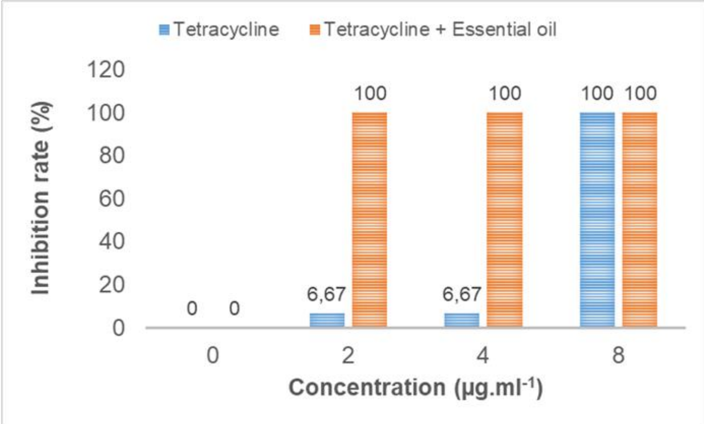
The major components of pepper rosemary essential oil are highlighted in the networks shown in Figure 3, interacting indirectly with the antibiotic amoxicillin through different signaling pathways. These compounds certainly contribute to the synergistic effect with the antibiotic amoxicillin for CoNS inhibition and disfavoring the antimicrobial resistance characteristic. Carvacrol has strong antibacterial activity against both Gram-positive and Gram-negative bacteria. Its activity against various strains has been reported, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumonia*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Enterobacter* spp. and *Serratia* spp. Studies have highlighted the possibility of its therapeutic use in fighting infections [56-57]. Research has revealed a synergistic action between the two monoterpenes, such as carvacrol and thymol, with the increased action being related to the greater interaction of the compounds with the bacterial membrane when combined [58-59]. As for Cymene and Terpinene, although there is controversial information about the antimicrobial action of these compounds, some studies claim that they may contribute to the antimicrobial activity of the oils [60-63], and they may be involved in some kind of synergism with other active compounds [64-65]. Reports on carvacrol, thymol, Cymene and Terpinene as β-lactamase inhibitors are still scarce in the literature. However, data from the literature indicate that the active site of β-lactamases has an affinity for small, hydrophobic molecules [66], such as some phytoconstituents present mainly in the essential oil of rosemary pepper, which suggests a protective action of the antibiotics by the constituents of the oils.

Figure 3: Chemical-protein interaction networks showing possible interactions between the antibiotic amoxicillin and bioactive compounds in rosemary essential oil. A - Network drawn up from all the bioactive compounds present in the composition of the pepper rosemary oil analyzed in this study and the antibiotic amoxicillin. B - Subnetwork made up of the oil's main constituents (Carvacrol, o-Cymene, γ-Terpinene and Thymol) and the antibiotic amoxicillin. C - Subnetwork drawn up from the compounds that interacted with histamine in the main network A, which was the molecule involved in the association of amoxicillin with the bioactive compound in pepper rosemary oil, Camphor.



About the antibiotic Tetracycline, it can be seen that at concentrations of 2 and 4 µg.ml-1, around 6.67% of the strains were inhibited at the respective doses, while 93.33% of the strains showed growth at low concentrations. At a concentration of 8 µg.ml-1, 100% of the strains were inhibited. When associating Tetracycline at different concentrations with the minimum inhibitory concentration of pepper rosemary oil, 100% of the strains of CoNS were inhibited, even at the lowest concentration of the antibiotic (Figure 4), showing the modulatory effect of the oil together with tetracycline in inhibiting microorganisms.

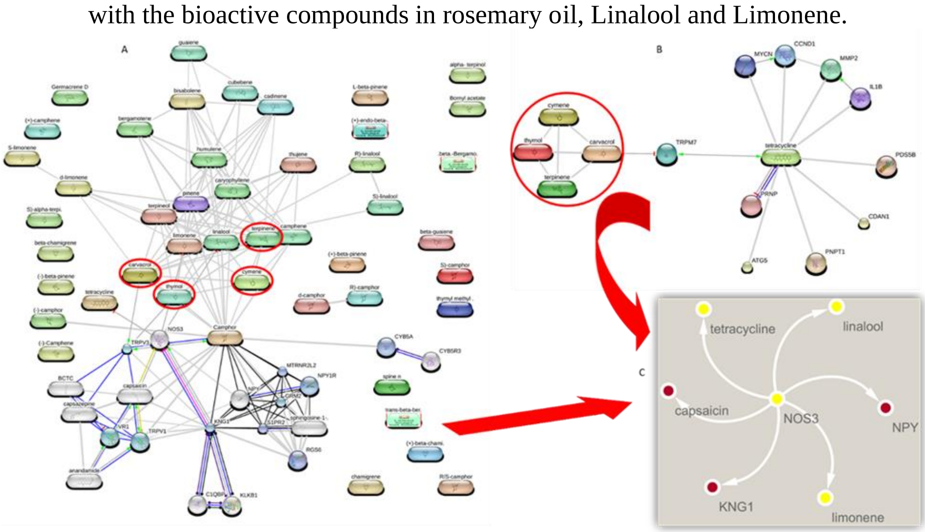
Figure 4: Inhibitory effect of the essential oil of rosemary pepper (*Lippia origanoides*) associated with the antibiotic tetracycline on strains of coagulase-negative *Staphylococcus*



