JOURNAL OF HERBAL MEDICINE 5 (2015) 77–85

Available online at www.sciencedirect.com

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

Original Research Article

Health attributes of an endemic orchid from Eastern Anatolia, *Dactylorhiza chuhensis* Renz&Taub. – *In vitro* investigations

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ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form
23 December 2014
Accepted 2 February 2015
Available online 10 February 2015

Keywords:
Dactylorhiza chuhensis Renz&Taub.
Antioxidant
Enzyme-inhibitory activity
Total phenolics

ABSTRACT

Phytochemical composition and potential health attributes of *Dactylorhiza chuhensis* Renz&Taub., an endemic orchid from Eastern Anatolia, were investigated. Lyophilized methanol-based extracts obtained from leaf, flower, stem and tuber were investigated for the presence of phenolic compounds [Folin–Ciocalteu assay and high performance liquid chromatography analysis (HPLC)], antioxidant capacities [ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assay] and enzyme-inhibitory activities [lipase, α-amylase, α-glucosidase and angiotensin converting enzyme (ACE)]. The tuber, used as a traditional remedy and utilized by the pharmaceutical industry, had the lowest content of total phenolics, inferior antioxidant and enzyme-inhibitory activities. The highest phenolic content (44.9 ± 0.8 mg GAE/g DW) was exhibited by the leaf extract, which also showed superior reducing (736.8 ± 16.2 μmol Fe^{2+}/g DW) and oxygen radical scavenging capacities (2715.8 ± 83.5 μmol Trolox E/g DW). The inhibitory activities of the leaf extract toward α-amylase, α-glucosidase and ACE were moderate. Applied at non-toxic concentrations, the leaf extract effectively reduced accumulation of nitric oxide (NO) in lipopolysaccharide (LPS)-activated hepatocellular carcinoma (HepG2) cells. Further studies towards potential utilization of *D. chuhensis* leaf as a source of physiologically active phytochemcials are justified.

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http://dx.doi.org/10.1016/j.hermed.2015.02.001

1. Introduction

The Orchidaceae family is one of the largest and widespread families in the plant kingdom with 26,567 species recorded worldwide (World Checklist of Selected Plant Families, Kew WCSP, 2011). The economic importance of orchids arises from their ornamental and therapeutic properties. Although predominantly known for their ornamental qualities, numerous orchid species are also used in food and pharmaceutical industries. Vanilla flavour derived from the fruit of orchid, belonging to the genus *Vanilla*, is an example of a widespread commercial application of orchid products.

High consumption of orchid tubers has been recorded for southern African countries. A meatless sausage "*chikanda*" made of Disa, Habenaria and Satyrium orchid tuber, traditionally consumed as a midday snack food, is a popular Zambian dish. The high popularity of chikanda sausage lead to a severe decline of orchid species in Zambia. Subsequently, a large export of edible orchid tubers from Tanzania to Zambia followed (Challe and Price, 2009).

In Mediterranean countries flour made of *Orchis mascula* and *Orchis molitaris* tuber is traditionally used for the preparation of a nutritional drink called 'salep'. The main component of orchid flour is glucomannan, a polysaccharide, comprising of mannose and glucose (Kaya and Tekin, 2001). Glucomannans are neutral water soluble fibers, reported to possess nutritive and demulcent properties (Kayacier and Dogan, 2006). Traditional preparation of orchid flour involves immersing washed tubers in boiling water to deactivate enzymes, removing the outer layer of skin and drying the tubers in the sun or in an oven. Dried tubers are ground into a fine yellowish powder, later used to prepare beverages (salep) and desserts (salep pudding and salep ice cream) (Kaya and Tekin, 2001). In Eastern Anatolia salep drink is still used by the locals to cure common colds, bronchitis and diarrhoea. Extensive use of *O. mascula* and *O. molitaris* tubers by the local population lead the plants near to extinction, and other orchid species are being explored for salep preparation. At present in Turkey salep is produced from approximately 120 taxa of orchids, representing the genera *Ophrys*, *Orchis*, *Himantoglossum*, *Serapias*, *Anacamptis*, *Compreria*, *Barlia*, *Dactylorhiza*, *Aceras*, and *Neotinea* (Sezik, 2002).

