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Antimicrobial, antioxidant and antimutagenic properties of *Iris albicans*

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ABSTRACT

This study investigated the antimicrobial, antioxidant and antimutagenic properties of ethanolic extracts of the aerial parts and rhizomes of *Iris albicans Lange*. While previous studies have examined the antimicrobial and antioxidant properties of *I. albicans*, to our knowledge, this is the first study to report on the antimutagenic activity of this plant.

Both aerial part and rhizome extracts exhibited limited antimicrobial activity against *Bacillus subtilis* ATCC 6633. IC_{50} values for the radical scavenging activity of the extracts were 8.8 and 11.1 mg/ml, respectively. Total antioxidant activity of the extracts (at 3.15 mg/ml) was 96.6 ± 0.07% and 97.2 ± 0.7%, respectively.

Antimutagenity was assessed using the Ames *Salmonella*/microsome mutagenicity test with the bacterial mutant strains *Salmonella typhimurium* TA98 and TA100. Antimutagenic activity was observed for the aerial part extract at 0.3 and 3 mg/plate concentrations and for the rhizome extract at 0.015, 0.15, and 1.5 mg/plate concentrations.

In sum, ethanolic extracts of *I. albicans* were found to possess antioxidant and antimutagenic properties that could have potential value in the field of medicine and industry; however, only limited antimicrobial activity was observed.

1. Introduction

Natural products represent a major source of chemical diversity that includes potentially innovative therapeutic agents for treating various conditions, including infectious diseases (Clardy and Walsh, 2004). It is estimated that two-thirds of the world's population continues to rely mainly on traditional plant-based medical remedies due to the limited availability and affordability of pharmaceutical medicines (Tagboto and Townson, 2001). In contrast to chemical drugs, medicinal preparations based on natural products present few side effects because they are able to maintain a biological balance without accumulating in the body (Zaman, 2005).

Avoiding exposure to or ingestion of mutagens and carcinogens is the best way to reduce the chance of mutation and, given the role of mutation in carcinogenesis, possibly the incidence of cancer (Kim et al., 2000). In spite of this, much attention has been focused on the chemoprevention of cancer, albeit with little success, whereas relatively little attention has been given to analyzing the protective role of substances found in medicinal plants (Shon et al., 2004). Many plant species, including herbs, exhibit preservative effects that suggest their tissues harbor antioxidative and antimicrobial constituents (Hirasa and Takemasa, 1998). Various plant species have also demonstrated antimutagenic properties that suggest a full range of prospective applications in human health (Ghazali et al., 2011).

Antioxidants are believed to play an important role in preventing the development of a wide variety of chronic diseases, including cancer, heart disease, stroke, Alzheimer's, rheumatoid arthritis and cataracts (Devare et al., 2012). Natural antioxidants, either in the form of raw extracts or their chemical constituents, have proven very effective in inhibiting destructive processes initiating from oxidative stress (Zengin et al., 2011).

Flavonoids are a large group of polyphenolic compounds that are ubiquitously present in plants as secondary metabolites. Recent studies have reported these compounds to be responsible for a variety of biopharmacological activity (Mahomoodally et al., 2005; Pandey, 2007). For example, isoflavanoids, a type of flavonoid, have

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been found to exhibit antioxidant, antibacterial, antiviral and anti-inflammatory activity (Cornwell et al., 2004; Dastigar et al., 2004). According to studies by Kumar and Pandey (2013) and Kumar et al. (2013) the antioxidant activity of flavonoids is mediated by the free-radical-scavenging and/or metal–chelating of functional hydroxyl groups within these compounds.

The iris (*Iridaceae*) plant is found throughout Eurasia, North Africa and North America (Rigano et al., 2009). Phytochemical studies have demonstrated the iris species to be rich in isoflavonoids (Wu and Xu, 1992; Farag et al., 1999), flavonoids (Farag et al., 1999), C-glycosylxanthones (Farag et al., 1999), quinones (Seki et al., 1994) and iridal triterpenoids (Krick et al., 1983).

Iris albicans Lange – known colloquially as cemetery iris, white cemetery iris, or white flag iris (and *cehennem zambağı* or *mezarlık zambağı* in Turkish) (Mathew, 1984) – is believed to have originated in Yemen and Arabia and is commonly found in many parts of the Mediterranean (Williams et al., 1997), including Turkey (Mathew, 1984). A phytochemical study by Mothana et al. (2010) demonstrated *I. albicans* to be rich in isoflavonoids and flavonoids.

Previous studies have evaluated the antimicrobial (Mothana et al., 2010), antioxidant (Mothana et al., 2010; Hacıbekiroğlu and Kolak, 2012) and cytotoxic (Mothana et al., 2010) activities of *I. albicans*. However, to date, there is no study in the literature reporting on the antimutagenic effects of *I. albicans*. Therefore, this study investigated the antimicrobial, antioxidant and antimutagenic activity of ethanolic extracts of the aerial parts and rhizomes of *I. albicans* in an effort to facilitate their use in phytomedicine and the pharmaceutical industry.

