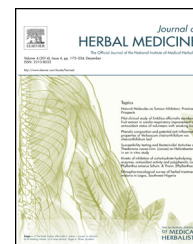




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Original Research Article

Phenolic composition and potential anti-inflammatory properties of *Verbascum cheiranthifolium* var. *cheiranthifolium* leaf

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ABSTRACT

Verbascum cheiranthifolium var. *cheiranthifolium* is a traditional remedy utilized in Eastern Anatolia, Turkey to cure a number of conditions, including rheumatism and inflammation. Lyophilized hydrophilic extract of *V. cheiranthifolium* leaf contained phenolic compounds and reducing sugars with luteolin hexoside as the dominating phytochemical and traces of apigenin, apigenin glucoside, chlorogenic acid, rosmarinic acid and quercetin glycosides. The extract, applied at non-toxic concentrations, suppressed the accumulation of nitric oxide (NO) in lipopolysaccharide (LPS)-activated murine macrophages (RAW 264.7) and hepatocellular carcinoma (HepG2) cells. The result suggests that *V. cheiranthifolium* phytochemicals downregulate the activity of the pro-inflammatory enzyme, inducible nitric oxide synthase (iNOS), which results in a decrease of NO production. These findings warrant future research towards an understanding of the mechanism of anti-inflammatory action of *V. cheiranthifolium*.

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1. Introduction

Indigenous plants, used traditionally for food and medicine by local populations, are a valuable source of phytochemicals required to maintain good health. Included in main meals, salads, soups, decoctions or herbal teas they contribute through their array of health-enhancing properties, including anti-inflammatory, anti-proliferative, cytotoxic, antioxidant,

chemopreventative or anti-diabetic properties, among others (Kaisoon et al., 2012; Neerghen-Bhujum, 2013).

Mullein is the common name of the genus of *Verbascum*, comprising approximately 250 species of flowering plants, native to Europe and Asia. The highest species diversity exists in the Mediterranean region. Multiple species are commonly used as nutritional supplements and remedies (Tatli and Akdemir, 2006). For example, *Verbascum thapsus* L. (common mullein) is traditionally used in Turkey to treat pulmonary

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problems, inflammation, asthma, spasmodic coughs, diarrhoea and migraine headaches (Turker and Camper, 2002; Turker and Gurel, 2005).

Verbascum cheiranthifolium Boiss. var. *cheiranthifolium* Boiss. of the Scrophulariaceae family is a biennial endemic herb of the genus *Verbascum*, known in Eastern Anatolia as masicerk or sigirkuyruğu, represented in Turkey's flora by its 228 species. The slender stem is yellowish, 30–120 cm tall, with multiple branches. Basal leaves are linear-lanceolate to oblong, 7–30 cm × 1.5–8 cm, blunt to acuminate, entire or rarely crenate; the petiole is 2–6 cm; upper cauline lanceolate to broadly ovate and suborbicular, rounded or subcordate at base. Inflorescence has many slender, erect-spreading branches, forming oblong to ovate panicle, with loose clusters of 2–7 flowers. In Eastern Anatolia infusion and/or decoction prepared from leaves of *V. cheiranthifolium* are extensively used to cure rheumatism, menstrual pains and haemorrhoids (Dalar and Konczak, 2012, 2013).

Although *V. cheiranthifolium* is among the most extensively used botanical in the East Anatolia region of Turkey, research on this plant is limited. Infusion or decoction made from the leaf is a common remedy, applied internally or externally. This suggests that water soluble phytochemicals are the physiologically active compounds. Limited research data are available regarding health attributes of *V. cheiranthifolium* herbal tea. Previous studies carried out by our research group revealed that *V. cheiranthifolium* extract has a relatively high level of phenolic compounds, and inhibits the activities of selected digestive enzymes (Dalar and Konczak, 2013). In order to understand the mechanisms of the molecular action of herbs and their products, it is necessary that their phytochemical composition be explored. Therefore, the objective of this study was to understand the phytochemical composition of hydrophilic extracts of *V. cheiranthifolium* and examine its potential physiological activities (anti-proliferative and anti-inflammatory) within a life cell.

2. Methods

2.1. Plant materials

V. cheiranthifolium var. *cheiranthifolium* (Scrophulariaceae) leaves with no apparent physical damage were harvested at the Güzeldere Path, Başkale, Van City, from May to August, 2010. The collected leaves were sealed in clean polythene bags and brought to the laboratory within a maximum of 3 h after harvest. The identity of plant material was confirmed by Dr Ömer Bingöl, Biological Sciences Department, Science Faculty, Yüzüncü Yıl University, Turkey and a voucher specimen stored in the university's herbarium (Herbarium code: VANF-163750; Collector code: MM204). Subsequently, the leaves were washed thoroughly with distilled water to remove surface dust and were left at room temperature in the dark until dry. After drying, the samples were ground into a fine powder using a laboratory mill and were stored at -20°C until analyzed.

2.2. Reagents

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich, Inc. (Sydney, Australia) and were of analytical or HPLC grade. Acarbose was purchased as 'Glucobay' from Bayer (Bayer Australia Ltd., Pymble, NSW). 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. (NSW, Australia). Penicillin and streptomycin were purchased from Invitrogen (Melbourne, Australia).

2.3. Cell lines

The cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured at 37°C in a humidified 5% carbon dioxide (CO_2) atmosphere in media containing 10% foetal bovine serum (FBS; Invitrogen Corporation, Carlsbad, CA, USA), 100 $\mu\text{g}/\text{ml}$ streptomycin and 100 units/ml penicillin (Invitrogen Corporation, Carlsbad, CA, USA). Hepatocellular carcinoma (HepG2) cells were cultured in Eagle's minimum essential medium (EMEM; Sigma-Aldrich). Murine macrophage (RAW 264.7) cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Corporation, Carlsbad, CA, USA). Experiments were conducted using cell cultures with less than 40 passages.

2.4. Preparation of lyophilized extract

The ground plant material was mixed with a 20-fold volume of acidified ethanol (80% ethanol, 19% H_2O and 1% of 0.1% trifluoroacetic acid, v/v/v), shaken for 2 h at room temperature (22°C) and centrifuged for 20 min at $15,320 \times g$ (10,000 rpm) at 4°C (Sorvall RC-5B; DuPont, Wilmington, DE, USA; rotor Beckman JA14 (137 mm) serial No. 02U8152, USA) with the supernatant collected. The extraction was repeated once more. The supernatants from the consecutive extractions were combined and the solvent evaporated under reduced pressure at 37°C using a rotary evaporator (Rotavapor R-205; Buchi, Switzerland). The derived fraction was dissolved in a minimum amount (5 ml) of purified water (Synergy UV, Millipore, Australia) and freeze-dried under a vacuum at -51°C to obtain a fine lyophilized powder.

2.5. Antioxidant testing

The antioxidant capacity was evaluated by the Folin-Ciocalteu (total phenolics, TP) method and the ferric reducing antioxidant power (FRAP) assay to estimate the total reducing capacities as well as by the oxygen radical absorbance capacity (ORAC) assay to estimate the oxygen radicals scavenging capacities, as previously described (Dalar et al., 2012). Total phenolics were quantified as mg gallic acid equivalent per gram dry weight of lyophilized extract (mg GAE/g DW), the FRAP values were expressed as micromoles Fe^{2+} per gram dry weight ($\mu\text{mol Fe}^{2+}/\text{g DW}$) and the ORAC values were expressed

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