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Chemical compositions and anti-influenza activities of essential oils from *Mosla dianthera*

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ABSTRACT

Ethnopharmacological relevance: *Mosla dianthera* as an aromatic herb is used in folk medicine for the treatment of cough, colds, fever, bronchitis, nasal congestion and headache.

Aim of the study: To characterize chemical compositions and to evaluate the anti-influenza effects of essential oils of *M. dianthera* (MDEO) in influenza virus A (IVA) infected mice.

Materials and methods: MDEO was obtained by hydrodistillation and analysed by gas chromatography–mass spectrometry (GC–MS). ICR mice were treated with MDEO for 5 consecutive days at doses of 90–360 mg/kg after post-infected. Levels of Serum IL-4 and IFN-γ were assayed by ELISA. Levels of MOD, SOD, TAOC and GSH-Px in lung tissue were determined by colorimetric method.

Results: GC–MS analysis revealed the presence of 29 components that account for 97.74% of phenolic sesquiterpenes and aromatic compounds. The major compounds were elemicin (16.51%), thymol (14.77%), β-caryophyllene (14.49%), iso-elemicin (9.22%), asarone (6.09%) and α-caryophyllene (5.26%). It had significant effects on decreasing lung viral titers, inhibiting pneumonia, reducing levels of serum IFN-γ and IL-4, and enhancing antioxidant activity in the lung tissue of IVA infected mice.

Conclusions: MPE could exhibit therapeutical effects in IVA infected mice as a suppressor of IVA replication and inflammatory mediators and a promoter of antioxidant potentials. Therefore, MDEO could provide a safe and effective therapeutic candidate for treatment of influenza and its subsequent viral pneumonia.

1. Introduction

Influenza as an acute contagious respiratory disease has afflicted humans for thousand years. Despite advances in the use of neuraminidase inhibitors (such as oseltamivir and zanamivir), emergence of influenza virus resistant is still a major problem and subsequent viral pneumonia remains the leading cause of morbidity and mortality in infected patients (Nelson et al., 2009; Baranovich et al., 2010). It takes much impetus for urgent need of available anti-influenza agents.

Mosla dianthera (Thunb.), belonging to the family *Labiatae*, is one of the important aromatic and medicinal species growing in East Asian. According to traditional Chinese medicine (TCM) theories, influenza is mainly invaded by wind-evil in the lung. It is believed by TCM physicians that diaphoresis is useful for ameliorating exterior symptom. Therefore, this herb is used traditionally as the antimicrobial, antiviral, anti-allergic, anti-inflammatory and diaphoretic agent for the treatment of cough, cold, fever, headache, bronchitis, sore throats and scabies (Chen et al., 1989; Lee et al., 2006). Until now, the majority of studies on this plant have focused on chemical compositions of volatile fraction (Kim et al., 2000). However, biological activities of essential oils of *M. dianthera* (MDEO) have not been studied previously. Therefore, this study was to investigate anti-influenza potentials of MDEO in experimental lethal influenza infection *in vivo*.

2. Materials and methods

2.1. Virus and reagent

Influenza A/PR/8/34 virus (H1N1 subtype) was donated by Professor Yi-yu Lu, Zhejiang Center for Disease Control and Prevention, China. The influenza virus A (IVA) in phosphate-buffered saline

Abbreviations: MDEO, essential oils of *Mosla dianthera*; IVA, influenza virus A; NC, normal control, mock-infected and saline-treated; MC, model control, influenza virus-infected and saline-treated; PC, positive control, infected mice treated with ribavirin; EO90, infected mice treated with MDEO at the doses of 90 mg/kg; EO180, infected mice treated with MDEO at the doses of 180 mg/kg; EO360, infected mice treated with MDEO at the doses of 360 mg/kg.

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(PBS), of which haemagglutination titer was 1024, was kept at −80 °C until use. Ribavirin granules (Lot. 091210) were purchased from Zhejiang O_2 Pharmaceutical Co., Ltd.

2.2. Plant material and extraction of essential oil

Aerial parts of *M. dianthera* were collected in August, 2008, from Pujiang, Zhejiang, China, and was identified by Associate Professor Bing Yu, Zhejiang Chinese Medical University. The dried herbs (2.0 kg) were soaked in distilled water for 30 min and then extracted by hydrodistillation in 5 volumes of water (v/w) for 1 h. The yield of essential oil from dried herb was 1.10 ± 0.07% (w/w). The oil mixture was dried by anhydrous sodium sulphate to remove the moisture and stored at 4 °C until use.

