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Antibacterial Activity of Monoterpenes (R) - (+) - *Citronellal* and (S) - (-) - *Citronellal* against *Escherichia coli* Strains

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ABSTRACT

Terpenes are a group of volatile natural substances, also known as volatile organic compounds, of plant origin and present in essential oils, which are the main therapeutic compounds obtained from medicinal plants, which have a variety of bioactive compounds, such as monoterpenes. In this case, stands out the monoterpene citronellal, a product of the secondary metabolism of plants. Studies with the chemical derivatives of citronellal and citronellol, both isolated from citronella oil, demonstrated good allopathic, antioxidant, herbicidal, antifungal and antimicrobial activity. Thus, the main objective of this work was to determine the antibacterial activity of (R) - (+) - citronellal and (S) - (-) - Citronellal monoterpenes against strains of *Escherichia coli*. For this, the broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). For the pharmacological tests, the substances were solubilized in DMSO and diluted in distilled water. By analyzing the CBM results it can be seen that for monoterpene (R) - (+) - citronellal the substance had a bactericidal effect, already for (S) - (-) - citronellal, the substance had a bacteriostatic effect to strains of *E. coli*. Based on these results it can be stated that the monoterpenes had a strong antibacterial effect against *E. coli* strains.

INTRODUCTION

Foodborne pathogens are diverse in nature and continue to be one of the major causes of public health problems in the world. They are responsible for considerable morbidity and mortality, costs of medical care and loss of productivity [1].

Pathogenic microorganisms (eg, bacteria, viruses, fungi and parasites) that invade host cells for reproduction cause infectious diseases. These diseases represent serious public health problems affecting a significant fraction of the world's population and, because of their socioeconomic aspect, represent one of the major challenges for the 21st century, especially in the poorest and most vulnerable regions of the world [2].

Bacterial resistance can cause infections that are very difficult to treat, remaining in place and favoring the proliferation of bacteria. The antibiotic should be prescribed rationally, based on a concrete diagnosis and not based only on epidemiological data of certain etiological agents responsible for certain infections. However, unnecessary and excessive consumption without this careful evaluation makes the development of this resistance more propitious, making it a serious problem in the treatment of infectious diseases. This event occurs in a greater proportion in hospital settings where the incidence of the use of these drugs is proposed in great quantity [3].

Bacterial infections can be caused by several species, and the main pathogens found in community and nosocomial infections are *Staphylococcus aureus* and *Escherichia coli* [4].

Escherichia coli is a Gram-negative rod-shaped bacterium with habitat in the intestinal tract. They can be immobile or mobile by scourges. The presence of fimbriae and other related structures plays an important role in the virulence of the bacteria. Some studies show that in relation to the problems caused by it, the main foods such as poorly cooked meats, mainly of bovine origin (hamburgers), cured sausages, lettuce seeds, unpasteurized fruit juices, cured cheese and raw milk [5].

E. coli is probably the most studied bacterium and one of the most commonly isolated in the clinical laboratory of microbiology. The strains of *E. coli* that are biologically significant for humans can be classified (based on genetics and clinical criteria) into 3 large groups: commensal, intestinal pathogens (enteric or diarrheal) and extraintestinal pathogens[6].

Since antimicrobial resistant bacteria present a challenge in the treatment of infections, the need to find new substances with antimicrobial properties to combat these microorganisms is well known[7].

In this context and due to ethnomedicinal use, phytotherapy is widely practiced. Among the medicinal plants most used by the population, few have proven action. However, traditionally consolidated popular use has been used as a guide for pharmacological research [8,9].

Medicinal plants have attracted the attention of researchers because they are a promising source of substances that can be used to control microorganisms. In the literature, there are several studies demonstrating its effects, in particular, antimicrobials on a great diversity of microorganisms, besides presenting an effective treatment on some resistant strains [10].

Essential oils are products derived from the secondary metabolism of plants, and can be extracted by all organs such as shoots, leaves, flowers, stems, twigs, roots and seeds and are stored in secretory, epidermal and trichome cells. They have gained more and more notoriety and aroused interest, among researchers from around the world, due to their innumerable properties [11].

Representative of a class of secondary metabolites, monoterpenes are the constituents of essential oils present in aromatic plant species. Its Biosynthetic origin derives from isoprene units, which are constituted by ten units of carbon[12].

Citronellal also belongs to the group of monoterpenoid alcohols and is the main component in the mixtures of terpenoid chemical compounds, giving the citronella oil its characteristic aroma of marked lemon [11].

It is one of the major substances of essential oils of aromatic plants [13], Citronellal has shown to have many activities, among them, we can exemplify antimicrobial action[14], allelopathic, antioxidant[15], herbicide[16] and insecticidal and repellent activity[17].

