Industrial Crops & Products 103 (2017) 274–282

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Research Paper

Essential oil composition and bioactivity variation in wild-growing populations of *Curcuma phaeocaulis* Valeton collected from China

Lanyue Zhang[a], Zhiwen Yang[a], Jingwen Wei[a], Ping Su[a], Wanyi Pan[a], Xi Zheng[b], Kun Zhang[a], Li Lin[c], Jian Tang[d], Yanxiong Fang[a], Zhiyun Du[a,*]

[a] Institute of Natural Medicine & Green Chemistry, School of Chemical Engineering and Light Industry, Guandong University of Technology, Guangzhou, 510006, China
[b] Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854, USA
[c] Allan Conney Biotechnology Company Ltd, Foshan, 528200, China
[d] Infinitus (China) Company Ltd, Guangzhou, 510006, China

ARTICLE INFO

Keywords:
Curcuma phaeocaulis valeton
Essential oils
Anti-tumor activity
Anti-inflammatory activity

ABSTRACT

The chemical compositions and bioactivities of essential oils from *Curcuma. phaeocaulis* Valeton rhizomes collected from twelve various locations in China are comparatively investigated. A total of 53 components are identified from essential oils by GC–MS, and the major compounds are 8,9-dehydro-9-formyl-cycloisolongifolene (15.55–46.24%), germacrone (8.88–21.21%), curlone (0.75–20.18%), α-caryophyllene (0.08–11.04%), curzerene (0.63–9.77%) and β-elemene (0.59–5.40%). Most of these essential oils have effective anti-fungus activities (IC_{50}, 153.33–580.09 μg/ml), and they also can inhibit the growth of bacteria (IC_{50}, 485.00–778.33 μg/ml) to some extent. They also exhibit different DPPH radical-scavenging activities (IC_{50}, 2.17–22.36 μg/ml), and the antioxidative activities of some oils are even better than Trolox C. The essential oils reveal good anti-inflammatory activity by markedly down-regulating the expression of COX-2 and TNF-α. The anti-tumor activities of essential oils against LNCaP and B16 cell lines are also studied, and some of them are excellent according to their IC_{50} values (20.36–245.30 μg/ml). These results indicate some essential oils from wild *C. phaeocaulis* grown in different areas have outstanding bioactivities, which makes them ideal candidates for natural functional nutrition, pharmaceutical, culinary and cosmetic additives.

1. Introduction

The genus *Curcuma* contains more than 70 species in the family Zingiberaceae, and they are widely distributed in the warm regions of Asia, Africa and Australia. There are more than 10 *Curcuma* species distributed in China, and *Curcuma phaeocaulis* Valeton, known as Pengezhu, Ezhu or Heihejianghuang, is one of those species (Wang et al., 2008). The rhizome of *C. phaeocaulis* is one of the commonly prescribed Chinese medical herbs, and it is named Rhizoma Curcumae in Chinese Pharmacopoeia (Chen et al., 2011). Rhizoma Curcumae has broadly been used to controll gastritis, reduce blood stasis and alleviate pain symptoms alone or in combination with other herbs. It has been reported that the dominant bioactive constituents of Rhizoma Curcumae are essential oil and curcumin, which have anti-inflammatory (Makabe et al., 2006), anti-tumor (Lu et al., 2012), and neuroprotective properties (Dohare et al., 2008). The essential oil from Rhizoma Curcumae has already been approved as a therapeutic remedy for disorders by the State Food and Drug Administration of China. The main compounds in essential oil, such as curzerene, α-caryophyllene, β-elemene, curdione, curlone and ar-tumerone, have shown anti-coagulant, anti-tumor, anti-microbial, anti-inflammatory and antioxidant activities (Tang and Eisenbrand, 1992; Sun et al., 2006).

As the secondary metabolites of herbs, the yield and quality of essential oils are influenced by factors such as original species, growth area, planting technique, soil nutrients, weather conditions, harvest time, etc. The agroclimatic conditions, including rainfall, temperature, humidity and sunlight, have a significant impact on the quality and production of oil constituents (Sanghamitra et al., 2015). *C. phaeocaulis* is widely distributed in southern regions of China including Yunnan, Hainan, Guangdong, Guangxi, Guizhou, Zhejiang and Sichuan provinces, so the chemical components of essential oils from different growth areas are significantly different. Because of this, the difference in components among various essential oils will lead to discrepancies in pharmacological functions. Hence, the essential oils isolated from different areas should be reasonably utilized based on their different components for better efficacy. However, the phytoconstituents and

* Corresponding author.
E-mail address: zhiyundu@foxmail.com (Z. Du).

http://dx.doi.org/10.1016/j.indcrop.2017.04.019
Received 26 December 2016; Received in revised form 9 April 2017; Accepted 11 April 2017

Table 1
Coordinates and locations of the collected *C. phaeocaulis* rhizomes in this study.

Province	District	Locality	Source	Longitude	Latitude	Accessions
Guangxi	Nanning	Xingning	Wild	108°22′	22°51′	CP1
	Wuzhou	Cangwu	Wild	111°33′	23°51′	CP2
Sichuan	Yibin	Junlian	Wild	104°31′	28°10′	CP3
	Panzhihua	Dongqu	Wild	101°43′	26°35′	CP4
Guizhou	Qiannan	Luodian	Wild	106°45′	25°25′	CP5
	Liupanshui	Shuicheng	Wild	104°57′	26°33′	CP6
Guangdong	Guangzhou	Panyu	Wild	113°40′	23°06′	CP7
	Shenzhen	Luohu	Wild	114°17′	22°57′	CP8
Yunnan	Kunming	Panlong	Wild	102°75′	25°14′	CP9
Hainan	Wanning	Xinglong	Wild	110°20′	18°73′	CP10
Zhejiang	Hangzhou	Xihu	Wild	120°16′	30°28′	CP11
Tianjin	Binhai	Kuining	Wild	117°12′	39°28′	CP12

subsequent bioactivities of *C. phaeocaulis* essential oil from various growing areas in China have not yet been comparatively investigated.

