Industrial Crops & Products 120 (2018) 180–186

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Antifungal evaluation of plant essential oils and their major components against toxigenic fungi

Haiwei Wang[a,b,1], Zhixin Yang[b,1], Guangyao Ying[a,c], Meihua Yang[a], Yujiao Nian[a,d], Fang Wei[a,c], Weijun Kong[a,*]

[a] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China
[b] College of Pharmacy, Heilongjiang University of Chinese Medicine, Harbin 150040, China
[c] College of Pharmacy, Jinzhou Medical University, Jinzhou 121001, China
[d] College of Traditional Chinese Medicine, Jilin Agricultural University, Changchun 130118, China

ARTICLE INFO

Keywords:
Plant essential oils
Toxigenic fungi
Antifungal activity
Minimal inhibitory concentration
Natural antifungal agents

ABSTRACT

Contamination of toxigenic fungi in foods, agricultural commodities and Chinese herbal medicines has posed serious threaten to human and animals' health and safety. Natural antifungal agents from plant essential oils (PEOs) and their active components have been candidates of interest. In this study, the antifungal activities of PEOs from 11 natural plants against three kinds of toxigenic fungi including *Aspergillus flavus*, *Penicillium viridicatum* and *Aspergillus carbonarius* were evaluated and compared through the determination of fungi zone diameter. Results showed that 4 PEOs of *Cinnamomum cassia Presl*, *Litsea cubeba*, *Cymbopogon martini* and *Thymus mongolicus Ronn* expressed stronger inhibitory effects and lower minimal inhibitory concentrations (MICs) against the three kinds of fungi, which would be the ideal antifungal candidates. Then, the chemical compositions of them were analyzed by an optimized gas chromatography-mass spectrometry method with *trans*-cinnamaldehyde and d-limonene, citral and citronellal, *trans*-geraniol, and carvacrol as their main active components, respectively. The six compounds with strong antifungal activity and small MICs on the three kinds of fungi might contribute largely to the inhibitory effects of the 4 selected PEOs containing them. The 4 selected PEOs and their 6 main active components exhibited wide application prospect as the novel natural antifungal alternatives for preventing fungal contamination in different matrices to assure their quality and safety. The use of PEOs with highly-volatile components is environment-friendly, convenient and highly-effective with minimal residues and low cost will get more interest in practice.

1. Introduction

For a long period, the security of foods, agricultural commodities and Chinese herbal medicines (CHMs) due to fungal contamination especially toxigenic fungi such as *Aspergillus flavus*, *Penicillium viridicatum* and *Aspergillus carbonarius* et al. (Asghar et al., 2017; Baquião et al., 2016; Geremew et al., 2016; Rundberget et al., 2004; Zhang et al., 2017) has attracted more and more consideration. One of the most important reasons is that these toxigenic fungi can naturally produce secondary metabolites-mycotoxins, which exhibit serious toxicities and considerable risks to the consumers (Edite Bezerra da Rocha et al., 2014). Among these mycotoxins, aflatoxins (AFs) mainly produced by *Aspergillus flavus* are toxic and carcinogenic metabolites, which have been classified as the Group IA carcinogen by the International Agency for Research on Cancer (IARC) (Al-Zoreky and Saleh, 2017; Iqbal et al., 2017; Yang et al., 2017). OTA mainly produced by *Aspergillus carbonarius* (Kanapitsas et al., 2016) and *Penicillium viridicatum* (Bragulat et al., 2008) is reported to be a nephrotoxin, an immune suppressant, as well as a carcinogen, and is reported to be related with Balkan Endemic Nephropathy (BEN) of human (Grollman and Jelakovic, 2007; Vecchio et al., 2012; Vrabcheva et al., 2004). Therefore, over the last few decades, exploring for effective, safe, and economic candidates for controlling or prohibiting fungal contamination and mycotoxins residue in the above-mentioned matrices has got increasing focus and interests of researchers.