On the other hand, due to the lack of knowledge of the possible existence of the toxic action, as well as its adequate indication, medicinal plants are often used incorrectly, not producing the desired effect[18].

Based on the information about the therapeutic potential of monoterpenes and the importance of combating infections caused by multiresistant bacteria, this innovative work aims to

evaluate the possible antibacterial activity of (R) - (+) - citronellal and (S) - (-) - Citronellal against strains of *Escherichia coli*.

MATERIALS AND METHODS

1.1 Place of Work

The experiments were carried out at the Microbiology Laboratory of the Rural Health and Technology Center (CSTR) of the Federal University of Campina Grande (UFCG), state of Paraíba, Brazil.

2.1 In vitro assays

2.1.1 Phyto-constituent

The (R)-(+)-citronellal and (S)-(-)-Citronellal monoterpenes were purchased from the Sigma-Aldrich Industry (São Paulo-SP). *For* the pharmacological tests, the substance was solubilized in DMSO and diluted in distilled water. The concentration of DMSO (dimethylsulfoxide) used was less than 0.1% v / v. The antimicrobial used in the tests as a positive control was chloramphenicol, purchased from Sigma-Aldrich® (São Paulo-SP).

2.1.2 Bacterial Species and Culture Media

Gram-negative bacteria were used: *Escherichia coli ATCC 8539, Escherichia coli 101, Escherichia coli 102, Escherichia coli 103, Escherichia coli 104*, previously isolated, identified and kindly provided by the Laboratory of Biochemistry, Genetics and Radiobiology (BioGer) of Molecular Biology Department (DBM), Center for Exact and Natural Sciences (CCEN), Federal University of Paraíba (UFPB), coordinated by Prof. Drª. Hilzeth de Luna Freire Pessôa. In the study of the antimicrobial activity a bacterial inoculum of approximately 1,5 x 10^8 CFU / mL standardized according to the turbidity of the 0.5 tubes of McFarland scale[19,20].

2.1.3 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the monoterpenes was determined by the broth microdilution technique[19,20]. Sterile, capped 96-well plates were used. At each well in the plate, 100 µL of the doubly concentrated Muller-Hinton liquid medium was added. Then 100 µL of the emulsion of each monoterpene at the initial concentration of 2048 µg / mL (also

doubly concentrated) was dispensed into the wells of the first row of the plate. The concentrations of 1024, 512, 256, 128, 64, 32, 16, 8 and 4 µg / mL were obtained by means of a two-fold serial dilution so that in the first row of the plate is concentration, and in the latter, the lowest concentration. Finally, 10 µL of the inoculum of approximately 1.5 x 108 CFU / mL of the bacterial species was added to the wells, where each column of the plaque refers to a bacterial strain, specifically.

At the same time, the same test was performed with the antibacterial chloramphenicol. A microorganism control was performed by placing 100 µL of the same doubly concentrated Muller-Hinton, 100 µL of sterile distilled water and 10 µL of the inoculum of each species into the wells. To verify the absence of interference in the results by the solvents used in the preparation of the emulsion, in the case of DMSO, a control was made in which 100 µL of the double concentrate broth, 100 µL of DMSO and 10 µL of the bacterial suspension were placed in the wells. A sterility control of the medium was also performed, where 200 µL of Muller-Hinton was placed in an orifice without the suspension of the bacteria.

The plates were aseptically closed and incubated at 35 ° C for 24-48hrs to be read. MIC for monoterpene and antibacterial will be defined as the lowest concentration capable of visually inhibiting the bacterial growth observed in the orifices when compared to control growth. The experiments were performed in duplicate.

2.1.4 Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of monoterpene was also determined for bacterial strains. After reading the MIC in 48 hours, aliquots of 20 µL were removed from each well of the microtiter plate that showed no bacterial growth and were transferred to wells of a new microtiter plate containing 100 µL of Muller-Hinton, devoid of any antimicrobial. The inoculated plates were aseptically closed and incubated at 35 ° C, and the MBCs were recorded after 48 h. The MBC was defined as the lowest concentration of monoterpene that resulted in visible inhibition of microorganism growth[21.22].

RESULTS AND DISCUSSION

Results concerning the antibacterial action of (S) - (-) - citronellal against *E. coli* strains are given in Table 1 and 2, where the (S) - (-) - Citronellal MIC was determined as 256 µg / mL for the strains tested. For CBM, it was determined as 256 µg / mL for the strain *Escherichia*

coli 104; 512 µg / ml for the strain *Escherichia coli* ATCC 8539; 1024 µg / mL for the strains *Escherichia coli* 101 and *Escherichia coli* 102 and without activity for the strain *Escherichia coli* 103.

