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Research paper

Antibacterial activity of *Plectranthus amboinicus* Lour (Lamiaceae) essential oil against *Streptococcus mutans*

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ABSTRACT

Introduction: Oral diseases, including caries and periodontal disease, are especially of concern to public health, where they affect a large part of the population, leading dental professionals to seek alternatives for their control. The aim of this work was to evaluate the chemical composition and antibacterial activity of the essential oil of *Plectranthus amboinicus* Lour (malvariço), a plant used by the local population for the treatment of diseases of the oral cavity, alone or as a mouthwash against a strain of *Streptococcus mutans*.
Methods: The essential oil obtained from the malvariço plant, obtained from two locations, was characterized by gas chromatography and evaluated for antibiotic activity. The essential oil was also tested for antibacterial-modifying activity in combination with mouthwash to determine any synergistic or antagonistic effect.
Results: The yield of the essential oils extracted from plants from Fortaleza was higher than that extracted from Crato plants (0.25 and 0.15%, respectively). The two samples of essential oil exhibited significant differences in composition, where the major compounds, carvacrol and germacrene D, varied. Antibacterial activity in the two samples was low or not detectable. The essential oil combined with mouthwash was effective in inhibiting bacterial growth, but this result was lower than with chlorhexidine alone ($p < 0.05$).
Conclusion: Our results demonstrated an antagonistic effect resulting from the association between the essential oil and mouthwash, indicating that this combination must be avoided and demonstrating the necessity of more studies about these combinations.

1. Introduction

Phytotherapy is based on the use of medicinal plants in their different pharmaceutical forms, where this approach encourages community development, solidarity and social participation. WHO reports that 80% of the world's population make use of medicinal plants or their products [1]. Brazil has advantages and opportunities for the development of this therapy. There is growing popular interest in medicinal plants, and Brazil's Health Ministry is promoting the use of phytotherapy in *Sistema Único de Saúde* (SUS; Unified Health System) [2].

Dental caries is an infectious disease with a high incidence in adults as well as children, and has been of concern to scientific communities and governments. The adoption of programs to ellaborate methods of prevention and lesion repair is important, especially in public health programs [2]. Currently, measures are sought for the biological control of dental plaque, where this control involves some microorganisms present in plaque (*Streptococcus* mutans and *Lactobacillus*, among others). According to an earlier study, there are around 500 species of bacteria that live in the mouth. To have an idea of what this means, saliva may contain from 43 million to 5.5 billion bacteria per milliliter, with an average of 750 million bacteria per milliliter [3].

Herbal products can be a therapeutic alternative, because besides the low cost, the plants can be grown in backyards and accepted and easily accessed by the public. It is a folk medicine alternative for future work in primary health care programs with school and community through actions of Oral Health Teams (OHT), which are part of the Family Health Program (FHP) [4]. Some essential oils have antimicrobial activity against a number of Gram-positive and Gram-negative bacteria, including species that are resistant to antibiotics and antifungals. Although new

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antibiotics have been produced by pharmaceutical companies in the past three decades, the resistance of microorganisms to these drugs has significantly increased. Antibacterial activity depends on the type, composition and concentration, processing and storage conditions of the essential oil and on the type of microorganisms and composition of the substrate [4]. *Plectranthus amboinicus* Lour (Lamiaceae), is commonly used such as in a mouthwash for the treatment of hoarseness, sore throat and mouth, and cough, and it has antibacterial properties when used as a decoction, infusion or tea [5].

Thus, this study aimed to investigate whether the essential oil from the leaves of malvariço (*P. amboinicus* L.) has an antibacterial effect alone or associated with a mouthwash, modulating the mouthwash activity against *S. mutans*, the main bacterium responsible for dental caries and present in saliva. This study could be of interest to dentistry but also for public health programs.

2. Material and methods

2.1. Collection of plant material

Fresh leaves of *P. amboinicus* L. were collected in two different places: in Crato and Fortaleza, in Ceará, Brazil. The plant material was identified by Dr. Maria Arlene Pessoa da Silva, and a voucher specimen was deposited with the respective identification number in the herbariums listed in Table 1.

