EVALUATION OF ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF CINNAMOMUM ZEYLANICUM, EUGENIA CARYOPHYLLATA, AND ROSMARINUS OFFICINALIS AGAINST STREPTOCOCCUS ORALIS

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INTRODUCTION

Streptococcus oralis is a Gram-positive coccus-shaped bacterium, considered a commensal bacterium which belongs to the mitis group. It is one of the first bacteria which begin to form the biofilm of dental plaque [1]. The research shows that this bacterium can interact with Porphyromonas gingivalis, which is considered one of the main causes of periodontal disease, the most common disease affecting the human oral cavity. In addition, it is an opportunistic bacterium that affects immunodeficient individuals and those with hematologic malignancies. In these individuals, it can create complications such as bacterial endocarditis, respiratory distress syndrome in adults, and streptococcal shock [2].

It is proven that advances in science and medicine have led to the development of many drugs of interest today. However, their use is not always rational and their long-term prescription has led to the so-callediatrogenic diseases, responsible for many adverse effects, even death. Thus, the misuse of antibiotics against various infections results in decreased efficiency due to increased resistance of bacteria [3-5]. This antibiotic resistance phenomenon is general and concerns all bacterial species including those of the oral cavity [6].

Furthermore, the other antibacterial agents used in the prevention and treatment of oral diseases, such as cetylpyridinium chloride, chlorhexidine, amine fluoride, or products containing such agents, are not devoid of toxicity [7], and side effects as in the case of ethanol (commonly used in mouthwashes) was observed in oral cancer [8].

Therefore, the search for alternative products continues, and natural phytochemicals isolated from plants used in traditional medicine are considered good alternatives to synthetic chemicals. Natural substances such as cinnamon bark oil and clove oil (cinnamaldehyde and eugenol) showed activity against oral bacteria [9].

In similar research of natural antimicrobial substances, we are interested in evaluating the antibacterial effect of the essential oils of Cinnamomum zeylanicum, Eugenia caryophyllata, and Rosmarinus officinalis against S. oralis which is a pathogen found in an unusual way and predominantly following a lack of hygiene in the oral cavity, in diabetic patients with periodontal disease.

METHODS

Essential oils

The three essential oils tested in the present study were provided by the Subnarôme Laboratory, Department of Food Science and Nutrition at the Institute of Agronomy and Veterinary Hassan II in Rabat, Morocco. They were extracted by hydrodistillation and stored at 4°C before use. The chemical composition of these essential oils was analyzed by a gas chromatograph. The percentage composition of these oils is shown in Table 1.

Bacterial strains and culture conditions

The tested bacterial strain was a Gram-positive bacterium: S. oralis, which was isolated from the oral cavity of diabetic patients with gingivitis. Bacterial strains were grown in blood agar medium and incubated at 37°C in a CO₂ incubator for 24 hrs. After incubation, the strains were identified by the API gallery.

The purity of the strain was verified by continuous cultures in blood agar medium.

Disc diffusion method

Antimicrobial activity was investigated by the disc diffusion method as already described [10]. The bacterial suspension was adjusted to a bacterial cell density of 1.0 × 10^8 CFU/mL (or 0.5 McFarland turbidity units). A sterile swab immersed in this bacterial suspension was used to inoculate the
entire surface of sheep blood agar; 5 µL of each essential oil was applied on a sterilized disc made from Whatman filter paper of 6 mm diameter [11], aseptically placed on the inoculated plates. Then, plates were incubated for 15 minutes at room temperature. Only one disc was tested per plate. After 24 hrs of incubation at 37°C in a CO₂ incubator, the inhibition zones were measured in millimeters. All experiments were done in triplicate. The average inhibition diameter was calculated to classify the essential oils as follows: S. oralis is not sensitive for a diameter <8 mm, moderately sensitive (+) for diameter of 8-14 mm, sensitive (++) for diameter of 14-20 mm, and very sensitive (+++) for a diameter >20 mm [10,12].

**Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

The broth macro-dilution method was employed to determine the MIC [13]. Serial dilutions of essential oil ranging from 20 to 0.15 µL/mL were prepared in test tubes containing Luria–Bertani broth with 0.15% agar. Each tube was inoculated with a bacterial suspension adjusted to 10⁶ CFU/mL. Controls containing medium with either microorganisms or the essential oil alone were included. The tubes were then incubated at 37°C for 24 hrs. MIC values were defined as the lowest concentrations of essential oil at which the absence of growth was recorded. To determine the MBC, 10 µL from tubes in which bacterial growth was not observed was spread on Mueller–Hinton agar and incubated at 37°C for 24 hrs. The MBC was defined as the lowest concentration of essential oil at which the incubated microorganism was completely killed [14]. Each test was performed in triplicate.

