BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Food Microbiology

Antimicrobial activities of six essential oils commonly used as condiments in Brazil against *Clostridium perfringens*

Marcela Radaelli[a], *Bárbara Parraga da Silva*[a], *Luciana Weidlich*[a], *Lucélia Hoehne*[b], *Adriana Flach*[c], *Luiz Antonio Mendonça Alves da Costa*[c], *Eduardo Miranda Ethur*[b],*

[a] *Centro de Ciências Biológicas e da Saúde, Centro Universitário UNIVATES Lajeado, RS, Brazil*
[b] *Centro de Ciências Exatas e Tecnológicas, Centro Universitário UNIVATES, Lajeado, RS, Brazil*
[c] *Departamento de Química, Universidade Federal de Roraima Boa Vista, RR, Brazil*

ARTICLE INFO

Article history:
Received 12 August 2014
Accepted 2 October 2015
Available online 2 March 2016

Associate Editor: Mariza Landgraf

Keywords:
Food-borne disease
Antimicrobial activity
Clostridium perfringens
Spices
Essential oils

ABSTRACT

Despite recent advances in food production technology, food-borne diseases (FBD) remain a challenging public health concern. In several countries, including Brazil, *Clostridium perfringens* is among the five main causative agents of food-borne diseases. The present study determines antimicrobial activities of essential oils of six condiments commonly used in Brazil, viz., *Ocimum basilicum* L. (basil), *Rosmarinus officinalis* L. (rosemary), *Origanum majorana* L. (marjoram), *Mentha* × *piperita* L. var. Piperita (peppermint), *Thymus vulgaris* L. (thyme) and *Pimpinella anisum* L. (anise) against *C. perfringens* strain A. Chemical compositions of the oils were determined by GC–MS (gas chromatography–mass spectrometry). The identities of the isolated compounds were established from the respective Kováts indices, and a comparison of mass spectral data was made with those reported earlier. The antibacterial activity was assessed from minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the microdilution method. Minimum inhibitory concentration values were 1.25 mg mL^{-1} for thyme, 5.0 mg mL^{-1} for basil and marjoram, and 10 mg mL^{-1} for rosemary, peppermint and anise. All oils showed bactericidal activity at their minimum inhibitory concentration, except anise oil, which was only bacteriostatic. The use of essential oils from these common spices might serve as an alternative to the use of chemical preservatives in the control and inactivation of pathogens in commercially produced food systems.

* *Corresponding author.*
E-mail: eduardome@univates.br (E.M. Ethur).
http://dx.doi.org/10.1016/j.bjm.2015.10.001

Introduction

Food-borne disease (FBD) is characterized by diverse symptoms that arise from consumption of contaminated foods or beverages. Despite the recent advances in food production technology and processing, FBD remains a major cause of morbidity and mortality, constituting both an important public health concern and a significant economic problem at a global level.[1–4]

One of the most common causes of FBD is the Gram-positive, anaerobic, spore-forming bacterium *Clostridium perfringens* (family Clostridiaceae), which is widely distributed in the environment and in foodstuffs. The spores of this bacterium, compared to the vegetative cells, are very robust and resistant to heating, drying, pH and certain toxic compounds. This allows the microorganism to persist until conditions become favorable for germination and growth. There are five strain-types of *C. perfringens*, designated as A–E. Each of them produces a unique spectrum of exotoxins. Type A strains of the bacterium cause food poisoning in the classical form, while the strains of type C cause necrotic enteritis – a disorder that can be severe and is often fatal but, fortunately, its occurrence is rare. The microorganism prefers substrates, such as meat products, poultry, and sauces, which contain high level of moisture and rich in protein. These are the main classes of food materials involved in occurrence of this disease. Specific factors that favor the spread of this agent are prolonged cooling and non-refrigerated storage, wherein sausage, canned fish, pate, cheese and fermented oyster provide ideal conditions for the development of the bacterium.[1,2,5]