To clearly understand the quality of rhizome essential oils from various locations (12 accessions) in China, we systematically analyzed the chemical compositions and bioactivities of these essential oils. In this study, using the steam distillation method, the compounds of essential oils are identified by GC–MS and the bioactivities, including anti-inflammatory, anti-microbial, anti-tumor and antioxidant activities, are studied.

2. Materials and methods

2.1. Plant materials and essential oil

The *C. phaeocaulis* rhizomes were collected from Guangxi, Hainan, Sichuan, Guizhou, Yunnan, Zhejiang, Tianjin and Guangdong provinces, from December 2015 through February 2016. Each sample collected from different growth regions were given a unique accession number (Table 1) for further extraction and analysis. Each of the fresh rhizomes were washed, air-dried and pulverized into powder. Then, the powder was placed in a Clevenger-type apparatus for steam distillation 3.5 h (Chinese Pharmacopoeia Committee, 2010). The essential oil was separated from the oil-water mixture and dried with small amount of anhydrous sodium sulfate, and then stored at 4 °C for subsequent experiments. The essential oil yield was calculated using the following equation:

Yield (%) (w/w) = Crude essential oil obtained (g)/Raw rhizome taken (g) × 100%

2.2. GC/MS analysis

Chemical composition of essential oil was analyzed by GC–MS (DSQ-II Ultra, Thermo Electron, UAS) with a 30 m DB-5MS capillary column (0.25-μm film thickness, Agilent, USA). Helium (flow-rate, 1.0 ml/min) was used as the carrier gas. The temperature was programmed as follow: initiated at 40 °C for 1 min, then increased to 280 °C by 3.0 °C/min and held at 280 °C for 5 min. The injector temperature was 250 °C, and the split ratio was 100:1. As for MS conditions, the electron energy was 70 eV, and the ion source temperature was 230 °C. The relative contents of the components were calculated by comparing its GC peak area to the total areas that are summed from all detected peaks. The retention indices (RIs) were calculated with a homologous series of *n*-alkanes (C_6-C_{40}) (Fattahi et al., 2016).

2.3. Minimum inhibitory concentration

These five strains, *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC15442), *Staphylococcus aureus* (ATCC6538), *Candida albicans* (ATCC10231) and *Saccharomyces cerevisiae* (GIM-2) provided by Guangdong Institute of Microbiology (Guangzhou, China), were used to estimate the antimicrobial activities of essential oils. The minimum inhibitory concentration (MIC) was determined by a broth microdilution method. The bacterial suspensions were added to microplates at a concentration of 5.0×10^5 cfu/ml. Then, different concentrations of essential oils solutions were added to the suspensions. After incubating at 37 °C for 24 h, microbial growth was revealed by the presence of turbidity, and the amounts of surviving organisms were determined by viable counts. The MIC of the essential oils resulted in a 90% decrease of inoculum viability (Angioni et al., 2004).

2.4. Antioxidative activities

The free radical scavenging activity of essential oils were utilized to illustrate its antioxidative capacity. Various concentrations of essential oils were added to 2.9 ml of a 0.004% (w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). After the mixture was incubated for 30 min in the dark (25 °C), the absorbance (A_{517}) was measured by a Shimadzu UV-300 spectrophotometer. The DPPH radical scavenging activity was estimated according to the following equation.

$$\text{Scavenging capacity } (\%) = 1 - \frac{A_{test} - A_{blank}}{A_{control}} * 100\%$$

where, A_{test} was the absorbance of DPPH solution with essential oil, $A_{control}$ was the absorbance of DPPH solution without essential oil, and A_{blank} was the absorbance of essential oil without DPPH solution.

The DPPH free radical-scavenging activity of essential oil was compared with Trolox C (a water-soluble analog of vitamin E). IC_{50} values (concentration of essential oil required to scavenge 50% of free radicals) were calculated by nonlinear regression using GraphPad Prism 5.0 software (López et al., 2008).

2.5. In vitro cytotoxic activity

The proliferation rates of prostate cancer cells (LNCaP) and melanoma cells (B16) in the presence of essential oils were determined by the colorimetric MTT assay (Basappa et al., 2015). The cells (2.5×10^4 cells/well) were seeded into 96-well microplates, and then essential oils with various concentrations were added into the plates and incubated at 37 °C for 24 h. After cells were incubated with MTT and maintained in a CO_2 incubator for 3 h at 37 °C in the dark, the absorbance was measured at 570 nm by a microplate reader. The inhibition ratio (I %) was evaluated using formula below,

$$I\,\% = [(A_{\text{ blank}} - A_{\text{ sample}})/A_{\text{ blank}}] \times 100\%$$

where solution without essential oil was used as blank (A_{blank}) and the solution containing essential oils was used as the sample (A_{sample}). The IC_{50} value of MTT assay was defined as the concentration of essential oils resulting in a 50% reduction of absorbance compared with blank