ARTICLE IN PRESS

Industrial Crops & Products xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

The synergistic effects of volatile constituents of *Ocimum basilicum* against foodborne pathogens

João Luiz Baldim[a], Juliana Grell Fernandes Silveira[b], Anelique Pinto Almeida[b], Patrícia Lunardelli Negreiros Carvalho[b], Welton Rosa[a], Jan Schripsema[c], Daniela Aparecida Chagas-Paula[a], Marisi Gomes Soares[a], Jaine Honorata Hortolan Luiz[a,*]

[a] Institute of Chemistry, Federal University of Alfenas, Alfenas, Brazil
[b] Faculty of Pharmaceutical Sciences, Federal University of Alfenas, Alfenas, Brazil
[c] Laboratory of Chemical Sciences, Federal University of North Fluminense, Rio de Janeiro, Brazil

ARTICLE INFO

Chemical compounds studied in this article:
Linalool (Pubchem CID: 6549)
Camphor (PubChem CID: 2537)
Fenchol (PubChem CID: 15406)
Alpha-trans-bergamotene (Pubchem CID: 86608)
Terpinen-4-ol (Pubchem CID: 11230)
Muurola-4(14),5-diene (Pubchem CID: 51351709)
Eucalyptol (Pubchem CID: 2758)

Keywords:
Ocimum basilicum
Essential oils
Foodborne pathogens
Multivariate statistical analysis
Gas chromatography

ABSTRACT

Foodborne diseases and antibiotic resistant bacteria have become a serious worldwide public health problem. In this context, this study was carried out to describe the chemical composition and antimicrobial activity of the essential oils from three Brazilian *Ocimum* species, since essential oils are considered safe. The essential oils were analyzed by GC–MS and the antimicrobial activities were determined by MIC assays against some pathogens. The essential oils from two samples exhibited effective antimicrobial activity against *Bacillus cereus* 100–200 µgnmL^{-1}. The concentration of the major compound, linalool (from 24.7% to 60.2%), was not the main feature for the antimicrobial activity. The development of a multivariate statistical method based on O2PLS-DA was carried out for the identification of the most effective combination of constituents to describe the efficiency of *O. basilicum* essential oils as antimicrobial agents against foodborne pathogens.

1. Introduction

Medicinal plants have been widely used by traditional medicine due to their many biological properties. Last decades scientific studies have recognized many biological properties of different plants, such as antioxidant, anti-inflammatory, antiviral and antibacterial that are related to the compounds inside vegetal cells. These studies also included the biological activities of essential oils (EOs). Due to these properties, EOs extracted from a variety of plants have been employed in the manufacture of cosmetics, cleaning products, fragrances, pesticides and antimicrobial agents associated to the food industry (Hyldgaard et al., 2012; Vieira et al., 2014).

EOs occur as a complex mixture of low molecular weight compounds which are mostly hydrophobic constituents. EOs contain diverse representative of volatile components comprising mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives such as alcohols, aldehydes, ketones, acids, phenols, ethers and esters. These compounds are encountered in all parts of the plant, however, their composition may vary in those parts. Other factors such as cultivation, soil, and climatic conditions can also determine the composition and quality of EOs within same species which are associated to their intraspecific variation (Maffei et al., 2011).

A small fraction of plants composition is associated with EOs, representing less than 5% of the vegetal dry matter. Accompanied by secondary metabolites, essential oils with the most diverse therapeutic applications are obtained from plants (Turek and Stintzing, 2013). Also in traditional medicine, several of these plants have been used as antibiotics, digestives, diuretics, sedatives, expectorants and other curative effects. These medicines are available as infusions, tablets and/or extracts in the market (Fornari et al., 2012). An increasing interest on

