ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 5, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

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

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Received: 13 January 2017, Revised and Accepted: 11 February 2017

ABSTRACT

Objective: *Streptococcus oralis* plays an important role in the biofilm formation of dental plaque and the occurrence of periodontal disease. The present study was conducted to evaluate in vitro antibacterial activity of three essential oils, namely, *Cinnamomum zeylanicum*, *Eugenia caryophyllata,* and *Rosmarinus officinalis* against *S. oralis*.

Methods: The antibacterial activity of essential oils was investigated by diffusion method using sterile discs (or aromatograms). The minimum inhibitory concentration (MIC) of essential oils showing important antibacterial activity was measured using the broth dilution method.

Results: Evaluation of the antibacterial activity of three essential oils as determined by the aromatogram technique showed that the essential oil of *R. officinalis* had no effect on *S. oralis*, while the latter was extremely sensitive to the other two essential oils, but with a higher efficiency of the essential oil of *C. zeylanicum* (42 mm diameter) than *E. caryophyllata* (20 mm diameter). Similarly, the MIC and minimum bactericidal concentration (MBC) were higher for the essential oil of *C. zeylanicum* than the essential oil of *E. caryophyllata*. The MBC/MIC ratio is of the order of 2. The essential oils studied therefore appear to exert bactericidal activity against *S. oralis*.

Conclusion: The findings suggest that essential oils of *C. zeylanicum* and *E. caryophyllata* may be used as an alternative to synthetic antibiotics.

Keywords: Essential oil, *Cinnamomum zeylanicum*, *Eugenia caryophyllata*, *Rosmarinus officinalis*, Antimicrobial activity, *Streptococcus oralis*.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i5.17072

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-called iatrogenic 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 CO_2 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 CO_2 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 14-20mm, and very sensitive (+++) for a diameter >20 mm [10,12].

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

The broth macrodilution 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 10^6 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.

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-pinene	20.62
Camphene	7.00
β-pinene	8.89
1,8 cineol	52.77
Camphor	6.64

C. zeylanicum: *Cinnamomum zeylanicum*, *E. caryophyllata*: *Eugenia caryophyllata*, *R. officinalis*: *Rosmarinus officinalis*

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.

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

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 22.0mm (+++), 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).

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*

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 bactericidal 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].

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 observed 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*.

ACKNOWLEDGMENTS

The authors would like to thank the Microbiology Laboratory Team of the National Institute of Hygiene, Rabat, Morocco, for their technical support.

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