Phytomedicine 24 (2017) 157–165

Contents lists available at ScienceDirect

Phytomedicine

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

Original Article

Eulophia macrobulbon – an orchid with significant anti-inflammatory and antioxidant effect and anticancerogenic potential exerted by its root extract

Roswitha Schuster[a], Laura Zeindl[a], Wolfgang Holzer[b], Nattakanwadee Khumpirapang[c], Siriporn Okonogi[c], Helmut Viernstein[a], Monika Mueller[a,*]

[a] Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstrasse 14, Vienna A-1090, Austria
[b] Department of Pharmaceutical Chemistry – Division of Drug Synthesis, University of Vienna, Althanstrasse 14, Vienna A-1090, Austria
[c] Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand

ARTICLE INFO

Article history:
Received 9 August 2016
Revised 7 November 2016
Accepted 22 November 2016

Keywords:
Eulophia macrobulbon
Cyrtopera macrobulbon
Anti-inflammatory
Cytotoxic
Antioxidant
Orchid

ABSTRACT

Background: The Orchidaceae family is one of the largest families of flowering plants. Orchids are widely used for the traditional herbal medicine, acting as aphrodisiac, antisepic, antimicrobial, anti-cancer agent, etc.

Purpose: This study was designed to elucidate the anti-inflammatory, antioxidant and cytotoxic potential of a 50% ethanolic extract of *Eulophia macrobulbon* roots (EME) *in vitro*, an orchid growing in Southern Asia. Furthermore, the main active compounds were isolated, and the bioactivity of the single constituents was determined.

Methods: The anti-inflammatory activity of EME and its compounds was evaluated by the secretion of pro- and anti-inflammatory cytokines and by the expression of inducible nitric oxide synthase (iNOS) in a lipopolysaccharide (LPS)-stimulated macrophage model, as determined by an enzyme linked immunosorbent assay (ELISA) and Western blot. Antioxidant activity was assessed using a DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) photometric assay. Cytotoxic effects were determined using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-assay.

Results: EME and its compounds significantly reduced the production of the proinflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), the expression of iNOS and subsequently increased the production of the anti-inflammatory cytokine interleukin 10 (IL-10) in LPS-stimulated macrophages. Additionally it could be demonstrated that EME is rich in radical scavengers. Furthermore, EME and its components showed notable cytotoxic effects on the human cervical adenocarcinoma cell line HeLa, the human colorectal adenocarcinoma cell line CaCo-2 and the human breast adenocarcinoma cell line MCF-7. The most active constituents were identified as 4-methoxy-9,10-dihydro-2,7-phenanthrenediol **(8)**, 4-methoxy-2,7-phenanthrenediol **(9)**, 1,5-dimethoxy-2,7-phenanthrenediol **(10)**, 1,5,7-trimethoxy-2,6-phenanthrenediol **(11)**, 1-(4-hydroxybenzyl)-4,8-dimethoxy-2,7-phenanthrenediol **(15)**.

Conclusion: Based on this data, EME provides various beneficial anti-inflammatory, antioxidant and cytotoxic attributes and may be used as herbal remedy in the pharmaceutical or food industries.

Abbreviations: AA, ascorbic acid; Abs, absorbance; ATCC, American type culture collection; APT, attached proton test; BBFO, broadband fluorine obersavtion; BSA, bovine serum albumin; CaCo-2, human colorectal adenocarcinoma cell line; COSY, correlated spectroscopy; DMEM, Dulbecco's modified Eagle medium; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picryl-hydrazyl-hydrate; ELISA, Enzyme linked immunosorbent assay; EME, Eulophia macrobulbon extract; FBS, Fetal bovine serum; HCl, hydrochloric acid; HeLa, human cervical adenocarcinoma cell line; HMBC, heteronuclear multiple bond correlation; HPLC, High performance lipid chromatography; HRMS, high resolution mass spectrometry; HSQC, heteronuclear single quantum coherence spectroscopy; IC50, half maximal inhibitory concentration; IL-6, Interleukin 6; IL-10, Interleukin 10; iNOS, Inducible nitric oxide synthase; LPS, Lipopolysaccharide; MeOH, methanol; MCF-7, human breast adenocarcinoma cell line; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NaOH, sodium hydroxide; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser enhancement spectroscopy; NSAID, Nonsteroidal anti-inflammatory drug; PBS, Phosphate buffered saline; SDS, Sodium dodecyl sulfate; TFA, trifluoroacetic acid; TLC, thin layer chromatography; TNF-α, Tumor necrosis factor alpha; UV, ultra violet; WHO, world health organization.