**RESULTS AND DISCUSSION**

**Essential oil composition**

As shown in Table 1, essential oils were chosen according to their chemical composition, in particular to their major components. The major compound of *C. zeylanicum* was cinnamaldehyde. Analysis of *E. caryophyllata* indicated eugenol and *R. officinalis* mainly contained cineole.

**Antibacterial activity of essential oils**

Results obtained with the disc diffusion assay regarding the growth of inhibition zones of the tested *S. oralis* strain are shown in Table 2.

Our results showed that essential oils from *C. zeylanicum* and *E. caryophyllata* were the most active of the oils tested against *S. oralis*, with average inhibition zones ranging from 42.0 to 22.0 mm (+++), while the essential oil of *R. officinalis* did not show antibacterial activity for this bacterium.

**MIC and MBC value determination**

Referring to the large inhibition zones observed with the disc diffusion method for two essential oils (*C. zeylanicum* and *E. caryophyllata*), the MIC values were determined with broth dilution assays (Table 3).

*C. zeylanicum* essential oil, mainly composed of aldehyde, was most efficient against *S. oralis* (0.625 µL/mL). The MIC of *E. caryophyllata* containing mainly eugenol was 1.25 µL/mL.

Concerning the MBC, in most cases, it was close to the MIC, indicating good bacterial activity against *S. oralis*, with an MBC-to-MIC ratio of the order of 2 for both essential oils.

Plants have been used by humans since antiquity to handle common infectious diseases. Some of these traditional treatments are always included as part of the usual treatment of various diseases [15,16].

These plants are an important reservoir of potential compounds, which have the advantage of having a big diversity in chemical structure and possessing a very wide range of biological activity [17,18].

Three essential oils were selected for their composition. Indeed, in the literature, it has been reported that essential oils containing mainly aromatic phenols or aldehydes presented major antimicrobial activity against respiratory tract pathogens [19,20].

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**Table 1: Chemical composition percentage of three essential oils**

| Component          | Essential oil (%) |
|--------------------|-------------------|
| *C. zeylanicum*    |                   |
| Cinnamaldehyde     | 74.4              |
| N-acetate          | 9.91              |
| Eugenylacetate     | 2.78              |
| Hydrocinnamylacetate | 1.14          |
| *E. caryophyllata* |                   |
| Eugenol            | 79.71             |
| β-caryophyllene    | 15.85             |
| Eugenylacetate     | 2.70              |
| *R. officinalis*   |                   |
| α-pineine          | 20.62             |
| Camphene           | 7.00              |
| β-pineine          | 8.89              |
| 1,8 cineole        | 52.77             |
| Camphor            | 6.64              |

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**Table 2: Inhibition zone diameters obtained with the three essential oils against *Streptococcus oralis***

| Essential oil   | Diameter in mm | Sensitivity |
|-----------------|----------------|-------------|
| *C. zeylanicum* | 42±0.5         | VS          |
| *E. caryophyllata* | 22±0.66      | S           |
| *R. officinalis* | 00             | R           |

R: Resistant, S: Sensitive, VS: Very sensitive, *C. zeylanicum*: *Cinnamomum zeylanicum*, *E. caryophyllata*: *Eugenia caryophyllata*, *R. officinalis*: *Rosmarinus officinalis*

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**Table 3: MIC and MBC of two selected essential oils against *Streptococcus oralis***

| Essential oil   | MIC (µL/mL) | MBC (µL/mL) | MBC/MIC |
|-----------------|-------------|-------------|---------|
| *C. zeylanicum* | 0.625       | 1.25        | 2       |
| *E. caryophyllata* | 1.25     | 2.5         | 2       |

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, *C. zeylanicum*: *Cinnamomum zeylanicum*, *E. caryophyllata*: *Eugenia caryophyllata*

Chromatographic analysis of the essential oils showed that the major compounds of *C. zeylanicum, E. caryophyllata, and R. officinalis* were, respectively, cinnamaldehyde (74.4%), eugenol (79.71%), and 1,8 cineole (52.77%); these components could be the active elements of these essential oils. These results are similar to those reported by other authors: Burdock in 1995 and Raynaud in 2006 [21,22].

The study of the antibacterial effect of essential oils by the standardized disc assay method showed that *R. officinalis* had no effect on *S. oralis*, while this bacterium was extremely sensitive to the essential oil of *E. caryophyllata* and that of *C. zeylanicum*.