The results concerning the antimicrobial action of (R) - (+) - citronellal against strains of *E. coli* are shown in Table 3 and 4, where the MIC50 was determined to be 512 µg / mL. CBM ranged from 256 µg / mL to 512 µg / mL for strains of *E. coli* 101 and 104, respectively, the others had no activity.

Table 1. Minimum inhibitory concentration (MIC) in µg / mL of monoterpene *(S) - (-) - Citronellal* against different strains of *Escherichia coli.*

	(S) – (-) Citronellal				
	Escherichia coli ATCC 8539	*Escherichia coli 101*	*Escherichia coli 102*	*Escherichia coli 103*	*Escherichia coli 104*
512µg/mL	+	+	+	+	+
256 µg/mL	+	+	+	–	+
128 µg/mL	+	–	–	–	+
Negative control	–	–	–	–	–
Positive control	+	+	+	+	+

(-) = There was no apparent inhibition of the strain
(+) = visible inhibition of the strain
(*) = no activity
Source: Own authorship

Table 2. Minimum Bactericidal Concentration (MBC) in µg / mL of the monoterpene *(S) - (-) - Citronelal* against different strains of *Escherichia coli.*

	(S) – (-) Citronelal				
	Escherichia coli ATCC	*Escherichia coli 101*	*Escherichia coli 102*	*Escherichia coli 103*	*Escherichia coli 104*
1024µg/mL	+	+	+	*	+
512 µg/mL	+	_	_	*	+
256µg/mL	_	_	_	*	+
Negative control	_	_	_	*	_
Positive control	+	+	+	*	+

(-) = There was no apparent inhibition of the strain
(+) = visible inhibition of the strain
(*) = no activity
Source: Own authorship

Table 3. Minimal Inhibitory Concentration (MIC) in µg / mL of the monoterpene *(R) - (+) - Citronellal* against different strains of *Escherichia coli*.

	(R) – (+)				
	Escherichia coli ATCC 8539	*Escherichia coli 101*	*Escherichia coli 102*	*Escherichia coli 103*	*Escherichia coli 104*
1024	+	+	+	+	+
512	+	+	-	-	+
256	-	+	-	-	+
128	-	-	-	-	-
Negative control	-	-	-	-	-
Positive control	+	+	+	+	+

(-) = There was no apparent inhibition of the strain
(+) = visible inhibition of the strain
(*) = no activity
Source: Own authorship

Table 4. Minimum Bactericidal Concentration (MBC) in µg / mL of monoterpene *(R) - (+) - Citronellal* against different strains of *Escherichia coli.*

(R) – (+) Citronelal					
	Escherichia coli ATCC 8539	*Escherichia coli 101*	*Escherichia coli 102*	*Escherichia coli 103*	*Escherichia coli 104*
1024 µg/mL	*	+	*	*	+
512 µg/mL	*	+	*	*	+
256 µg/mL	*	+	*	*	-
Negative control	*	-	*	*	-
Positive control	*	+	*	*	+

(-) = There was no apparent inhibition of the strain
(+) = visible inhibition of the strain
(*) = no activity
Source: Own authorship

Sartoratto et al. (2004) suggest that antimicrobial activity is classified as strong when, for essential oils, they have MICs up to 500 µg / mL, moderate for MICs of 600 to 1500 µg / mL and weak for MICs above 1500 µg / mL[23].

Thus, according to the results of the (R) - (+) - citronellal and (S) - (-) - citronellal monoterpenes, they can be considered strong inhibitors against the strains *Escherichia coli*, since it presented an MIC50 (Inhibitory Concentration Minimally capable of inhibiting the growth of 50% of the strains) of 512 µg / mL and 256 µg / mL, respectively.

According to Hafidh et al. (2011) for a compound to be considered bactericidal or bacteriostatic according to the Minimum Bactericidal Concentration (MBC) should be equal to or twice as large as the MIC or the MBC should be greater than twice the MIC, respectively. By analyzing the CBM results it can be seen that for monoterpene (R) - (+) - citronellal the substance had a bactericidal effect, already for (S) - (-) - citronellal, the substance had a bacteriostatic effect to strains of *E. coli*[24].

The results obtained in this study corroborate with the data obtained with the racemic mixture of Citronella monoterpene, which revealed to have numerous activities, among them antimicrobial action[14].

CONCLUSION

In view of the results obtained, it was possible to observe that the (R) - (+) - citronellal and (S) - (-) - citronellal monoterpenes presented relevant results. In view of this, they may be considered as promising for the treatment of many diseases caused by the *E. coli* bacterium, however further studies are necessary to elucidate mechanisms and standards of efficiency and effectiveness.

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