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Potential Antibacterial Action of α-Pinene †

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Abstract: The indiscriminate use of antibiotics generates several problems for human health, the main one being bacterial resistance. The abuse of antibiotics is responsible for reducing the effectiveness of medicines, making it difficult to treat diseases and representing a major obstacle for researchers and health professionals. Natural alternatives have been widely studied, such as essential oils and their phyto-constituents, in order to verify their antibacterial action. This research aimed to identify the antibacterial activity of α-pinene. This is a descriptive study, using a qualitative approach, with methodological experience based on an integrative review. The bibliographic survey was carried out in the LILACS and MEDLINE, though the Virtual Health Library, PubMed and Web of Science databases, using the following search strategies: Anti-bacterial agents AND α-pinene for the PubMed and VHL databases, and (Antibacterial agents AND pinene) and (Antimicrobial AND α-pinene) on the Web of Science. After reading the articles in full, 10 works were selected. α-pinene was relatable, including its positive mix and its association with antimicrobials. The article points out that α-pinene has wide potential in antimicrobial therapy in order to inhibit the growth of bacteria as an isolated result or as a synergist of antibiotics. However, they are bactericidal and bacteriostatic when against bacterial strains. Therefore, it is concluded that it is relevant to develop scientific research to analyze the effectiveness of this compound to the most diverse microorganisms that affect human health.

Keywords: antibacterial activity; α-pinene; pinene; microbiology

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1. Introduction

The discovery of the first antibiotic, penicillin, by Alexander Fleming in 1928 revolutionized the history of science and enabled the advancement of the medical industry and the development of new antibiotics [1]. However, bacterial resistance of some strains is still an obstacle for conventional antibiotics. In addition, poor management of infection control practices allows bacterial proliferation. Thus, high morbidity and mortality due to the positive selection of multidrug-resistant pathogens related to the irrational use of these drugs constitute challenges to public health [2].

The indiscriminate use of antibiotics has led to the development of microorganisms resistant to drug therapies. Thus, the use of alternative approaches in the fight against bacterial pathologies has been shown to be a viable line of research [3]. In this context, natural compounds, based on their chemical structures and biological properties, constitute one of the main sources for the discovery of new drugs [4]. Among these compounds, terpenes stand out as active pharmaceutical ingredients, as their antimicrobial effects are known as a result of their action on the function and structure of microbial cell walls and membranes [5].

Among the monoterpene compounds is pinene, which has two active isomers, one of which is α-pinene, with the chemical formula $C_{10}H_{16}$. It is present in several essential oils and many studies have analyzed the antibacterial activity of α-pinene, as well as its potential to modulate antimicrobial resistance [6]. In this sense, the present work aimed to identify the antibacterial activity of α-pinene.

2. Methodology

2.1. Study

This is a descriptive-exploratory study, using a qualitative approach, with methodological artifice based on integrative review. This article questions the following research element: What is the understanding that the current literature has about the antibacterial action of α-pinene in different strains of bacteria? A bibliographic survey was conducted in three research bases, using different search strategies. For LILACS and MEDLINE, the search occurred through the BVS. In PubMed, the search strategy used was: Antibacterial agents AND α-pinene. In the Web of Science, two search strategies were designed: (Antibacterial agents AND pinene) and (Antimicrobial AND α-pinene), used alone.

2.2. Inclusion and Exclusion Criteria

Full-text studies published in the last five years (2017–2022), written in Portuguese, English and Spanish, addressing the antibacterial effect in different strains of α-pinene compound bacteria were included. Reviews of literature, books, editorials, dissertations and theses were excluded from this review. Studies that presented analysis of chemical composition and antibacterial activity of different essential oils were disregarded from the final analysis.

2.3. Selection and Analysis of Studies

The studies were selected manually and blindly by the researchers M.F.d.A.B. and R.d.L.S. by title and abstract, and were read in full. It is emphasized that J.P.d.A.C. was responsible for the analysis of conflicting articles. The steps followed the PRISMA flowchart, 2009, adapted by the authors. For organization purposes, the articles were categorized and analyzed by a data collection instrument. This instrument was organized according the authors, year of publication, database in which they were found, studied compound, their concentration, bacterial strains evaluated, presence of isolated action of α-pinene and use of other antibiotics.

3. Results and Discussion

Initially, 1637 articles were found in electronic search databases without the use of filters. After applying the filters, 299 works remained; 35 in the VHL, 42 in PubMed and 222 in the Web of Science. A total of 16 studies were excluded because they were literature reviews, leaving 283 articles. After reading the titles and abstracts, nine articles were selected in a convergent manner, with two duplicates being found, and four chosen from among the divergent ones. At the end of the complete reading of the texts evaluated for eligibility, 10 studies were selected for the composition of the study.

For organization purposes, the articles are detailed in Table 1, in which the authors, year of publication, bacterial strains evaluated, type of assay used and sensitivity to α-pinenoand active concentrations are presented.

Table 1. Presentation of the synthesis of the results found in the selected articles.