2. Materials and methods

2.1. Plant material

Aerial parts and rhizomes of *I. albicans* were collected from Mugla, Turkey. Specimens were air-dried at room temperature for 7 days, and stored for analysis at a later date. A voucher specimen was taxonomically identified by Mehtap Donmez Sahin and deposited in the University of Usak Herbarium in Uşak, Turkey (Herbarium No: 1115).

2.2. Preparation of ethanolic extracts

Air-dried plant samples were extracted with ethanol (Merck) using a Soxhlet apparatus. Extracts were evaporated, extracted in ethanol/water (1:1, v/v) and stored in sterile opaque glass bottles under refrigerated conditions until used.

2.3. Microbial strains

Bacillus subtilis ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10239 were used in determining antimicrobial activity. *Salmonella typhimurium* TA98 and TA100 strains were used in determining mutagenicity and antimutagenicity. The bacterial stock cultures were inoculated in nutrient broth and incubated at 37 °C for 12–16 h with gentle agitation prior to use (Oh et al., 2008).

2.4. Antimicrobial activity

Antimicrobial activity of the extracts was evaluated using standard paper disc-diffusion assays as detailed elsewhere (Collins et al., 1995; Murray et al., 1995; Ugur et al., 2010). Zones of inhibition against the tested microorganisms were measured for both the aerial part and rhizome extracts at 100 mg/ml. Ethanol/water (1:1, v/v) was used as a negative control, and penicillin (10 U), ampicillin (10 μg), amoxicillin + clavulanic acid (20 μg/10 μg), imipenem (10 μg), cefoperazone (75 μg) and nystatin (30 μg) discs were used as positive controls.

2.5. Antioxidant activity

2.5.1. DPPH free-radical-scavenging activity

Antioxidant activity of the extracts was determined based on their ability to react with stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radicals (Yamasaki et al., 1994). Aerial part and rhizome extracts were prepared at concentrations of 1.25, 2.5, 5 and 10 mg/ml in 1:1 ethanol/water (v/v), and 50 μl of each preparation was added to 5 ml of DPPH solution (0.004% in ethanol). Solutions were incubated at room temperature for 30 min. For each solution, absorbance at 517 nm was measured, and percentage of inhibition and the concentrations required to scavenge 50% of DPPH (IC_{50}) were calculated. BHT and ascorbic acid were used as positive controls.

2.5.2. Total antioxidant activity (β-carotene-linoleic acid assays)

Total antioxidant activity was evaluated using β-carotene-linoleic acid assays (Jayaprakasha and Jaganmohan Rao, 2000). A total of 0.5 mg β-carotene dissolved in 1 ml chloroform was mixed together with 25 μl of linoleic acid and 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate). The chloroform was completely evaporated using a vacuum evaporator, and the resulting solution was diluted with 100 ml of oxygenated water. For each extract, a 2.5 ml aliquot was transferred to a separate test tube containing a 0.5 ml sample at a concentration of 3.15 mg/ml in 1:1 ethanol/water (v/v). The same procedures were repeated with BHT (0.5 mg/ml), ascorbic acid (0.5 mg/ml) and a 1:1 ethanol/water (v/v) blank. Tubes were incubated at 50 °C, and absorbance was measured every 30 min until the color of the β-carotene disappeared in the control (2 h). Following incubation, absorbance of all solutions was measured at 490 nm.

The bleaching rate (R) of β-carotene was calculated using the formula $R = \ln(a/b)/t$ where, ln = natural log, a = absorbance at time 0 and b = absorbance at time t (120 min). Antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using the formula $AA = [(R_{Control} - R_{Sample})/R_{Control}] \times 100$.

2.6. Mutagenic and antimutagenic activity

Mutagenicity and antimutagenicity of the extracts were evaluated using the Ames *Salmonella*/microsome mutagenicity assay performed with the mutant strains *S. typhimurium* TA98 and TA100. Both strains were analyzed according to Mortelmans and Zeiger (2000) for histidine and biotin requirements alone and in combination, rfa mutation, excision repair capability, presence of plasmid pKM101 and spontaneous mutation rates.

2.6.1. Viability assays and determination of test concentrations

Cytotoxic doses of the extracts were determined according to Mortelmans and Zeiger (2000). The toxicity of the extracts toward *S. typhimurium* TA98 and TA100 was determined as described in detail elsewhere (Santana-Rios et al., 2001; Yu et al., 2001).

2.6.2. Mutagenicity and antimutagenicity tests

Sub-cytotoxic doses (aerial part extracts at 0.03, 0.3, and 3 mg/plate and rhizome extracts at 0.015, 0.15, and 1.5 mg/plate) were used to assess *I. albicans* mutagenicity and antimutagenicity. Extracts were examined using the plate incorporation method (Maron and Ames, 1983) described in detail by Sarac and Sen (2014). The known mutagens 4-nitro-o-phenylenediamine (4-NPD) 3 μg/plate) and sodium azide (NaN_3) (8 μg/plate) were used as positive controls for *S. typhimurium* TA98 and *S. typhimurium*