* Corresponding author at: Federal University of Alfenas, Rua Gabriel Monteiro da Silva 700, Alfenas, MG, Brazil.
E-mail addresses: jotaelebaldim@gmail.com (J.L. Baldim), juugrell@hotmail.com (J.G. Fernandes Silveira), anelique@gmail.com (A.P. Almeida), patrikapnc@gmail.com (P.L.N. Carvalho), welton_rosa@hotmail.com (W. Rosa), jan.schripsema@gmail.com (J. Schripsema), da.chagaspaula@gmail.com (D.A. Chagas-Paula), marisigs@gmail.com (M.G. Soares), jaine.luiz@unifal-mg.edu.br, jainehh@gmail.com (J.H.H. Luiz).

https://doi.org/10.1016/j.indcrop.2017.12.016
Received 26 August 2017; Received in revised form 1 December 2017; Accepted 5 December 2017
0926-6690/

Please cite this article as: Baldim, J.L., Industrial Crops & Products (2017), https://doi.org/10.1016/j.indcrop.2017.12.016

EOs of plants have been observed due to its cost-effective and low side effects.

Regarding the natural purpose of the production of EOs, their constituents present a diverse range of biological properties such as protection against microbial infections, pest attack, coloring, scent or as pollinators attractants (Yap et al., 2014).

In this context, *Ocimum* species, which are cultivated worldwide and commonly used as medicinal and culinary herbs, have their EOs associated with interesting antimicrobial properties (Opalchenova and Obreshkova, 2003). *O. basilicum*, basil or sweet basil is considered as “king of the herbs” and was originally found in tropical and subtropical Asia. *Ocimum* species are annual and perennial herbs, mostly native of tropical and warm temperate regions. The genus *Ocimum*, member of the family Lamiaceae, comprises more than 150 species with a large number of varieties (Saran et al., 2017). The differences in the constitution of EOs depend on the weather conditions (Hussain et al., 2008), immediate microbiome and plants diversity, and specimens which lead to intraspecific variations. The constitution of EOs from *O. basilicum* point to linalool, linolen, camphor, eugenol, methyl eugenol, α-terpineol, methyl cinnamate, and estragole, as major constituents. The minor constituents comprise pinenes (α and β), β-bergamotene, thujone, germacrene D, fenchone, α-humulene, eucalyptol, terpinenes (α and γ), and guaienes (α and δ) (Bouzouita et al., 2003; Opalchenova and Obreshkova, 2003). Some of these compounds are considered antimicrobial agents, as in the case of linalool, pinenes (α and β), and ;1;eugenol (Cosentino et al., 1999; Karapinar and Sahika, 1987; Rivas da Silva et al., 2012). Some of these minor compounds are considered effective agents against Gram-positive and Gram-negative strains, as in the case of ϒ-terpinene, 1,8-cineole, camphor, germacrene D (Lang and Buchbauer, 2012).

Studies on EOs and organic extracts of basil are related to the screening and identification of the cultivars that have different antimicrobial properties (Flanigan and Niemeyer, 2014; Kuorwel et al., 2011; Lee and Scagel, 2009). Furthermore, the influence of EOs as antimicrobial agents has become notorious due to foodborne outbreaks noticed worldwide (Varona et al., 2013). Nowadays, the high incidence of foodborne diseases makes the bacterial food contamination of great concern (Wang et al., 2017). Foodborne pathogens produce toxins, such as emetic toxin (*Bacillus cereus*), and enterotoxins (*Staphylococcus aureus*) which are indicated to be the primary cause of foodborne outbreaks resulting in a worldwide public health problem (Cremonesi et al., 2014). Medicinal and culinary herbs have demonstrated their importance as food preservatives against pathogens that produce those toxins (Hyldgaard et al., 2012). Foodborne cases are constantly reported as human infections, as in the case of European Union with almost 5200 outbreaks, over 43,183 infected humans and almost 6000 hospitalizations resulting in 11 deaths. In the United States, the number of cases are exorbitant, reaching 9,4 million episodes per year, almost 56,000 hospitalization and 1351 deaths (Martinovic et al., 2016). Besides, long-term ingestion of foods contaminated with toxins can be associated with some illnesses, the literature reported that some mycotoxins produced by fungi such as aflatoxins, ochratoxin A, and others can cause autoimmune diseases, some are teratogenic and carcinogenic (Hossain et al., 2016; Garcia et al., 2009).

