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Full Length Research Paper

Antioxidant and antimicrobial activities of extracts from tubers and leaves of *Colchicum balansae* Planchon

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In this study, we examined the antioxidant and antimicrobial properties of *Colchicum balansae* Planchon (CB). The solvent extracts were prepared from CB tubers and leaves. In addition, free radical scavenging activities were also determined. Result of this study show that leaves extracts of CB exhibited higher antioxidant activity than tuber extracts with all types of solvent. The highest antioxidant activity efficiency was determined in extract leaf-ethanol (64%) and the least efficiency in extract tuber-benzine (14.5%). All extracts of CB tubers and leaves have effective free radical scavenging and reducing power. The highest free radical scavenging activity was determined in extract leaf-benzine (68.35%), this activity was followed by acetone (61.23%), methanol (58.67%) and ethanol extracts (54.74%) respectively. The highest radical scavenging activity was determined in extract tuber-benzine (61.28%), and the least efficiency in extract tuber-ethanol (20.48%). In addition, all extracts of CB tubers and leaves were determined as pyrocatechol equivalents. The results showed that *Colchicum* ethanol extract had a weak inhibitory effect against tested bacteria. *S. aureus* ATCC 25923 was more sensitive against ethanol extract (10 mm inhibition zone). When comparing the antimicrobial activity of the control antibiotics, the ethanol extract exhibited lower antimicrobial activity.

Key words: Antimicrobial, antioxidant, *Colchicum balansae*, radical scavenging activity.

INTRODUCTION

Free radicals are responsible for aging and causing various human diseases. A study shows that antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases. By donating hydrogen radicals, the primary radicals are reduced to nonradical chemical compounds and are then converted to oxidize antioxidant radicals (Jadhav et al., 1995; Yamaguchi et al., 1998). This action helps in protecting the body from degenerative diseases. Epidemiological studies have shown the beneficial effects of diets rich in vegetables, fruits and grain products in reducing the risk of cardiovascular disease and certain cancers (Beecher, 1999). The principal agents responsible for the protective effects could be the presence of antioxidant substances that exhibit their effects as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers and metal ion chelators (Okawa et al., 2001).

Antioxidant activity is important in view of the free radical theory of aging and associated diseases (Lee et al., 2000). Geophyte species are often considered as a source of carbohydrates Unlike seed storage proteins, very little is known about the reserve proteins of the underground storage organs of geophytes (Gulmaraes et al., 2001). In addition to their storage function, proteins can play additional roles in the plant, e.g. enzymatic (Tonon et al., 2001; Rosahl et al., 1987), inhibitory (Yeh et al., 1997; Hou et al., 1999), antibacterial (Flores et al., 2002) or antifungal ones (Flores et al., 2002, Terras et al., 1993). The storage proteins can be defined as proteins whose major role is to act as stores of nitrogen, sulphur and carbon (Shewry, 2003).

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Many kinds of alkaloids have been identified in *Colchicum* and *Merendera* plants. The major alkaloid of *Colchicum* is colchicine. The use of colchicine for treatment of gout was propounded by different researchers. Moreover, colchicine has an inhibitory effect on the growth of certain tumours in plant and animals. Colchicine acts on the mitotically active cell producing metaphasic arrest, often resulting in a doubling of the chromosome number and giving rise to polyploids. *Colchicum* species contain poisonous alkaloids, such as colchicine. When these poisonous alkaloids are accidentally ingested by humans and animals, they cause very serious health problems such as serious liver damage and finally death. All parts of *Colchicum* species have been shown to contain colchicine, but seeds and corms contain more colchicine than other plant parts. Numerous studies have been carried out by different researchers on colchicine and other chemical constituents of *Colchicum* species (Düşen and Sümbül, 2007).

MATERIAL AND METHOD

Plant materials and chemicals

Different parts (leaves and tubers) of *Colchicum balansae* were collected from the natural environment of Muğla province from Turkey in October 2008. *Colchicum* specimens were dried according to standard herbarium techniques and preserved in the Pamukkale University Herbarium (PAMUH).

