Industrial Crops & Products 107 (2017) 90–96

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Chemical composition and anticancer activity of *Elsholtzia ciliata* essential oils and extracts prepared by different methods

Lauryna Pudziuvelyte[a], Mantas Stankevicius[b], Audrius Maruska[b], Vilma Petrikaite[d,e], Ona Ragazinskiene[c], Gailute Draksiene[a], Jurga Bernatoniene[a,*]

[a] Department of Drug Technology and Social Pharmacy, Lithuanian University of Health Sciences, Eivenių str. 4, LT-50161, Kaunas, Lithuania
[b] Department of Biology, Vytautas Magnus University, Vileikos str. 8, LT-44404, Kaunas, Lithuania
[c] Kaunas Botanical Garden of Vytautas Magnus University, Kaunas, Lithuania
[d] Department of Drug Chemistry, Faculty of Pharmacy, Lithuanian University of Health Sciences, LT-44307, Kaunas, Lithuania
[e] Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius University, LT-10222, Vilnius, Lithuania

ARTICLE INFO

Keywords:
Elsholtzia ciliata
Anticancer activity
Essential oil
Ethanolic extracts
Hydrodistillation
GC–MS

ABSTRACT

E. ciliata (Lamiaceae) is very interesting and promising herb mainly for chemical composition and pharmacological activities. The aim of this study was to determine chemical composition of the essential oils of fresh, frozen and dried herbal materials of *E. ciliata* and compare different extraction methods This is the first study on composition of *E. ciliata* volatile compounds from fresh, frozen and dried herbal samples. The samples were prepared by hydrodistillation (HD), extraction with polar solvent-ethanol (ESE) and dynamic headspace solid-phase micro extraction (SPME) and analyzed by gas chromatography-mass spectrometry (GC–MS) method. A total of 48 compounds were identified by GC–MS. Dehydroelsholtzia ketone, elsholtzia ketone, sesquiterpenes β-bourbonene, caryophyllene, α-caryophyllene, germacrene D and α-farnesene were identified and found to be predominant compounds in SPME composition of the fresh, frozen and dried herbal samples. The major amounts of ketones (dehydroelsholtzia and elsholtzia) were determined in dried herbal samples where they made up 24.94% ($p < 0.05$) and 71.34% ($p < 0.05$) of the SPME composition. Artemisia ketone was determined only in fresh herb. No previous report exists regarding this ketone in *E. ciliata* fresh, frozen and dried herbal materials or essential oil. There were 26 components identified in the essential oil obtained by HD. The main compounds of this essential oil were dehydroelsholtzia ketone (78.28%) and elsholtzia ketone (14.58%).

Essential oil showed antiproliferative activity on three tested cancer cell lines (human glioblastoma (U87), pancreatic cancer (Panc-1) and triple negative breast cancer (MDA-MB231)) *in vitro*. EC_{50} (half maximal effective concentration) values of essential oil against those cells were in the range of 0.017–0.021%. The viability of human normal fibroblasts exposed to the same concentrations of the essential oil was statistically significantly higher compared to the viability of cancer cells ($p < 0.05$). The extracts did not show any effect on U87 cells, and only slightly decreased MDA-MB231 cell viability (up to 76.4%) at the highest concentration of 10 mg/mL.

The impact that different extraction methods have on the yield of the essential oil from the tested herbal materials requires a further research. It would enable production of pharmaceutical forms enriched by these essential oils, which are notable for their anticancer activity.

1. Introduction

Essential oils are considered to be one of the most important substances in plants and to possess antimicrobial, antiviral, antifungal, antioxidant and anti-inflammatory activities (Bey-Ould Si Said et al., 2016; Raut and Karuppayil, 2014; Teixeira et al., 2013; Buchbauer, 2010). Essential oils are complex mixtures of various terpenes and their oxygenated derivatives such as alcohols, phenols, ketones. Essential oils are usually obtained by HD, steam distillation or solvent extraction (Bakkali et al., 2008; Longaray Delamare et al., 2007; Raut and Karuppayil, 2014). For the experiments herbal materials of *E.ciliata* grown in Lithuania (Europe) were chosen. A flowering plant *Elsholtzia ciliata* (Thunb.) Hylander of the family Lamiaceae is native to Asia and is also found in Europe, Africa, North America, India (Guo et al., 2012; Korolyuk et al., 2002). *Lamiaceae* family plants are well known for their antiviral, antimutagenic, chemotherapeutic, antimicrobial, antioxidant

* Corresponding author.
E-mail addresses: jurgabernatoniene@yahoo.com, jurga.bernatoniene@lsmuni.lt (J. Bernatoniene).

http://dx.doi.org/10.1016/j.indcrop.2017.05.040
Received 11 January 2017; Received in revised form 19 May 2017; Accepted 21 May 2017

and anti-inflammatory activities (Raut and Karuppayil, 2014). *E. ciliata* is widely used in folk medicine for antibacterial, anticancer and anti-inflammatory properties (Tian, 2013). In traditional Chinese medicine the herb has been used to treat the common cold, headaches, pharyngitis, fever, edema, diarrhea, rheumatic arthritis, digestion disorders, nephritises (Guo et al., 2012; Sung et al., 2011). The pharmacological activities, such as antibacterial, antiviral, antioxidant, anti-inflammatory, diuretic and anti-obese, of extracts and pure compounds from *E. ciliata* are under investigation (Guo et al., 2012; Sung et al., 2011). The major chemical constituents in *Elsholtzia* are flavonoids, phenylpropanoids, phytosterols, cyanogenic glycosides and triterpenes (Guo et al., 2012; Liu et al., 2012).