In addition, we showed that cinnamon presented higher activity against *S. oralis*. This result is consistent with other studies that reported that the essential oil of *C. zeylanicum* containing cinnamaldehyde (an aromatic aldehyde) showed higher activity than that of *E. caryophyllata* [20,23,24].

Moreover, the essential oil of *E. caryophyllata* containing an aromatic phenol (clove containing eugenol) was less active (+++) than *C. zeylanicum*. These results could be directly linked to the structures of the major aromatic phenols from clove essential oil. In fact, essential oils containing the aromatic phenols, carvacrol and thymol, were more efficient (+++) against *Streptococci* [23]. These phenolic compounds are deemed to have great antibacterial activity [25-29].

The differences between our results and previous studies could be due to the fact that the composition of essential oils is not strictly defined.
but is a complex mixture of organic substances, varying in quality and quantity [30-32].

Indeed, previous studies showed that the essential oil of C. zeylanicum was more effective than that of E. caryophyllata on the oral microbiota [33].

The antibacterial activity of the oils selected was studied by determining the MIC and MBC. In this study, MIC results were reliable with the inhibition zone diameters measured with the disc diffusion method, C. zeylanicum being the more effective essential oil followed by the essential oil of E. caryophyllata. The MBC/MIC ratio is of the order of 2. According to Guinoiseau [34], both essential oils studied appear to exert a bactericidal effect against S. oralis [35], but investigations such as pharmacokinetic and pharmacodynamic studies are needed to characterize the antibacterial activity in vivo and their clinical efficacy [36].

CONCLUSION

We show interesting antibacterial activity of two essential oils against S. oralis isolated from the oral cavity of diabetic patients with gingivitis, particularly C. zeylanicum essential oil, but we need further investigations to evaluate the bactericidal properties in practical applications on clinical strains and to assess the potential for therapeutic application. As there is no evidence for the potential clinical use of these essential oils, further research is needed to determine whether they could efficiently substitute antibiotics or, perhaps, be used in combination.

Indeed, this preliminary result may be a basis for launching other studies on the action of these active ingredients on this pathogenic strain in vivo.

