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An antioxidant and antimicrobial activities of Inula helenium

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ABSTRACT: - The antioxidant activities of methanol (ME), ethanol (EE), water (WE) and ethyl acetate (EAE) extracts of four Inula helenium L. taxa (I. helenium ssp. orygalis (Boiss.) Grierson, I. helenium ssp. pseudohelenium Grierson, I. helenium ssp. turcarasemosa Grierson and I. helenium ssp. vanensis Grierson) were investigated. The phosphomolybdenum assay, β-carotene–linoleate bleaching and DPPH radical scavenging activity were used to evaluate the antioxidant capacity. The total phenolic contents determined by Folin-Ciocalteu assay of the extracts ranged from 4.18 to 102.91 mg gallic acid equivalents (GAE)/g dry extract. The extracts showed considerable effect on reducing the oxidation of β-carotene. The highest radical scavenging activity was obtained for ME of I. helenium ssp. orygalis in DPPH assay. ME, EE and EAE of four Inula helenium taxa showed significant antibacterial activity against 13 bacteria tested. WEs had no inhibitory effect against bacteria tested except for I. helenium ssp. orygalis. EE of I. helenium ssp. orygalis was only effective against C. albicans.

Keywords: Inula helenium; antimicrobial activity; antioxidant activity; DPPH

I INTRODUCTION

Phenolics are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants (Dai and Mumper, 2010). Plant polyphenols comprise a diversity of compounds, among which flavonoids and several classes of nonflavonoids are usually distinguished. More than 4000 flavonoids have been identified in plants, and the list is constantly growing (Cheynier,2005). Flavonoids have many diverse functions including defense, UV protection, auxin transport inhibition, allelopathy, and flower coloring (Buer,2010). They have been reported to possess many useful properties, including anti-inflammatory, oestrogenic,enzymeinhibition,antiallergic, antioxidant, vascular and cytotoxic antitumour activities (Buer, 2010; Cushnie and Lamb, 2005). The antimicrobial effects of polyphenols have also been widely reported and there is an increasing interest in this topic because plant polyphenols could represent a source of new anti-infective agents against antibiotic-resistant human pathogens (Ferrazzano et al., 2011).

Oxygen metabolism continuously generates small amounts of reactive oxygen species (ROS). ROS are normally produced during physiologic processes such as cellular respiration and inflammatory defense mechanisms. Generation of ROS beyond the antioxidant capacity of a biological system gives rise to oxidative stress. Oxidative stress plays a role in the pathogenesis of several diseases. Increasingly, plant polyphenols are becoming the subject of medical research due to their marked effects in the prevention of various oxidative stress associated diseases such as cancer.

II MATERIALS AND METHODS

2.1. Chemicals

Folin-Ciocalteu reagent, DPPH, sodium carbonate, gallic acid, ascorbic acid, nutrient agar, nutrient broth, malt extract agar and malt extract broth were purchased from Merck (Darmstadt, Germany). The other chemicals And solvents used in this experiment were analytical grade, also purchased from Merck.

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2.2. Plants

The collection information of the I. helenium taxa, which are individually numbered, is listed below:1. I. helenium ssp. orgyalis, between Eflani and Daday,Kastamonu,1137m,41^{O}26'28"N–33^{O}17'65"E, 05.07.2011 (MYP 872). (E) 2. I. helenium ssp. pseudohelenium, at Kırşehir- Çiçekdağ crossroads, towards the Çiçekdağ, 2.km,Kırşehir,1070m,39^{O}17'21"N–34^{O}07'25"E,06.09.2010 (MYP 950).
They were identified by senior taxonomist Prof. Dr. Ahmet AKSOY from Akdeniz University, Department of Biology. The voucher specimens were deposited at the Herbarium of the Department of Biology, Erciyes University, Kayseri, Turkey. I. helenium ssp. orgyalis and I. helenium ssp. vanensis are endemic (E) to Turkish flora (Davis, 1975).

2.3. Extraction

Dried aerial parts of the plants at room temperature were crushed in a coffee grinder for 2 min. At 15 s intervals, the process was stopped for 15 s to avoid over-heating the sample. Powdered plant samples (10 g) were separately extracted using a Soxhlet type extractor with 100 mL methanol,ethanol,water and ethyl acetate.

III DETERMINATION OF ANTIOXIDANT ACTIVITY

3.1 Phosphomolybdenum assay

The antioxidant activities of the plant extracts were determined by the phosphomolybdenum method of Prieto et al. (1999). 0.4 mL of the plant extract (1 mg/mL) was mixed with 4 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95 ^{O}C for 90 min. After the samples had cooled to room temperature, the absorbance of the green phosphomolybdenum complex was measured at 695 nm. In the case of the blank, 0.4 mL of solvent was used in place of the sample. The antioxidant activity was determined using a standard curve with ascorbic acid solutions as the standard. The mean of three readings was used, and the reducing capacities of the extracts were expressed as mg of ascorbic acid equivalents (AAE)/g extract.