Therapeutic preparations made of orchids are also used in traditional Chinese medicine (Bulpitt et al., 2007) and in traditional Indian medicine, Ayurveda (Giri et al., 2012a). In India an orchid *Habenaria edgeworthii* is used for preparation of a popular tonic, chyawanprash. The tonic is used to cure coughs, colds, anaemia and to strengthen vitality (Giri et al., 2012a).

Eastern Anatolia is home to a large number of endemic orchids. *Dactylorhiza chuhensis* Renz&Taub. is an endemic plant growing in a restricted area of Van city in the Eastern Anatolia Region. The plant is locally known as 'kartolik', 'şepirze' and 'kulîlka qamişa'. The herb is robust (15–45 cm), the leaves are oblong, lanceolate (7–13 cm × 1–5 cm) and the flowers are rose-purple. The plant produces one edible tuber (roughly the size of one small potato) and the entire plant is removed in the harvesting process. The tuber is collected in spring, air dried in the dark and ground to obtain a fine powder for later use; the aerial parts are not used. Utilization of the aerial part, especially the leaf, may bring additional economical benefits.

Orchids accumulate a large number of diverse phytochemicals, responsible for their pharmacological activities, however research on phytochemical composition of edible and medicinal orchids and their physiological activities has been limited to only a few (Hossain, 2011). Therefore, this study aimed at the evaluation of *D. chuhensis* leaf as a potential source of physiologically active phytochemicals for therapeutic applications. The antioxidant capacities and total phenolic content of lyophilized extracts prepared from tuber, stem, leaf and flower of *D. chuhensis* were evaluated. Subsequently, inhibitory activities towards selected digestive enzymes and potential anti-inflammatory properties of leaf extract were investigated.

2. Materials and methods

2.1. Plant materials

Plants were harvested in the Eastern Anatolia Region of Turkey (Van city, Chuh path, from Hoşap town to Başkale County; 38°09′04.17″ N and 043°59′19.54″ E; 2307 m) during May–August, 2010. Plant samples with no apparent physical damage were collected from Chuh path, Başkale, Van City. The plant material, sealed in pre-cleaned polythene bags, was brought to a laboratory within a maximum of 3 h after harvest. The identity of the plant material was confirmed by Dr. Sinan İşler at the Biological Sciences Department, Science Faculty, Yüzüncü Yıl University, Turkey, and a voucher specimen stored in the university's herbarium (Herbarium code: VANF-4418). Tubers, stems, leaves and flowers of fresh plants were separated, washed with distilled water to remove surface dust and left at room temperature in the dark until dry. Subsequently, samples were ground into a fine powder using a laboratory mill and stored at −20 °C until analysis.

2.2. Reagents

Unless otherwise stated, all chemicals were purchased from Sigma–Aldrich, Inc. (Sydney, Australia) and were of analytical or HPLC grade. Soluble starch, iodine reagent and colouring reagent (Glucose C2) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sodium carbonate was purchased from Ajax Chemicals (Sydney, Australia). Acetic acid and sodium hydroxide were purchased from Ajax Finechem Pty. Ltd., Sydney, Australia. Folin–Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). α-Amylase from porcine, intestinal acetone powders from rat, angiotensin converting enzyme (ACE) from rabbit lung, porcine pancreatic lipase (type II), orlistat and captopril were purchased from Sigma–Aldrich, Inc. (USA). Acarbose was purchased as 'glucobay' from Bayer (Bayer Australia Ltd., Pymble, NSW).

2.3. Cell lines

All cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured at 37 °C in a humidified 5% carbon dioxide (CO_2) atmosphere