2.2. Extraction of oil and preparation of solutions for antibacterial assay

The two sets of leaves of *P. amboinicus* L. were extracted separately. The leaves were triturated in small pieces to enhance the contact surface with the solvent, and 400 g were placed in a glass flask, submersed in distilled water and boiled for 2 h in a Clevenger type oil extractor. The water/oil mixture obtained was separated, treated with anhydrous sodium sulfate and filtered. The essential oil was diluted in DMSO (dimethyl sulfoxide) to a concentration of 10 mg/mL and then diluted with distilled water to a concentration of 1024 μg/mL.

2.3. Phytochemical characterization

The identification of the classes of secondary metabolites in the essential oil of *P. amboinicus* L. (EOPA) was done using a Shimadzu QP2010 series GC/MS system, equipped with an Rtx-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness); helium was the carrier gas at 1.5 mL/min, and flow rate was 0.8 mL/min, using split mode. The injector and detector temperatures were 250 and 200 °C, respectively. The column temperature was programmed from 35 to 180 °C at 4 °C/min and then 180–250 °C at 10 °C/min. The mass spectrometer was operated at an ionization energy of 70 eV. The identification of individual components was based on their fragmentation in the NIST 8 mass spectral library and by comparison with the literature data.

2.4. Bacteriological material

The bacterial strains utilized were *S. mutans* INCQS 00446 and NCTC 10449 (ATCC 25175), isolated from dental caries. The bacterial strains were maintained on slants with heart infusion agar (HIA, Difco Laboratories Ltda). Before the assay, the cells were grown for 24 h at 37 °C in brain heart infusion broth (BHI, Difco Laboratories Ltda.).

Table 1
Botanical families, species and voucher number of the plants used in this study.

Family	Species	Number	Herbarium
Lamiaceae	*Plectranthus amboinicus* (Lour) Spreng.	#26433	Dárdano Andrade Lima, URCA Crato, CE
Lamiaceae	*Plectranthus amboinicus* (Lour) Spreng.	# EAC40080	Prisco Bezerra, UFC Fortaleza, CE

2.5. Inoculum preparation

The bacteria were first grown on BHI solid medium and then transferred to test tubes containing 5 mL of sterile saline. The bacterial suspension was diluted in saline to a concentration of 10^5 CFU/mL according to the McFarland nephelometer scale. In microdilution tests, 10% BHI was the medium used.

2.6. Drug

Chlorhexidine was obtained as a mouthwash (Colgate PERIOGARD®, 0.12% chlorhexidine) (Colgate-Palmolive Company) and used at an initial concentration of 1200 μg/mL

2.7. Antibacterial and modulatory assay

MIC (minimum inhibitory concentration) was determined using a broth microdilution assay, with an inoculum of 100 μL of bacterial suspension in BHI broth (10^5 CFU/mL) in 96-well microtiter plates. Prior to bacterial inoculum, essential oil samples (100 μL) were added to the microplate and serially diluted twofold, giving final concentrations of 512–9 μg/mL. Mouthwash was likewise diluted to give final concentrations of 600–0.6 μg/mL. The plates were incubated at 35 °C for 24 h. Afterwards, viable cells were stained with resazurin and the color change was determined at 630 nm using an ELISA plate spectrophotometer (Termoplate®). MIC was recorded as the lowest concentration needed to inhibit bacterial growth.

To investigate the modulatory effect of the essential oils, the method proposed by Refs. [6,7] was used, where the oils were tested at subinhibitory concentration (MIC/8). Eppendorf tubes were prepared each containing 1.5 mL of 10% BHI, 150 μL of the bacterial inoculum and the natural product at a concentration of MIC/8. The positive and negative controls were performed: briefly and respectively, a control without any inhibitor (with 100% of growth) and other without microorganism (with 0% of growth). The ELISA plate was completed by adding 100 μL of this solution to each well. Then, 100 μL of drug was mixed well to the first, proceeding in the broth microdilution series. The concentrations of the EOPA (essential oil of *P. amboinicus*) and the mouthwash gradually decreased from 600 to 0.6 μg/mL. The plates were incubated and analyzed using the same method previously described. All experiments were performed in triplicate and the results expressed as geometric mean.

2.8. Statistical analysis

Statistical analysis was carried out using two-way ANOVA followed by Student-Newman–Keuls test, with GraphPad Prism 6.0, and $p < 0.05$ was considered significant.

3. Results

P. amboinicus L. is rich in essential oils. The chemical composition of the essential oil of *P. amboinicus* L. (EOPA) was determined by gas chromatography coupled to mass spectrometry (GC/MS), and as a result the components were identified by