3.2 β-Carotene bleaching assay

The extracts ability to inhibit the bleaching of the β-carotene–linoleic acid emulsion was determined (Cao et al 2009). β-carotene (10 mg) was dissolved in 10 mL of chloroform ($CHCl_3$). An aliquot (0.2 mL) of this solution was added to a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed using a rotary evaporator at 40 ^{O}C for 5 min. Distilled water (50 mL) was slowly added to the residue and mixed vigorously to form an emulsion. The emulsion (5 mL) was added to a tube containing 0.2 mL of the extract solution. The test emulsion was incubated in a water bath at 50 ^{O}C for 2h, at which point the absorbance was measured at 470 nm. In the negative control, the extract was substituted with an equal volume of ethanol. BHT (Butylated hydroxytoluene) was used as the positive control.

3.3 DPPH Assay

The hydrogen atom or electron donation abilities of the plant extracts were measured by bleaching the purple coloured DPPH (2,2-diphenyl-1- picrylhydrazyl) methanol solution.This spectrophotometric assay uses the DPPH stable radical as a reagent (Lee et al 1998). Fifty microliters of various concentrations (0.1-2 mg/mL) of the plant extract in the same solvent were added to 1 mL of 0.1 mM DPPH methanol solution. After a 30 min incubation period at room temperature, absorbance was read against a blank at 517 nm. IC_{50} (the concentration required to scavenge 50% DPPH free radicals) values of the plant extracts were determined graphically. The same procedure was repeated with BHT as a positive control. The measurements were performed in triplicate, and the results were averaged.
Radical scavenging activity was expressed as a percentage inhibition of the DPPH radical and was calculated by the

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following equation:
Inhibition% = (A_{blank} – A_{sample} /A_{blank}) x 100
where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

Salmonella typhimurium NRRLE 4463, Staphylococcus aureus ATCC 29213, Yersinia enterocolitica ATCC 1501, Candida albicans ATCC 1223 and Saccharomyces cerevisiae BC 5461.

3.4 Agar-well diffusion method

Antimicrobial activity assays of the extracts were carried out using the agar-well diffusion method (Sagdic et al 2009). Each microorganism was suspended in sterile nutrient broth. Test yeasts (C. albicans, S. cerevisiae) were suspended in malt extract broth. Suspensions of microorganisms, adjusted to 10^6-10^7 colony-forming units (cfu)/mL, were placed in flasks containing 25 mL of sterile nutrient or malt extract agar at 45 ^{o}C. The mix was poured into Petri plates (9 cm in diameter). The agars were then allowed to solidify at 4 ^{o}C for 1 h. The wells (5 mm in diameter) were cut from the agar. The extracts were prepared at 1%, 2.5%, 5% and 10% concentrations in the same solvent and 40 µL of the extract solutions were then applied to the wells. The absolute methanol, absolute ethanol, water and ethyl acetate without herb extract were used as a control. Y. enterocolitica, C. albicans and S. cerevisiae were incubated at 25 ^{o}C for 24-48 h in an inverted position. The other microorganisms were incubated at 37 ^{o}C for 18-24 h. At the end of the incubation period, all plates were examined for any zones of growth inhibition, and the diameters of these zones were measured in millimeters.

3.5 Statistical analysis

SAS (1988) statistical software was used for data analysis. The comparative analyses between means were conducted using the Tukey multiple range test. Data were subjected to analysis of variance (Two-way ANOVA). Bivariate correlations were analyzed by Pearson's test using SPSS 10.0 (1965) on Windows.

IV RESULTS AND DISCUSSION

This study determined the antioxidant and antimicrobial activities of the extracts (ME, EE, WE and EAE) of four I. helenium taxa growing Turkish flora. The percentage yields of extracts ranged from 1.53 to 27.66 (w/w) (Table 1). Among the extracts of plants, WEs had highest yields while EAEs had lowest yields.

V CONCLUSION

The results of the present study showed that the extracts of four I. helenium taxa, of which two are endemic, contain high amount of phenolic compounds and exhibited the clear antioxidant and antimicrobial activity. Total phenolic contents, antioxidant and antimicrobial activities of I. helenium ssp. turcarasemosa, I. helenium ssp. vanensis, I. helenium ssp. pseudohelenium, I. helenium ssp. orygalis growing in Turkey have been reported here for the first time. Hence, I. helenium taxa could be a good source of antioxidant and antimicrobial agents in foods, pharmaceuticals and cosmetic preparations. Further studies are warranted for the isolation and also in vivo studies are need for better understanding their mechanism of action as antioxidant and antimicrobial agents.

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