Many of the spices commonly used in food not only improve palatability and flavor but also assist in the preservation of the food itself. The antimicrobial activity of such spices is bestowed mainly by the essential oils that contain terpenoids hydrocarbons, alcohols, aldehydes, ketones, phenolics and their derivatives. The quantity, quality, and chemical profiles of the essential oils derived from a single plant species can vary considerably according to the geographic origin, climatic conditions, soil composition, the part of the plant used, and the age and season when the material is collected. Besides, drying process and storage time can alter, quantitatively, and qualitatively, the essential oil composition.[6] While the antimicrobial activities of essential oils are well reported, the mechanisms of their action are not yet fully understood, although there might be several different microbial target sites.[7,8]

In the present study, the essential oils of some of the spices most commonly used in Brazil, namely, *Ocimum basilicum* L. (basil), *Rosmarinus officinalis* L. (rosemary), *Origanum majorana* L. (marjoram), *Mentha* × *piperita* L. var. Piperita (peppermint), *Thymus vulgaris* L. (thyme) and *Pimpinella anisum* L. (anise), were assessed with regard to their antimicrobial activities against *C. perfringens* strain A. The criteria for selection of plants were their popular use as spices, availability to common people, and ensuring that organic production methods have been used in accordance with the Law: 10,831.[9] The chemical compositions of the essential oils were determined by GC–MS (gas chromatography–mass spectrometry), and minimum inhibitory and bactericidal concentrations were determined using the microdilution method.

Materials and methods

Plant materials

Dried, fragmented leaves of basil, rosemary, marjoram, peppermint, and thyme and dried fruits of anise were purchased commercially in São Paulo, Brazil, in October 2013. All samples were acquired in accordance with the terms of expiry mentioned on the labels.

Extraction of essential oils

Dried leaves of basil, marjoram, peppermint, rosemary and thyme, and the dried fruits of anise were powdered (10–20 mesh) and samples were extracted by hydro-distillation (plant:water ratio 1:10, w/v) for 3.5 h in a modified Clevenger apparatus. The oily phase was removed, dried over anhydrous sodium sulfate, and stored in a freezer at –20 °C. Microbiological analyses were performed seven days after the extraction.

Chemical compositions of essential oils

Essential oil samples were submitted for GC–MS analysis to the Laboratory of Chromatography, Energy Research Center, Federal University of Roraima, Boa Vista, RR, Brazil. The analyses were performed using Shimadzu GC2010 system with an autoinjector AOC-20i and Plus mass detector QP2110, and equipped with an HP5-MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm). The chromatographic conditions were as follows: carrier gas, helium at a flow rate of 1.02 mL min^{-1}; oven temperature programmed initially at 60 °C and increased to 310 °C at a ramp of 3 °C min^{-1}; injector temperature, 220 °C; injector mode in split ratio of 1:20 with 2 mL min^{-1} purge; MS interface temperature, 280 °C; ion source temperature, 260 °C; and ionization energy, 70 eV. The oil samples (15 mg) were dissolved in 1.5 mL of purified ethyl acetate and 1 μL volume of that was injected for analysis. The isolated compounds were identified by their respective Kováts retention indices determined in reference to a series of *n*-alkanes, and verified by a comparison of mass spectral data with those obtained using pure standards and with those reported in the literature,[10] and eventually by comparing their mass spectra with the GC–MS spectral library (Wiley 8 and FFNSC 1.2 libraries).

For GC-FID, HP-5 MS column (30 m × 0.25 × 0.25 μm) at same temperature as that of GC–MS, using hydrogen and nitrogen carrier gas, was used. The FID temperature was 260 °C. The relative compositions of the oils were calculated from the peak areas (uncorrected for specific response factors) of the isolated compounds.

Antimicrobial activities of essential oils

The minimum inhibitory concentration (MIC) and the MBC of the oil samples were assessed against type A strain of