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Research paper

In vitro cytotoxic, antibacterial, anti-inflammatory and antioxidant activities and phenolic content in wild-grown flowers of common daisy—A medicinal plant

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ABSTRACT

Bellis perennis L. is a medicinal plant in the family Compositae. It has been used as a remedy for wounds, rheumatism, eczema, eye diseases, inflammation and tonsillitis in folk medicine. In the present study, 19 different extracts and two fractions were obtained from wild-grown flowers, leaves and/or *in vitro*-grown leaves of common daisy by using different solvents and extraction methods. Biological activities of these extracts and fractions were assessed using selected bioassays: cytotoxic activity, disc diffusion assay, radical scavenging activity (DPPH), total phenolic content, oxygen radical absorbing capacity (ORAC) and 2′,7′-dichlorofluorescin-diacetate (DCFH-DA) cell-based assays. The cytotoxic activity of extracts and fractions was investigated against human lung carcinoma (A-549) and colon adenocarcinoma (DLD-1) cells. *In vitro*-grown leaf extracts showed the highest cytotoxic activity against selected cell lines. Moreover, *n*-butanol (*n*-BuOH) and ethyl acetate (EtOAc) fractions of flowers exerted high levels of cytotoxic activity. The MeOH extract and the EtOAc fraction of flowers exhibited broad-spectrum antibacterial activity against *Streptococcus pyogenes*,*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterobacter cloacea*. The strongest antioxidant activity was found in the EtOAc fraction of flowers with the highest amount of phenolic content and ORAC value. The MeOH extract of flowers showed strong anti-inflammatory activity on RAW 264.7 macrophages. The amount of the chosen 22 phenolic compounds in dichloromethane (DCM), MeOH extracts, *n*-BuOH and EtOAc fractions of field-grown flowers was detected using LC–ESI–MS/MS. The results of these studies support the potential use of *B. perennis* for wounds, rheumatism, inflammation, cancer and eye diseases.

1. Introduction

Many plant materials have been used as herbal teas or other homemade remedies for many years in traditional medicine (Dzhambazov et al., 2002). They have been generous sources of medicines because they generate a host of secondary bioactive compounds; most of which presumably evolved as chemical defenses against bacterial infections (Cox and Balick, 1994). Determination of the biological activities of medicinal plants with bioassays is beneficial for the standardization and quality control of heterogeneous plant secondary metabolites (McLaughlin et al., 1998). Screening studies for medicinal plants are also important because folkloric usage of these plants gains some scientific justification.

Bellis perennis L. (common daisy) is a medicinal plant species in the Compositae family (Panda, 2004; Davis, 1975), which has been known as a popular wound-healing plant in Europe since ancient times (Karakas et al., 2012a). The aerial parts of *B. perennis* have been used for the treatment of rheumatism, (Morikawa et al., 2011), common cold (Cakılcıoglu et al., 2010) and headache (Uzun et al., 2004). They are utilized for their expectorant, sedative and anti-inflammatory activities (Siatka and Kasparova, 2010) in traditional medicine. Some traditional usages of *B. perennis*, such

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as for their wound healing (Karakas et al., 2012a) and sedative activities (Karakas et al., 2011), have been verified with controlled experimental animal studies. The aerial parts (especially flowers) of *B. perennis* have been also used as an herbal tea against breast and uterus cancer (Duke et al., 2002). Li et al. (2005) indicated the cytotoxic activities of six triterpenoid saponins from the roots of *B. perennis* against HL-60 human promyelocytic leukemia cells. Furthermore, Karakas et al. (2015) showed the moderate antiproliferative activity of MeOH extract obtained from *B. perennis* flowers on MCF-7 human breast cancer and HepG2/C3A human hepatocellular carcinoma cells.

Although common daisy has many areas of usage in traditional medicine, there are no reports of some of the important biological activities for field-grown flowers, leaves and *in vitro*-derived leaves in the literature. In the present study, the cytotoxic activity of wild-grown leaf, *in vitro*-grown derived leaf, and flower extracts was investigated against human lung cancer (A-549) and human colon cancer (DLD-1) cell lines with the resazurine reduction test. The antibacterial activity of *B. perennis* extracts and fractions was evaluated against ten different bacterial strains. The antioxidant activity of 12 different *B. perennis* extracts [with hexane, dichloromethane (DCM), MeOH and water] of wild-grown flowers, leaves and *in vitro*-derived leaves was examined using four different antioxidant methods; free radical scavenging activity (DPPH), total phenolic content (Folin-Ciocalteau), oxygen radical absorbing capacity (ORAC) and direct antioxidant assays [cell-based assay using 2′.7′-dichlorofluorescin-diacetate (DCFH-DA)]. Additionally, the phenolic content of wild-grown flowers of *B. perennis* was detected by LC–ESI–MS/MS analysis. The current research indicates, to our knowledge for the first time, the antiproliferative, antibacterial and antioxidant activities of *B. perennis* using different extracts obtained from different extraction methods, different plant parts and sources, and bioassays.