Physical and chemical preservatives such as ultraviolet and ɣ-rays irradiation, sulphur and aluminium phosphide fumigation, etc, have been introduced to inhibit the growth of fungi. Nevertheless, due to the potential decomposition reactions and residues, as well as their own potential toxicity, and potential undesirable biological effects on

* Corresponding author.
E-mail address: kongwj302@126.com (W. Kong).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.indcrop.2018.04.053
Received 14 January 2018; Received in revised form 1 March 2018; Accepted 19 April 2018

human health, these strategies have been forbidden in many official documents (da Cruz Cabral et al., 2013; Li et al., 2016; Prakash et al., 2015). Recently, some plant essential oils (PEOs) and their active components with strong and broad-spectrum antifungal properties as the succedaneums to protect foods, agricultural commodities and CHMs from fungal contamination in a safe, non-residual, biodegradable and environment-friendly way have been taken into consideration in some aspects (Prakash et al., 2012a,b; Viuda-Martos et al., 2011). Several studies have shown the antifungal and antimicrobial properties of PEOs from *Syringa Linn.*, *Cinnamomum cassia Presl*, *Litsea cubeba*, *Cymbopogon martini*, *Foeniculum uulgare Mill*, etc, and they were of great value in foods, cosmetics, and cigarettes (da Cruz Cabral et al., 2013; Li et al., 2016; Soylu et al., 2006; Van Haute et al., 2016; Velluti et al., 2004; Zuzarte et al., 2013). It could be concluded that most of the reported bacteria or fungi were not toxigenic and could not produce mycotoxins. The relationships of the antifungal and antimicrobial properties and the internal compositions of PEOs were not explained. In addition, the antifungal effects of PEOs from the above-listed CHMs on the toxigenic fungi such as *Aspergillus flavus*, *Penicillium viridicatum* and *Aspergillus carbonarius* have not been clarified from then on. Therefore, evaluation on the antifungal activities of EOs from some widely-distributed and easy-to-obtain plants on the toxigenic fungi widely-found in the foods, agricultural commodities and other CHMs matrices that are easily to be contaminated by toxigenic fungi to explore natural and highly-effective mould inhibitor is still urgently needed.

As a proof of concept, this study aimed to firstly evaluate the inhibitory activities of 11 kinds of PEOs from anticipated candidates including *Cinnamomum cassia Presl*, *Litsea cubeba*, *Cymbopogon martini*, *Thymus mongolicus Ronn*, *Syringa Linn.*, *Lavendula angustifolia Mill.*, *Foeniculum uulgare Mill*, *Citrus reticulata Banco*, *Mentha haplocalyx Briq.*, *Allium sativum* and *Artemisia argyi* on the toxigenic fungi of *Aspergillus flavus*, *Aspergillus carbonarius* and *Penicillium viridicatum* by using the modified *in vitro* inhibition zone assay through fumigation mode. Then, the minimal inhibitory concentrations (MIC) of the PEOs with stronger antifungal activities on the selected fungi were determined with the same method. The chemical compositions of PEOs with stronger antifungal activities were analyzed by an optimized gas chromatography-mass spectrometry (GC–MS) method. Then, the inhibitory activities of main active components in the selected PEOs were assessed and compared to elucidate the relationship between the active components and their antifungal properties. The schematic representation for this study was presented in Fig. 1. This work will provide powerful references for exploring highly-effective, safe, and economic antifungal agents including PEOs together with some active compounds with low cost and wide source for inhibiting or controlling fungal contamination and mycotoxins residue in a large number of foods, agricultural commodities and CHMs matrices with high edible or medicinal values to assure their quality and safety for clinical application.

2. Materials and methods

2.1. Materials

Plant essential oils extracted from *Cinnamomum cassia Presl*, *Litsea cubeba*, *Cymbopogon martini*, *Thymus mongolicus Ronn*, *Syringa Linn.*, *Lavendula angustifolia Mill.*, *Foeniculum uulgare Mill*, *Citrus reticulata Banco*, *Mentha haplocalyx Briq.*, *Allium sativum* and *Artemisia argyi* were purchased from Jiang Xi XueSong Natural Medicinal oil Co., Ltd. (Jiangxi, China).