* Corresponding author.
E-mail address: monika.mueller@univie.ac.at (M. Mueller).

http://dx.doi.org/10.1016/j.phymed.2016.11.018
0944-7113/

Introduction

Cancer, diabetes, rheumatoid arthritis and atherosclerosis – these diseases are related to oxidative stress, dysfunctional regulatory mechanism of inflammation and cell development and are, among many others, a major health problem worldwide (Coussens and Werb, 2002; Haffner, 2006; Libby et al., 2002). Inflammation is primarily a beneficial response which protects and defends the body from harmful stimuli such as pathogens, toxins, allergens and damaged cells and tissues. It is controlled by the secretion of pro- and anti-inflammatory cytokines and mediators. If the regulatory mechanism appears to be dysfunctional, inflammation can become detrimental to tissues. In this manner, chronic inflammation plays an important role in various diseases. According to the World Health Organization (WHO), cancer, diabetes, coronary heart diseases and asthma, which are all related to chronic inflammation and/or cell differentiation and/or oxidative stress, belonged to the top 10 causes of death in 2012 (WHO, 2012). Medical treatment (*i.e.* nonsteroidal anti-inflammatory drugs; NSAIDS, glucocorticoids, anti-cancer agents) exerts beneficial curative effects, but usually also shows various side effects in long term use including ulcers, immunosuppression, edema and weight gain (Bjarnason et al., 1993; Coghlan et al., 2003; Schäcke et al., 2002; Sostres et al., 2010). Natural products, which exert complementary biological effects, may be an alternative in prophylaxis and treatment of chronic inflammation, cell differentiation and related disorders (Mueller et al., 2016; Schuster et al., 2016).

Over the years, various studies have reported different mechanisms for anti-inflammatory, antioxidant and cytotoxic effects of herbal medicines (Luo et al., 2010; Mensor et al., 2001; Mueller et al., 2010). The Orchidaceae family is natures largest family and most extravagant group of flowering plants with a great diversity in shape, size and color of their flowers (Dressler, 1993). Orchids are primarily grown as ornamentals but they also have a long history as source of herbal remedies in traditional medicine all over the world. The Chinese were the first who described orchids for medicinal use since 2800 BC., followed by the Indians in 2000–600 BC (Bulpitt, 2005; Hossain, 2010). The list of traditional uses of orchids for the treatment of various diseases is very long. Hossain (2010) presents a good overview on the great variety on medicinal orchids and summarized their traditional uses (*i.e.* cancer, tuberculosis, cholera, eczema, stomach disorders, arthritis, menstrual disorders, inflammations, pain, diarrhea, asthma, etc.), popular orchid preparations (*i.e.* Shi-Hu, Tian-Ma, Bai-Ji, Vanilla, Salep) and important phytochemicals in orchids (alkaloids, flavonoids, anthocyanins, sterols, stilbenoids, etc.). Despite of the incredible range of therapeutic uses, pharmacological and toxicological studies of Orchids are very rare. Ho and Chen (2003) report the anti-cancer effect of moscatilin, a phytochemical derived from the species *Dendrobium* on different cancer cell lines. Chinsamy et al. (2014) determined the anti-inflammatory potential of different *Eulophia* species. However most of the studies on Eulophia species examined the antioxidant activity (Kumar et al., 2013; Narkhede et al., 2012; Tatiya et al., 2013).

