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Investigation of antibacterial activity of new classes of essential oils derivatives

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ABSTRACT

Essential oils (EOs) have deserved much attention in the past decades for their antimicrobial activity, since many of them have demonstrated efficacy against food-borne pathogenic and spoilage microorganisms. Moreover, they have potential application in animal nutrition as multifunctional feed supplements, avoiding or diminishing the use of antibiotics in livestock. However, low solubility and bioavailability as well as volatility and marked aromatic note are important limitations in food and feed applications. In this study we present the synthesis, characterization and evaluation of the antibacterial activity of new thymol, carvacrol and menthol derivatives. The new compounds have been designed to overcome the limitations of the precursors, such as poor water solubility and volatility, still maintaining a good antimicrobial profile. We evaluated the activity of the synthetized compounds against pathogens causing important foodborne diseases, *i.e. Clostridium perfringens*, *Salmonella typhimurium*, *Salmonella enteritidis* and *Escherichia coli*. The low MICs and MBCs values for some of the studied compounds, combined with water solubility and negligible cytotoxicity towards HT-29 human cells, confirmed the potential use for EOs derivatives in the food industry.

1. Introduction

Microorganisms causing food spoilage are a major concern for the food industry and the extension of shelf-life is an on-going demand for both retailers and consumers. Such extension is mainly achieved by technological improvements and addition of synthetic food preservatives. Natural products in general are an alternative to synthetic preservatives, and among them, essential oils (EOs) are typical antimicrobial agents without harmful residues. Since the 1990s, they have been widely studied for their antimicrobial activity and many EOs (e.g. thyme, oregano, cinnamon, horseradish) and their components have demonstrated antimicrobial efficacy against food-borne pathogenic and spoilage microorganisms (Arsi et al., 2014; Bakkali & Idaomar, 2008; Burt, 2004; Calo, Baker, Park, & Ricke, 2015; Kim & Rhee, 2016; Lang & Buchbauer, 2012; Pinheiro et al., 2015; Tajkarimi, Ibrahim, & Cliver, 2010). Another interesting area of application for EOs is animal nutrition. The prophylactic use of antibiotics in the livestock industry to obtain improvements in growth, feed consumption and decreased mortality caused by bacterial diseases has been a common practice for decades, especially for swine and poultry. However, the concern over the transmission and the proliferation of resistant bacteria via the food chain has led to the ban of the feed use of antibiotic growth promoters in livestock within the European Union since 2006. A wide range of EOs have the potential to act as multifunctional feed supplements for animals. Some EOs, in fact, are reported to have multiple actions in monogastric animals, including effects on performance, digestive systems, lipid metabolism, prevention of tissue oxidation and modulation of microbial

Abbreviations: EOs, essential oils; TGA, thermogravimetric analysis; HIA, heart infusion agar; BHI, brain heart infusion; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; GI%, growth inhibition percent.

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populations (Liang et al., 2013; Mitsch, Ko, Gabler, Losa, & Zimpernik, 2004; Zhaikai, Sai, Hongliang, & Xiangshu, 2015).

EOs antimicrobial activity has been attributed mainly to phenolic compounds (Pesavento et al., 2015), such as carvacrol and thymol (Cherallier, 1996; Lambert, Skandamis, Coote, & Nychas, 2001; Valero, 2006; Xu, Zhou, Ji, Pei, & Xu, 2008). They are additives generally recognized as safe and they are widely used as food preservatives (Goñi et al., 2009; Lv, Liang, Yuan, & Li, 2011). They can be directly incorporated into or coated onto packaging films, in order to enhance shelf-life (Calo, Crandall, O'Bryan, & Ricke, 2015). However, their low solubility and bioavailability limit the cytotoxic potential on bacteria, virus, fungi and parasites and their delivery is still a challenge (Kaur, Darokar, & Ahmad, 2014; Suntres, Coccimiglio, & Alipour, 2015). Additionally, the volatility and marked aromatic note of a lot of EOs, which are appreciable features in applications such as aromatherapy or perfume production, are conversely major limitations in food and feed applications. In fact, high concentrations are needed to ensure food safety, but effective concentrations usually result in negative flavour and in sensory changes, which discourages the consumption. The purpose of this study is the synthesis, characterization and evaluation of the antibacterial activity of new carvacrol, thymol and menthol derivatives (Fig. 1). The compounds have been designed with the aim of overcoming limitations, such as poor water solubility and volatility, still maintaining a good activity against pathogens. In this way, it would be possible to exploit the antibacterial properties of EOs active principles (i.e., menthol, carvacrol, thymol), but with more manageable compounds. Compounds **1**–**8** were synthetized and fully characterized and their activity against *Clostridium perfringens*, *Salmonella typhimurium*, *Salmonella enteritidis* and *Escherichia coli* is presented.