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REFERENCES

1. Feur E, Labeyrie C, Boucher J, Eid A, Cabut S, Dib S, et al. Health indicators among high school teenagers, Val-de-Marne, France in 2005: Overweight, dental caries and risk of depression. Wkly Epidemiol Bull 2007;4:29-31.
2. Maeda K, Nagata H, Kuboniva M, Ojima M, Tsukaza O, Naoto M, et al. Identification and characterization of Porphyromonas gingivalis customer proteins bound to Streptococcus oralis glyceroldehyde-3-dehydrogenase. Infect Immun 2013;81(3):753-63.
3. Singer RS, Finch R, Wegener HC, Bywater R, Walters J, Lipstich M. Antibiotic resistance the interplay between antibiotic use in animals and human beings. Lancet Infect Dis 2003;3(1):47-51.
4. Bisht R, Katiyar A, Singh R, Mittal P. Antibiotic resistance – A global issue of concern. Asian J Pharm Clin Res 2009;2:34-9.
5. Sharma P, Mack JP, Rojtman A. Ten highly effective essential oils inhibit growth of methicillin resistant Staphylococcus aureus and methicillin sensitive Staphylococcus aureus. Int J Pharm Sci 2013;5(1):52-4.
6. Bidani P, Chandad F, Grenier D. Risks of bacterial resistance associated to systemic antibiotic therapy in periodontics. J Can Dent Assoc 2007;73(8):721-5.
7. Rodrigues F, Lehmann M, do Amaral VS, Reguly ML, de Andrade HH. Genotoxicity of three mouthwash products, cepacol, peroguard, and plax, in the Drosophila wing-spot test. Environ Mol Mutagen 2007;48(8):644-9.
8. Lachenmeier DW. Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. J Occup Med Toxicol 2008;3:26.
9. Saeki Y, Ito Y, Shibata M, Sato Y, Okuda K, Takaoz I. Antimicrobial action of natural substances on oral bacteria. Bull Tokyo Dent Coll 1989;30(3):129-35.
10. El Amri J, El Badayou K, Zair T, Bouhab H, Chakir S, Aloua T. Study of the antibacterial activity of the essential oils of Teucrum capitatum L. and the extract of Silene vulgaris on different strains tested. J Appl Biosci 2014;82:7481-92.
11. Dulger B, Ugurlu E. Evaluation of antimicrobial activity of some endemic Scrophulariaceae members from Turkey. Pharm Biol 2005;43(3):275-9.
12. Franchomme P, Jollos R, Penoél D. L’Arômatherapie Exactement. Limoges, France: Roger Jollos; 2001.
13. Bouhid S. Antimicrobial and antioxidant activities of essential oils. Doctoral Thesis. Faculty of Science, University Abdelmalek Essaadi, Tetouan, Morocco; 2009.
14. Smith-Palmer A, Stewart J, Fyfe L. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett Appl Microbiol 1998;26(2):118-22.
15. Rios JL, Reicco MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol 2005;100:1 Suppl 2:80-4.
16. Rollinger JM, Haupt S, Stuppern H, Langer T. Combining ethnomicrobiology and virtual screening for lead structure discovery: COX-inhibitors as application example. J Chem Inf Comput Sci 2004;44(2):480-8.
17. Anbukumaran A, Ambikapathy V, Panneerselvam A. Preliminary phytochemical and antimicrobial activity of leaves of Azima tetracantha Lam. World J Pharm Sci Life 2016;2:127-32.
18. Shayou H, Talhi H, Talha I, Amghar S, Hilali A. Evaluation of the genotoxicity of essential oil from Origanum compactum benth. in human lymphocytes. Asian J Pharm Clin Res 2016;9(2):274-6.
19. Inouye S, Takizawa T, Yamaguchi H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J Antimicrob Chemother 2001;47(5):565-73.
20. Inouye S, Yamaguchi H, Takizawa T. Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. J Infect Chemother 2001;7(4):251-4.
21. Burdock GA, editor. Fenaroli’s Handbook of Flavor Ingredients. 3rd ed., Vol. I. Boca Raton, Florida: CRC Press; 1995. p. 134.
22. Raynaud J. Prescription and Advice in Aromatherapy. Paris: Lavoisier Technique et Documentation; 2006. p. 86-214.
23. Fabio A, Cermelli C, Fabio G, Nicolletti P, Quaglio P. Screening of the antibacterial effects of a variety of essential oils on microorganisms responsible for respiratory infections. Phytother Res 2007;21(4):374-7.
24. Bouhdid S, Abrini J, Baudoux D, Manresa A, Zhirhi A. Essential oils of oregano compact and cinnamon: Antibacterial potency and mechanism of action. J Pharm Clin 2012;31(3):141-8.
25. Ulhe A, Gorris LG, Smid EJ. Bactericidal activity of carvacrol towards the food-borne pathogen Bacillus cereus. J Appl Microbiol 1998;85(2):211-8.
26. Ulhe A, Kets EP, Smid EJ. Mechanisms of action of carvacrol on the food-borne pathogen Bacillus cereus. Appl Environ Microbiol 1999;65(10):4606-10.
27. Ettayebi K, El Yamani J, Rossi-Hassani BD. Synergistic effects of nisin and thymol on antimicrobial activities in Listeria monocytogenes and Bacillus subtilis. FEMS Microbiol Lett 1999;183:191-5.
28. Ulhe A, Slump RA, Steging G, Smid EJ. Antibacterial activity of carvacrol toward Bacillus cereus on rice. J Food Prot 2000;63(5):620-4.
29. Shayou H, Boumazda A, Hilali A, Amghar S. Chemical composition and antibacterial and antioxidiant activities of Thymus sativareoiss. essential oil. Int J Pharm Pharm Sci 2016;8(10):183-7.
30. Bruneton J. Pharmacognosie Phytochimie, Plantes Médicinales. 4th ed., Paris: Lavoisier Technique et Documentation; 2009. p. 483.
31. Dormann HJ, Deans SG. Antibacterial agents from plants: Antibacterial activity of plant volatile oils. J Appl Microbiol 2000;88(2):308-16.
32. Delaquis PJ, Stanich K, Girard B. Antibacterial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int J Food Microbiol 2002;74:1 Suppl 2:101-9.
33. Gupta C, Kumari A, Garg AP, Catanzaro R, Marotta F. Comparative study of cinnamon oil and clove oil on some oral microbiota. Acta Pharmaceutici Ind 2009;2:80-4.
34. Guinoiseau E. Antibacterial Molecules Derived from Essential Oils: Separation, Identification and Mode of Action. France: Life Sciences, University of Corsica; 2010. p. 65-70.
35. Mayaud L, Carricoa A, Zhirhi A, Aubert G. Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics. Lett Appl Microbiol 2008;47:167-73.
36. Pankey GA, Sabath LD. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of gram-positive bacterial infections. Clin Infect Dis 2004;38(6):864-70.