Essential oils accumulating herbal materials are used to produce various pharmaceutical forms such as tablets, capsules, microcapsules, ointments, creams or gels. Usually materials used in production are air dried; however, using fresh or frozen materials could also be suitable. There has not been a research done yet comparing chemical composition of essential oils from freshly collected and from frozen herbal materials, and it is not known what effect the material preparation has on the volatile composition. The aim of this study was to investigate the effects of different methods of material preparation and extraction on chemical composition of volatile compounds from *E. ciliata*. Also, one of the objectives was to evaluate the toxicity and anticancer activity *in vitro* of the plant extracts as well as the essential oil.

2. Materials and methods

2.1. Plant material

E. ciliata aerial parts were collected in Vilnius, Lithuania, in July 2016 and were purchased as fresh and dried herbs from "Žolynų namai" (Vilnius, Lithuania). The herbs were identified by Dr. Prof. Nijole Savickiene, Medical Academy, Lithuania University of Health Sciences, Kaunas, Lithuania. A voucher specimen (L 17710) was placed for storage at the Herbarium of the Department of Drug Technology and Social Pharmacy, Lithuanian University of Health Sciences, Lithuania. Fresh and dried materials were mechanically ground in a laboratory mill to a homogenous powder or paste. A sample of fresh herb was frozen in a freezer (−18 °C) until preparation of extracts and SPME by GC–MS method.

2.2. Isolation of the essential oil

30 g dried *E. ciliata* sample was mixed with 500 mL bi-distilled water and submitted to HD for 4 h using a Clevenger-type apparatus (European pharmacopoeia). A yellow colored oil with specific aroma was obtained. The essential oil was collected with water and stored in a refrigerator at +4 °C until needed.

2.3. Preparation of ethanolic extracts

Fresh, frozen and dried powdered materials (Fig. 1) of plant's areal parts (0.5 g each) were extracted for 24 h with 20 mL 96% ethanol in TiterTek shaker (Germany). The extracts were filtered through a paper filter and 0.22 μm pore size PVDF membrane filter and stored at +4 °C in a refrigerator until GC–MS analysis.

2.4. Dynamic headspace SPME

Samples for gas chromatography analysis were prepared using SPME. Extraction of *E. ciliata* volatiles was performed on 65 μm PDMS/DVB (polydimethylsiloxane/divinylbenzene) Stable Flex fibre (Supelco, Bellefonte, USA). 10 mg of fresh, frozen and dried samples were added into 10 mL vials and placed in the AOC-5000 autosampler. The samples were thermostated for 10 min at 40 °C and the fiber was exposed in the headspace.

2.5. GC–MS analysis

The analysis was carried out using a GCMS-QP2010 system (Shimadzu, Tokyo, Japan). For separation of volatiles a low polarity RTX-5MS (Restec, USA) (30 m × 0.25 mm i.d. × 0.25 μm film thickness) GC column was used. The oven temperature gradient started at 60 °C and was raised to 150 °C at 5 °C/min, and then raised to 280 °C at 20 °C/min and was held at it for 3 min. The carrier gas helium (99.999%, AGA Lithuania) was used at a flow rate of 1.2 mL/min. The injector temperature was kept at 230 °C in a split mode (1:20). The mass detector electron ionization was 70 eV. The ion source and interface temperatures were set at 220 °C and 260 °C correspondingly.

The compounds preliminary were identified by mass spectra library search (NIST, v1.7) and by comparing with the mass spectral data from literature (Adams, 1995). The repeatability of solid-phase micro extraction – gas chromatography mass spectrometry method was evaluated by injecting the same sample three times. The relative standard deviation for the peak area was 5.15%.

2.6. Cell lines

Anticancer activity was tested on three selected cancer cell lines: human glioblastoma (U87), pancreatic ductal adenocarcinoma (Panc-1) and triple-negative mammary gland adenocarcinoma (MDA-MB231). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) high glucose (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1% antibiotics (10,000 units/mL penicillin and 10 mg/mL streptomycin) (Gibco) at 37 °C in a humidified atmosphere containing 5% CO_2. Human fibroblasts were grown in a Medium 106 with Low Serum Growth Supplement (LSGS) (Gibco) supplemented with 1% antibiotics (10,000 units/mL penicillin and 10 mg/mL streptomycin) (Gibco) at 37 °C in a humidified atmosphere containing 5% CO_2. All cell lines were grown to 70% confluence and trypsinized with 0.125% TrypLE™ Express solution (Gibco) before passage. They were used until passage 20.

2.7. Determination of cell viability

Cell viability was studied using the method of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma). 100 μL of cells were seeded in 96-well plates in triplicates (5000 cells/well for U87, MDA-MB231 and human fibroblasts cells, and 3000 cells/well for Panc-1 cells) and incubated at 37 °C for 24 h. Then serial dilutions of different extracts and the essential oil were made in microplates. The essential oil before the experiment was mixed with 10% of Tween 80 to enhance its solubilization in culture medium. In order to avoid the essential oil evaporation, 0.05% of methylcellulose (Sigma) was added into the medium. Cells treated only with medium containing the same concentration of ethanol (in the case of extracts) or Tween 80 (in the case of essential oil) served as a negative control. Only medium without cells was used as a positive control. After 72 h incubating at 37 °C, 10 μL of MTT was added in each well. After 3 h the liquid was aspirated from the wells and discarded. Formazan crystals were dissolved in 100 μL of DMSO, and absorbance was measured at a test wavelength of 490 nm and a reference wavelength of 630 nm using a multidetection microplate reader. The experiments were repeated three times independently.

2.8. Statistical analysis

Data are presented as mean ± SEM. Statistical analysis was performed by using Student's *t*-test. A value of $p < 0.05$ was taken as the level of significance.