Author (Year)	Bacterial Strains	Type of Test Used	Sensitivity to A-Pinene	Active Concentrations
Ložienė et al. (2018)	*S. aureus* ATCC 29213 * *E. coli* ATCC 25922 **	Microdilution in broth	Positive	-
Leite-Sampaio et al. (2022)	*E. coli*, EPEC e ETEC **	Microdilution in broth	Weak or none	MIC ≥ 1024 µg/mL for (+)-α-pinene + sulfamethoxazole + trimethoprine
Sieniawska et al. (2017)	*M. tuberculosis* ***	Serial dilution	Positive Negative	MIC = 0.475 µg/mL for α-pinene + rifampicin MIC = 16 to 125 µg/mL for α -pinene + ethambutol; MIC = 32 to 125 µg/mL α-pinene + isoniazid
Shih et al. (2020)	Nonspecific	Standard total plate count	Positive	MIC = 0.03125 g/100 mL, 0.0625 g/100 mL e 0.125 g/100 mL
Wang, Chen e Hou (2019)	*E. coli* ** *S. enterica* ** *S. aureus* *	Dilution in agar with minor modifications	Positive	MIC = 0.686 mg/mL MIC = 0.686 mg/mL MIC = 0.420 mg/mL
Araújo et al. (2021)	*S. aureus* 1199 *	Serial dilution	Negative	MIC = between 20 and 40 µg/mL for α-pinene + ethidium bromide; between 50 and 75 µg/mL for α-pinene + norfloxacin
Melkina et al. (2021)	*E. coli* K12 MG1655, JW3914-1, JW3933-3, QC868 e QC871 **	Agar diffusion	Weak to ≤5 mg (≤6 µL) (+)-α-Pinene	-
Šimunović et al. (2020)	*C. jejuni* NCTC 11168 **	Microdilution in broth	Weak	Overall MIC for (-)-α-pinene alone = 2000 mg/mL
Eduardo et al. (2018)	*E. coli* ATCC (25922) ** *S. aureus* ATCC 25923 *	Disk diffusion, broth microdilution and bacterial killing kinetics	Positive	Inhibition halos = 12 mm at a concentration of 160 µL/mL Inhibition halos = 11 mm at a concentration of 160 µL/mL
Amaral et al. (2020)	*E. coli* ATCC 25922 **	Broth microdilution and modified disk diffusion	Positive in synergism with other antibiotics	Inhibition halos for (+)-α-pinene = 13 mm at a concentration of 160 µL/mL

Gram stain: * gram-positive, ** gram-negative, *** does not aplly. Source: Own authorship, 2022.

After screening the studies, it was observed that 40% obtained positive results for the isolated antibacterial action of α-pinene. Of these, the strains worked were *E. coli* ATCC, *S. aureus* ATCC and *S. enterica*, revealing their susceptibility to the compound [3,7–9]. However, strains of *C. jejuni* NCTC, *S. aureus* 1199B and Mycobacterium tuberculosis were not sensitized with the action of α-pinene [5,10,11].

One of the main mechanisms of action of α-pinene is the heat shock generated by direct contact with *E. coli* strains, through the modification of the DnaKJE-σ32 complex, and this subunit is responsible for the synthesis of heat shock promoters [12]. In addition to this mechanism, when evaluating the antibacterial activity of α-pinene in species of bacteria commonly found in food, values of 0.686 mg/mL were obtained for *E. coli*, 0.686 mg/mL for *S. enterica* and 0.420 mg/mL for *S. aureus* for MIC. This demonstrates the more effective action of monoterpene on gram-positive bacteria, given that gram-negative bacteria have

lipopolysaccharides that block the penetration of hydrophobic compounds, in this case α-pinene. However, the work does not describe the types of strains used [7].

In studies in which essential oils were used, the result was positive [3,13]. In the essential oils extracted from *Juniperus communis*, when α-pinene is isolated with different enantiomeric concentrations, the antibacterial activity of this substance can be determined through the broth microdilution method. It was revealed that, in the strains of *E. coli* and *S. aureus*, the pure α-pinene compound with the highest concentration of the positive enantiomer was more effective than the oil with the positive form also predominant, as it presented lower MIC, demonstrating that the other components of essential oil chemicals can interfere with α-pinene activity [3].

The same result is seen when analyzing the antibacterial activity of Pistacia essential oil against Heliobacter pylori, using the microdilution and disc diffusion method. Regarding the composition of the oil, α-pinene corresponded to 93.17% of the total. The zones of inhibition ranged from 26 to 35 mm, while the MIC ranged from 275 to 1100 μg/mL. Thus, α-pinene can be considered as the main agent responsible for the antibacterial activity [13]

α-pinene has low antimicrobial action against Campylobacter Jejuni, even at high concentrations, but it is able to modulate the quorum sensing of this microorganism, as well as the colonization of chicken hosts when administered at subinhibitory concentrations [11]. When testing the antibacterial activity of the negative enantiomer of α-pinene against strains of this same bacterium, with the MIC defined as the amount necessary for the compound to reduce the fluorescence to white, the low levels of antibacterial activity of the monoterpene were confirmed, having considering that the MIC was considered very high [14]. Furthermore, α-pinene demonstrated low antimicrobial action against multidrug-resistant *E. coli*, while against enteropathogenic and enterotoxigenic serotypes, activity was not observed [15].

It was noted in most articles that tests of the synergism of α-pinene with antibiotics were performed [5,10–12,15,16] Among them, research exclusively focused on this association was carried out realizing that in fact the compound potentiated the antibacterial effect of different antibiotics, as well as induced cross-resistance in some drugs [16].

4. Conclusions

The present study demonstrates that α-pinene has antibacterial properties when applied to certain microorganisms. However, it was evidenced that its effectiveness is directly linked to its concentration, the interaction with certain bacterial strains and in some cases the concomitant action of antibiotics, acting in the latter case as a synergist potentiating the drug.

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