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Proceeding Paper

Potencial Antibacterial Action of Alpha-Pinene †

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Abstract: The indiscriminate use of antibiotics generates several for human health, the main one being bacterial resistance, responsible for making medicines of medicines, making it difficult to treat diseases and representing a major obstacle to the study and professional health problems. Natural alternatives are being improved, such as essential oils and their phyto-constituents, in order to verify their antibacterial action. This research aimed to identify the antibacterial activity of alpha-pinene. This is a descriptive study, with a qualitative approach, with methodological experience based on an integrative review. The bibliographic survey was carried out in the LILACS and MEDLINE search bases, through the Virtual Health Library, PubMed and Web of Science, through the search strategy Anti-bacterial agents AND alpha-pinene for the Pubmed and VHL databases and (Antibacterial agents AND pinene) and (Antimicrobial AND alpha-pinene) on the Web of Science. At the end of reading the articles in full, ten works were selected. Alpha-pinene was relatable, as was its positive mix and associated with its antimicrobials. The article points out that alpha-pinene has a 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 the development of scientific research that analyzes the task of this compound to the most diverse microorganisms to human health is relevant.

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

2. Metodology

2.1. Study

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

2.2. Inclusion and Exclusion Criteria

Full-text studies published in the last five years (2017–2022), in the following languages: Portuguese, English and Spanish, addressed the antibacterial effect in different strains of alpha-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 means of a collection instrument in which the authors are presented, year of publication, database in which they were found, studied compound, their concentration, bacterial strains evaluated, whether isolated action of alpha-pinene and if other antibiotics were used.

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. 16 studies were excluded because they were literature reviews, leaving 283. After reading the titles and abstracts, 9 articles were selected in a convergent manner, with two duplicates being found, and 4 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 were detailed in Table 1, in which the authors, year of publication, bacterial strains evaluated, type of assay used, sensitivity to alpha-pinenoand active concentrations are presented.

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

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Author (Year)	**Bacterial Strains**	**Type of Test Used**	**Sensitivity to Alpha-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 alpha-pinene + ethidium bromide; between 50 and 75 μg/mL for alpha-pinene + norfloxacin
end				
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 alpha-pinene. Of which, 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 alpha-pinene [5,10,11].

One of the main mechanisms of action of alpha-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 alpha-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 alpha-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]. The essential oils extracted from Juniperus communis, when alpha-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 alpha-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 essential oil chemicals can interfere with alpha-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, alpha-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, alpha-pinene can be considered as the main agent responsible for the antibacterial activity [13]

Alpha-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 alpha-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, alpha-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 alpha-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 demonstrated that alpha-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.

5. Patents

Author Contributions: Conceptualization, M.F.d.A.B.; methodology, J.P.d.A.C., R.d.S.L. and M.F.d.A.B.; validation, S.B.F. and T.R.d.M.; formal analysis, T.R.d.M.; investigation, M.F.d.A.B., R.d.S.L. and J.P.d.A.C.; resources, S.B.F.; data curation, T.R.d.M.; writing—original draft

preparation, M.F.d.A.B., R.d.S.L., J.P.d.A.C. and T.R.d.M.; writing—review and editing, M.F.d.A.B. and J.P.d.A.C.; visualization, M.F.d.A.B.; supervision, S.B.F.; project administration, M.F.d.A.B. All authors have read and agreed to the published version of the manuscript.

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