2. Materials and methods

2.1. Chemistry

All reagents and solvents were commercially available. NMR spectra were recorded on Bruker AVANCEIII (FT; 400 MHz, ^{1}H; 75 MHz, ^{13}C{^{1}H}). Chemical shifts (δ) for ^{1}H and ^{13}C{^{1}H} NMR spectra were referenced using internal solvent resonances and were reported relative to tetramethylsilane (TMS). FTIR spectra (4000–700 cm^{-1}) were recorded on a Nicolet Nexus spectrophotometer equipped with a Smart Orbit HATR accessory (diamond crystal). Melting points (mp) were determined using an Electrothermal melting point or a Köfler apparatus and are uncorrected (see Table 1). For **3**–**7** mass spectra were acquired in EI mode (positive ions) by mean of a DEP-probe (Direct Exposure Probe) mounting on the tip a Pt-filament with a DSQII Thermo Fisher apparatus equipped with a single quadrupole analyzer. The analyses were conducted in flash mode with an amperage gradient of 100 mA/s up to 1000 mA, correspondingly to an estimated temperature of 1000 °C. ESI mass of **8** was registered by using a Waters

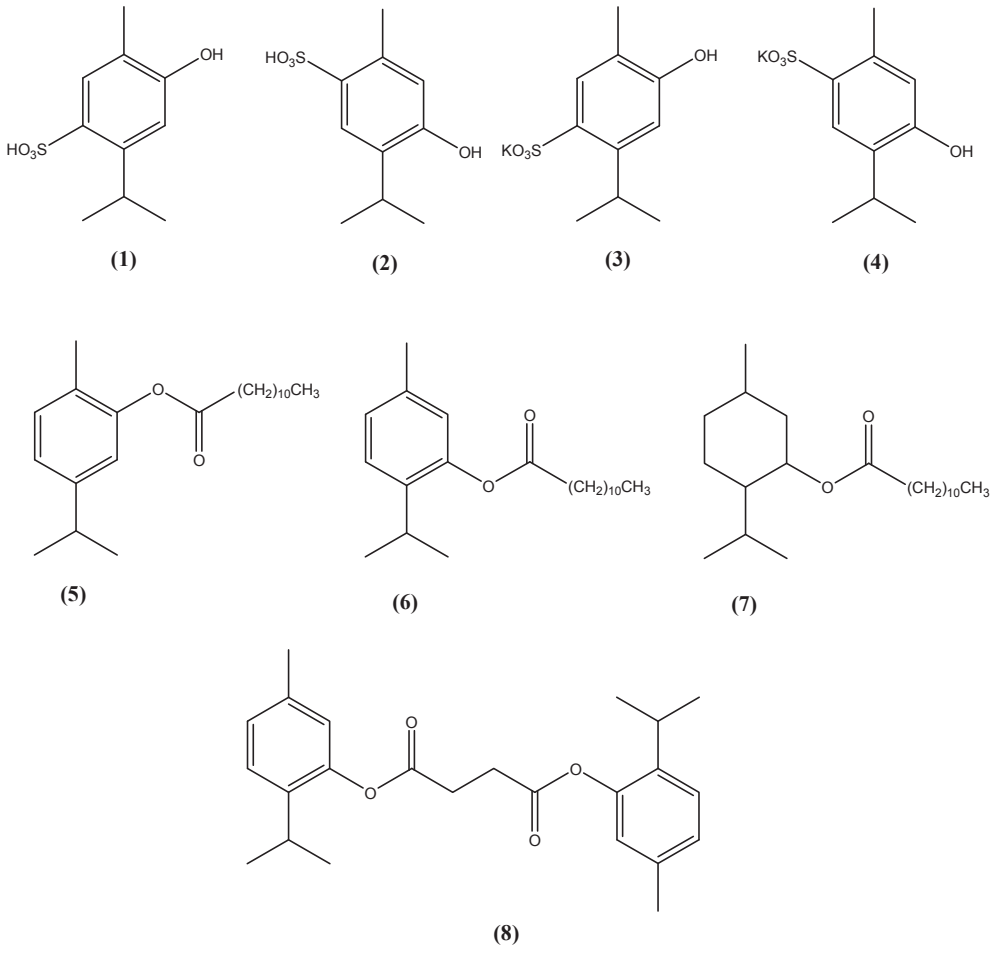

Fig. 1. Schematic representation of carvacrol, thymol and menthol derivatives **1**–**8**.