2.3. GC–MS analysis

GC–MS analysis of essential oils of *M. dianthera* was carried out according to the method previously reported (Kim et al., 2000) with slight modifications. Analyses were performed on a mass spectrometer (GC–MS, SHIMADZU QP-5000) equipped with a SE 54 capillary column (30 m × 0.32 mm × 0.5 μm) using helium as the carrier gas with the flow rate of 1.0 ml/min. The oven temperature programmed from 50 °C (1 min) to 230 °C at a rate of 10 °C/min and then holding at 230 °C for 10 min. Injector and detector temperatures were both 230 °C. Electron impact ionization (EI^+, 70 eV) in the scan range of 50–550 *m*/*z* was used for all samples. The constituents of the oils were identified by matching their mass spectra with reference spectra in the computer library and also by comparing their Kovat retention indices, which were determined by co-injection of the sample with a solution containing the homogous series of C_8–C_{20} *n*-alkanes as reported before (Cao et al., 2009; Sereshti et al., 2011). The percentage composition was computed from the GC peak areas.

2.4. Animals and experimental design

SPF ICR mice weighing 18–22 g were purchased from Animal Experimental Center, Zhejiang Chinese Medical University, China. Experiments were carried out in accordance with local guidelines for the care of laboratory animals of Zhejiang Chinese Medical University, and were approved by the ethics committee for research on laboratory animal use of the institution [No. SCXK (zhe) 2008-0116].

The mice were anesthetized with diethyl ether, intranasally challenged with mouse-adapted 10 × LD_{50} (50% lethal dose) IVA in a volume of 30 μl PBS. These infected mice were divided into MC group, ribavirin-treated group and MDEO-treated groups (each group had 10 mice). MDEO or ribavirin was orally administered daily for 5 days after infection, while mice in the MC group and NC group were only given saline at the same intervals. All mice were observed daily for changes in weight and for any death.

2.5. Biochemical analysis and histopathology

After day 5 post-infection, all mice were sacrificed to monitor histological changes and challenge dose of the virus in the lungs of IVA-infected animal by calculating lung index and HA titer as our previous reports (Wu et al., 2010; Yu et al., 2010). Blood samples were collected and centrifuged at 3000 × *g* for 20 min to obtain serum. The levels of serum IFN-γ and IL-4 were determined using ELISA kits (Boster Biotech. Inc., Wuhan, China). Levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and total antioxidant capacity (TAOC) in lung tissue were determined by colorimetric method using commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China). Simultaneously, lung tissues were put in a buffer solution of 10% formalin and embedded in paraffin. Six to ten 4-μm-thick sections were prepared in a noncontiguous way and dyed with hematoxylin–eosin; stained areas were viewed using an optical microscope.

Table 1
Chemical compositions of essential oil from *Mosla dianther* analyzed by GC–MS.

Compound	Kovat retention indices	Relative content (%)
α-Terpinene	1020	0.27
D-Limonene	1029	0.33
p-Cymene	1035	1.31
γ-Terpinene	1062	1.69
Linalool	1098	0.26
Terpinen-4-ol	1175	0.22
L-Carvone	1191	1.21
β-Cubebene	1219	3.25
Thymol	1294	14.77
α-Cedrene	1302	0.63
β-Caryophyllene	1348	14.49
Thymol acetate	1423	0.86
α-Caryophyllene	1435	5.26
β-Farnesene	1458	0.85
Germacrene D	1486	0.38
α-*trans*-Bergamotene	1499	2.30
β-Bisabolene	1510	4.81
α-Farnesene	1576	2.62
Elixene	1603	0.53
β-Sesquiphellandrene	1669	2.40
β-Elemene	1694	0.42
δ-Cadinene	1811	1.06
Caryophyllene oxide	1902	0.44
Eugenol methyl	2030	0.58
Elemicin	2089	16.51
Myristicin	2147	4.23
iso-Elemicin	2205	9.22
Asarone	2213	6.09
Apiol	2287	0.75
Chemical classes		
Monoterpene hydrocarbones		4.45
Oxygenated monoterpenes		1.69
Sesquiterpene hydrocarbones		38.15
Oxygenated sesquiterpene		0.44
Aromatic compounds		53.01
Total		97.74

2.6. Acute toxicity

This protocol was performed as reported previously (Atsamo et al., 2011). After overnight fasting, groups of ten mice were administered MDEO in graded doses up to 4.32 g/kg body weight, while the control group received water. The groups were observed for 7 days and at the end of the experiment mortality was recorded for each group.

2.7. Statistical analysis

All parameters were recorded for individuals within all groups. All data were shown as mean ± S.D. Statistical comparisons of data were carried out using the ANOVA and *t*-test of the SPSS 18.0 system. A value of $P < 0.05$ was considered significant.

3. Results and discussion

Several natural products have taken attention as one of the alternative therapies for minimal adverse effects and multiple targets in preventing and treating influenza (Wang et al., 2006; Liu et al., 2008; Kurokawa et al., 2010; Wu et al., 2011). A number of studies have shown that physiological activities of components extracted