Eulophia macrobulbon (E. C. Parish & Rchb. f.) Hook. f. (Syn: Cyrtopera macrobulbon, Graphorkis macrobulbon, etc.) is an terrestrial growing, up to 45 cm long orchid, which blooms in the spring and can be found naturally in Thailand, Laos, Vietnam, Myanmar and Cambodia at elevations around 700 m (Grant, 1895). According to the local Thai folk, the plant is traditionally used as a medicinal remedy for the treatment of gangrene and insect bites. To the best of our knowledge, there are no scientific studies on *Eulophia macrobulbon* yet.

In the current study, the main active compounds of *E. macrobulbon* roots are isolated and the anti-inflammatory and antioxidant potential of *E. macrobulbon* roots and its pure components, as well as the cytotoxic effect on three different cancer cell lines (HeLa, CaCo-2, MCF-7) are elucidated *in vitro*. Furthermore, the most active constituents are identified.

Materials and methods

Chemicals and reagents

Acetonitrile (HPLC grade) was purchased from Promochem (Wesel, Germany). Acetone, dimethyl sulfoxide (DMSO), absolute ethanol, butanol, 1-propanol, methanol (MeOH), hydrochloric acid (HCl), formic acid, disodium hydrogen phosphate, potassium chloride, sodium chloride, sodium dodecyl sulfate (SDS), sodium hydroxide (NaOH) and Tween 20 were obtained from Merck (Darmstadt, Germany). Dulbecco's minimum essential medium (DMEM), fetal bovine serum (FBS), L-glutamine and a penicillin/streptomycin mixture were obtained from Life Technologies (Carlsbad, CA, USA). Macrophage RAW 264.7, HeLa, CaCo-2 and MCF-7 cells were purchased from American Type Culture Collection (ATCC-TIB-71, ATCC-CCL-2, ATCC-HTB-37, ATCC-HTB-22). MTT, LPS from *E. coli*, trifluoroacetic acid (TFA), dihydrogen phosphate, DPPH and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (Vienna, Austria). An ELISA kit was obtained from eBioscience (San Diego, CA, USA). Rabbit polyclonal anti-iNOS was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Goat Anti-Rabbit IgG, DyLight™ 800 was obtained from Thermo Fisher Scientific (Rockford, IL, USA). All other chemicals and reagents used for this work were purchased from Sigma-Aldrich (Vienna, Austria) and Merck (Darmstadt, Germany).

Extraction of the plant material

Fresh roots of *E. macrobulbon*, were collected from the area of Chiang Mai province, Thailand in July 2012. The plant voucher specimens (no. 023208) were deposited at the Herbarium of the Faculty of Pharmacy, Chiang Mai University and authenticated by the CMU staff botanist. After washing, the plants were cut into small pieces, dried in a circulating oven at 55 °C and ground into fine powder, which was macerated with 50% ethanol for 24 h at room temperature. After filtration (Whatman No.1, Sigma-Aldrich) the solvent was evaporated in a rotary evaporator at 40 °C to remove the ethanol. The water was removed by lyophilization in a Heto Power Dry LL3000 freeze-dryer (Thermo Scientific, Waltham, MA, USA) and the crude extracts (EME) were stored at room temperature in the dark until use. For the assays of anti-inflammatory and cytotoxic effects, the residues were freshly dissolved in 50% ethanol at a concentration of 100 mg/ml.

Preparative high performance liquid chromatography (HPLC)

Separation of the EME was performed by preparative HPLC using a Thermo Scientific Ultimate 3000 system, connected to a Hypersil GOLD™ column (5 µm, 21.2 mm × 250 mm, Thermo Scientific). The mobile phase consisted of eluent A (trifluoroacetic acid/acetonitrile/water 0.1:5:94.9 v/v) and eluent B (trifluoroacetic acid/acetonitrile 0.1:99.9 v/v). The elution program set as followed: 0.5% B from 0 to 5 min, 17.5% B from 5 to 30, 50% B from 30 to 35 min, 100% B from 35 to 41, 0.5% B from 41 to 48 min, with a flow rate of 15 ml/min and UV detection at 254 nm. The single fractions were collected in repeated separations, the solvent was evaporated and the remaining water was removed by freeze-drying (Heto Power Dry LL3000). The dry extracts were stored at room temperature until use.