2. Materials and methods

2.1. Plant material and extract preparation

Aerial parts (flowers and leaves) of wild-grown *B. perennis* were collected from Gölköy, Bolu/Turkey in May 2011. Identification of the species was made by Prof. Dr. Arzu Ucar Turker (collection number AUT-1909) and the specimen was deposited at Abant Izzet Baysal University (AIBU) Herbarium, Bolu, Turkey. *In vitro*-derived leaves of *B. perennis* were collected from plantlets that were propagated with established protocol in plant tissue culture laboratory (Karakas and Turker, 2013). Three different sources of plant (wild-grown flowers, wild-grown leaves and *in vitro*-derived leaves) were dried in an incubator at 37 °C for three days. Five different types of extraction methods (soxhlet, water bath, decoction, infusion and liquid-liquid extraction) were performed for flowers, and the Soxhlet extraction method was used for wild-grown leaves and *in vitro*-derived leaves.

2.1.1. Soxhlet extraction

Three different plant materials [wild-grown flowers (70 g), wild-grown leaves (70 g) and *in vitro*-derived leaves (55 g)] were weighed for Soxhlet extraction. The dried powdered plant parts from *B. perennis* were alternately extracted with hexane (at 65–70 °C), DCM (at 55–60 °C), MeOH (at 60 °C) and water (at 80 °C) using a Soxhlet apparatus (700 ml of each solvent for 24 h). The extracts were filtered and pooled.

2.1.2. Water bath extraction

The powdered flowers of *B. perennis* (20 g) were extracted with hot water (250 ml), cold water (250 ml), ethanol (EtOH; 200 ml), methanol (200 ml) or acetone (200 ml) in a water bath at 45 °C for 12 h and then filtered.

2.1.3. Decoction

The powdered flowers of *B. perennis* (10 g) were boiled in 100 ml of distilled water for 1 h and the obtained decoction was filtered. The same procedure was repeated three times and the results of the three successive extractions were collected and combined.

2.1.4. Infusion

Pour 100 ml boiling distilled water over 10 g of powdered flowers of *B. perennis,* mix and leave for 1 h at room temperature; the resulting infusion was filtered and the procedure was repeated three times as described above. Crude aqueous extracts were lyophilized.

2.1.5. Liquid-liquid extraction

The powdered flowers of *B. perennis* (400 g) were comprehensively extracted with MeOH (3:l, 60 °C, three times, 1.5 h each time) followed by MeOH-H_2O 80:20 with heating. The extracts were filtered and pooled. After evaporation of MeOH in vacuum, the aqueous phase was extracted successively with DCM (3 × 500 ml), EtOAc (5 × 500 ml) and saturated *n*-butanol (*n*-BuOH) with H_2O (60:40; 5 × 500 ml) using liquid-liquid extraction. The *n*-BuOH and EtOAc phases were separated and decanted.

Aqueous extracts were evaporated using a lyophilizator at −55 °C. All other solvents were evaporated under low pressure at 40 °C using a rotary evaporator. The designation of the extracts, name of the extracts, part used, and name of the extraction method for each extract are given in Table 1.

2.2. Human cancer cell lines and culture conditions

The human lung carcinoma (A-549), colon adenocarcinoma (DLD-1) and normal skin fibroblast (WS-1) cell lines were provided from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cell lines were cultured in Dulbecco's minimum essential medium (DMEM) with Earle's salts. A 10% fetal bovine serum, a solution of vitamins, sodium pyruvate and non-essential amino acids (all at a 1:100 v/v dilution of supplied solutions), penicillin (100 IU/ml) and streptomycin (100 μg/ml) were added to the culture medium. Cells were maintained at 37 °C in a humidified environment containing 5% CO_2 (Legault and Pichette, 2007).

2.3. Cytotoxicity by resazurin assay

The cytotoxic activity of extracts obtained from wild-grown flowers, wild-grown leaves and *in vitro*-grown leaves was analyzed using resazurin on an automated 96-well Fluoroskan Ascent F1™ plate reader (Labsystems) as described by O'Brien et al. (2000). Etoposide was used as a positive control. Cytotoxic activities of tested extracts were showed as means ± standard deviation and indicate the concentration inhibiting 50% of cell growth (IC_{50}). Each study was carried out three times in triplicate.

2.4. Antibacterial activity assay

The disc diffusion assay (Kirby-Bauer Method) was used to evaluate the antibacterial activities of 19 different extracts obtained from *B. perennis*, with some modifications (Karakaş et al., 2012b). Ten bacterial strains were used in the bioassay: the Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 14028), *Serratia marcescens* (ATCC 8100), *Proteus vulgaris* (ATCC 13315), *Enterobacter cloacae* (ATCC 23355) and *Klebsiella pneumoniae* (ATTC 13883), and the Gram-positive bacteria *Streptococcus*