Trans-cinnamaldehyde, citral, *trans*-geraniol, carvacrol d-limonene and citronellal were bought from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China).

The aflatoxigenic *Aspergillus flavus* lyophilized powder (CGMCC 3.4410) and *Penicillium viridicatum* lyophilized powder (CGMCC 3.4038) were purchased from the China General Microbiological Culture Collection Center (Beijing, China). And ochratoxigenic *Aspergillus carbonarius* (ITEM-5222) lyophilized powder was supplied by Agri-Food Toxigenic Fungi Culture Collection (ITEM) of the Institute of Science of Food Production, CNR (Bari, Italy). All the powders were dissolved in 0.5 mL of sterile water (121 °C, 20 min). *A. flavus* and *A. carbonarius* spores were cultured on the Salt Czapek Dox Agar medium (SCDA; Quingdao Hope Bio-Technology Co., Ltd., Qingdao, China) and *P. viridicatum* spore was cultured on the Potato Dextrose Agar Medium (PDA; Beijing Aoboxing Bio-Technology Co., Ltd., Beijing, China) at constant temperature and humidity (28 °C, 95% relative humidity (RH)) respectively.

2.2. Chemicals and reagents

Chromatographic-grade hexane was purchased from Thermo Fisher Scientific (Beijing, China). Other reagents and chemicals were all analytical grades and obtained from Beijing Chemical Works (Beijing, China).

The Czapek Dox Agar (CDA) medium prepared by dissolving 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KCl, 0.01 g $FeSO_4$, 30 g sucrose, 15 g agar in 1000 mL of distilled water (pH6.8 ± 0.2) was purchased

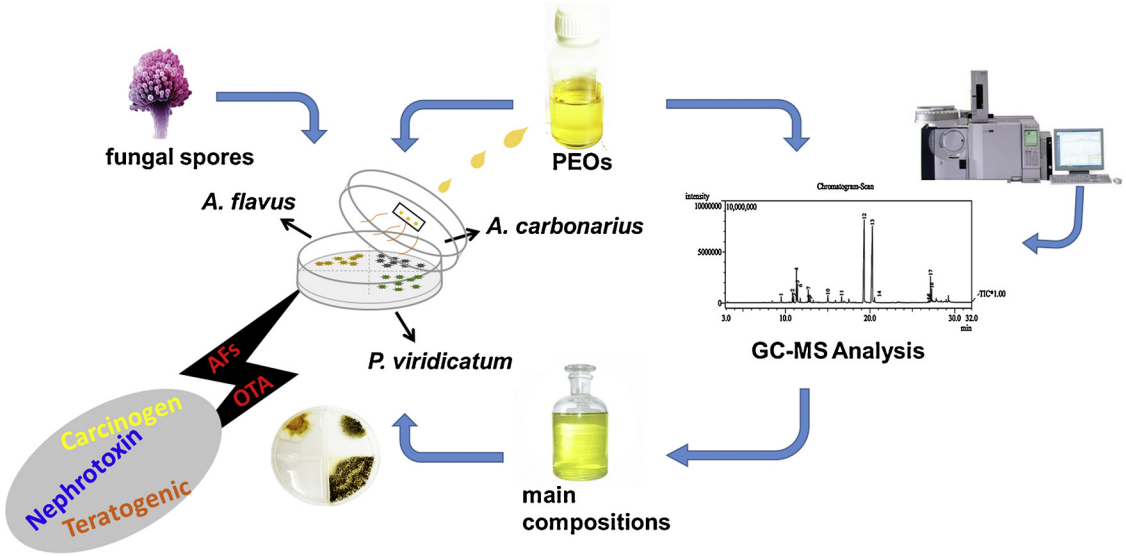

Fig. 1. Schematic representation for antifungal evaluation of plant essential oils and their main active components.