In the in silico analyses shown in Figure 5, the interaction between the antibiotic tetracycline and the bioactive compounds in rosemary oil, linalool and limonene, was observed through the nitric oxide synthase 3 (NOS3) molecule. Nitric oxide (NO) is a molecule with a defense function against bacterial biofilms, produced by NO synthase (NOS) [67]. The production of nitric oxide is considered the most pronounced antimicrobial mechanism of macrophages [68]. Based on the bioinformatic analyses obtained in the present study, it is suggested that the bioactive compounds in the oil may potentiate the action of tetracycline through interaction with the NOS3 enzyme. Studies show that phenolic compounds such as linalool and limonene are important anti-inflammatory agents because they modulate the expression of pro-inflammatory enzymes such as nitric oxide synthase [69] and cytokines [70]. The literature cites linalool [71] and D-limonene [72] among numerous substances obtained from medicinal plants as potentially antimicrobial phytoconstituents. Limonene is a monoterpene widely dispersed in nature, mainly in the peels of citrus fruits [73]. In a previous study, limonene was found to have antimicrobial activity against strains of *C. albicans*, *E. coli* and *S. aureus* [72], which are considered to be microorganisms resistant to available synthetic antimicrobials. Linalool has been used successfully as a sedative and its bactericidal and fungicidal properties are being studied [71, 74].

The presence of the oil's main constituents in the chemical-protein interaction networks indicates that these bioactive compounds may also have a synergistic effect with the antibiotic tetracycline. Functional studies are needed to prove the isolated and combined action of the constituents of pepper rosemary oil in potentiating the action of antibiotics.

Figure 5: Chemical-protein interaction networks showing possible interactions between the antibiotic tetracycline and bioactive compounds in the essential oil of pepper rosemary. A - Network drawn up from all the bioactive compounds present in the composition of the pepper rosemary oil analyzed in this study and the antibiotic tetracycline. B - Subnetwork made up of the oil's main constituents (Carvacrol, o-Cymene, γ-Terpinene and Thymol) and the antibiotic tetracycline. C - Subnetwork made up of the compounds that interacted with NOS3 in the main network A, which was the molecule involved in the association of tetracycline with the bioactive compounds in rosemary oil, Linalool and Limonene.



The use of essential oils has been widely used as alternative solutions to conventional antimicrobials. The mechanism of action of oils on microorganisms, as they have hydrophobic compounds, permeabilize the cell membrane, altering protein synthesis and ATP production, leading to cytoplasmic alterations due to changes in intracellular pH [8]. In this context, the essential oil of rosemary (Lippia origanoides) can be used as a potential antimicrobial in conjunction with antibiotics, since it showed synergistic interactions between the oil and beta-lactam antibiotics, against antimicrobial-resistant CoNS.

Controlling strains of CoNS is essential since this group of microorganisms is associated with subclinical udder infections, raising the somatic cell count (SCC) of milk and causing the animal's production to drop [75]. Inhibiting this group of microorganisms is essential in order to avoid possible problems for public health [76], especially when drinking fresh milk or dairy products that have not been processed correctly, leading to food poisoning.

The sensitivity of microorganisms to the action of essential oils and the modulating effect associated with antibiotics is still the subject of studies. Further studies are needed to examine each component of the essential oil separately, as well as different combinations of phytochemicals, in order to ascertain whether the chemical constituents have a modulating action in isolation or in association.

IV. Conclusion

It is concluded that the essential oil of pepper rosemary is capable of potentiating the activity of the antibiotics amoxicillin and tetracycline against CoNS of clinical origin. These results indicate that this natural product contains phytochemicals that could be used as antimicrobial adjuvants in the treatment of infections caused by CoNS bacteria resistant to the antibiotics conventionally used in dairy farming.

Considering that bacteria have a difficult ability to adapt to volatile oils, as they are complex mixtures of bioactive substances, this association between synthetic molecules and natural products is capable of reaching various targets in the bacterial cell, acting through different mechanisms of action and cooperating synergistically to inhibit bacterial growth and combat resistance. There is a need to individually study combinations of volatile oils and antimicrobials capable of promoting synergistic effects, confirming the mechanisms of action that prevent bacterial growth and/or death by carrying out functional studies. Once the synergistic effect of pepper rosemary oil and beta-lactams has been confirmed, this combination could be used as the basis for developing an alternative therapy to combat difficult-to-control infections caused by resistant bacteria.

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