In this regard, EOs reduce or prevent the proliferation of pathogens acting as antimicrobial agents. This effect leads to the diminishment of pathogenic species consequently decreasing the secretion of toxins, such as staphylococcal enterotoxin, from *Staphylococcus aureus*; cereulide, from *Bacillus cereus*; listeriolysin O, from *Listeria monocytogenes*; salmonella enterotoxin, from *Salmonella* sp; and exotoxin from *Pseudomonas aeruginosa* (Martinovic et al., 2016).

Besides the effective antimicrobial activity of compounds produced by *Ocimum* species, they are also used for other purposes including as medicinal and culinary herbs considered an inexhaustible source of EOs combined with high commercial value (Silva et al., 2016). Its constituents are strongly associated to the antimicrobial activity, in some cases specifically to occurrence of the major constituent linalool which could affect the collective antimicrobial properties of EOs (Herman et al., 2016).

Based on these evidences the aim of this study was to evaluate the potential of EOs of *O. basilicum* collected under different conditions against the foodborne pathogens *S. aureus*, *B. cereus*, *L. monocytogenes*, *Salmonella* sp., and *P. aeruginosa* for the detection of the most efficient combination of constituents to the antimicrobial activities. The strategies adopted in this investigation were based on multivariate statistical analyses (MSA) to the identification of essential components of EOs for the antimicrobial property. *Ocimum* species are considered a sustainable source of EOs with high aggregated value, depending on its constituents. For this reason, we investigated chemical composition and antimicrobial activity of *Ocimum* EOs collected in Alfenas-Brazil in different seasons because these species present great variations in their EOs composition and these variations imply in the EOs quality and their applications.

2. Materials and methods

2.1. Plant materials

O. basilicum species were collected at Alfenas, in the Southwest region of Minas Gerais − Brazil. Ten samples of basil collected under different weather conditions were obtained for this study. Two variations of basil were studied: *Ocimum basilicum* named Green Basil (GB: GB1 21°25′44″S 45°56′46″W and GB2 21°25′08″S 45°56′53″W) and *Ocimum basilicum* var. *purpurascens* named Purple Basil (PB 21°25′59″S 45°57′58″O). To each sample, a code was associated according to the day of collection, average temperature, and precipitation conditions in conformity with the data obtained for the weather datacenter at Federal Institute of Minas Gerais, for the region of Alfenas (Table 1**)**. Besides, amount of aerial parts and essential oils are described in Table 1.

The species were authenticated by Vinicius Politi Duarte and Gabriela Azevedo Rocha from Institute of Natural Sciences of the Federal University of Alfenas under the supervision of Professor Dr.

Table 1
Sample specification, collection date, weather conditions, amount of the aerial parts of plants (g), amount of oils (mg) and yielding of the EOs of O. basilicum species.

Sample	Collection date	Temperature (°C)	Monthly precipitation (mm)	Mass (g)	Oil (mg)	% m/m
GB1a	11/1/2013	20.2	152.0	440	640.7	0.14
GB1b	2/7/2014	22.5	75.0	403.9	1131.6	0.28
GB2a	6/9/2014	16.2	12.5	554.3	224.4	0.04
GB2b	10/13/2014	21.2	55.0	307.5	139.9	0.04
GB2c	11/24/2014	21.1	183.2	192.2	120.9	0.06
GB2d	12/4/2014	21.0	350.0	181.5	152.8	0.08
GB2e	3/3/2015	20.0	255.0	142.1	33.2	0.23
PB1	2/5/2014	22.5	75.0	172.3	259.6	0.15
PB2	6/3/2014	16.2	12.5	225.5	152.3	0.07
PB3	12/4/2014	21.0	350.0	125.1	12.5	0.01