Tween-20, methanol, ethanol, benzene, acetone, β-carotene, chloroform, linoleic acid were obtained from E. Merck (Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) were obtained from sigma Chemical Co. (St. Lois, MO). All other chemicals and solvents were analytical grade.

Extraction of sample

After the plant is collected when it has flowered, its tubers and leaves were dried, chopped up with a blender, and prepared for the experiment. In this study 10 g of the plant and 100 ml of solvent (Merck) were used for every sample (Darwish et al., 2002). These extractions were prepared using different solvents (methanol, ethanol, acetone and benzine). The mixture was extracted after being heated in a vibrating water bath at 55ºC. The extract obtained was filtered through filter paper (Whatman No: 1), and the solvents were evaporated in a rotary evaporator at 48 – 49°C. The water in each extract was frozen in Freeze-drying machine and then drawn out.

Measurement of antioxidant activity

β-Carotene bleaching assay

Total antioxidant activity of extracts *C. balansae* Planchon and standards (BHT) was measured according to the method of Velioglu et al. (1998), Lu and Foo (2000) and Amin and Tan (2002). One mililitre of β-carotene solution (0.2 mg/ml chloroform) was pipetted into a round-bottom flask (50 ml) containing 0.02 ml of linoleic acid and 0.2 ml of 100% Tween 20. The mixture was then evaporated at 40°C for 10 min by means of a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100 ml of distilled water. The distilled water was added slowly to the mixture with vigorous agitation to form an emulsion. For control, 0.2 ml of solvent (methanol-70%, ethanol-70%, acetone and benzine) was placed in test tubes instead of the extract. The tubes were then gently mixed and placed at 45°C in a water bath for 2 h. Absorbance of the samples was measured at 470 nm using a spectrophotometer (Shimadzu UV–1601, Japanese) at initial time (t=0) against a blank, consisting of an emulsion without β-carotene. Standards at the same concentration with samples were used as comparison. 0.2 ml of 70% methanol, 70% ethanol, acetone and benzine in 5 ml of the above emulsion was used as the control. The measurement was carried out at 30 min intervals. All determinations were performed in triplicate.

Antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene by using a slightly modified version of the formula from Jayaprakasha et al. (2001). According to Jayaprakasha, Singh and Sakariah (2001), the time for A_t and A°_t were at 180 min. In this method, as the absorbance was measured at 120 min, A_t and A°_t were at 120 min.

$$AA = \left[1 - \frac{A_0 - A_t}{A^{\circ}_0 - A^{\circ}_t}\right] x\,100$$

where A_0 and A°_0 are the absorbance values measured at initial time of the incubation for samples and control respectively, while A_t and $A^{½}_t$ are the absorbance values measured in the samples or standards and control at t = 120 min.

Free radical scavenging activity

Effect of *C. balansae* Planchon extracts on DPPH radical was measured based on the method modified by Lu and Foo (2000) and Lai et al. (2001). An aliquot of 200 µl of *C. balansae* Planchon extract (0.62 - 4.96 mg/ml) and BHT (0.04 - 1.28 mg/ml) were mixed with 800 µl of 100 mM Tris - HCl buffer (pH 7.4). The mixture was then added to 1 ml of 500 µM DPPH. This was made up to a DPPH final concentration of 250 µM. The mixture was shaken vigorously and left to stand at room temperature for 30 min in a dark room.

Absorbance at 517 nm was measured using a UV-spectrophotometer until the reading reached a plateau. The capability of *C. balansae* Planchon extracts to scavenge the DPPH radical was calculated by using the following equation:

$$\text{Scavenging effect \%} = 1 - \left(\frac{A_t\ 517\ nm}{A_o\ 517\ nm}\right) x\,100$$

IC50 value was determined from the plotted graph of scavenging activity versus the concentration of *C. Balansae* Planchon extracts,

Table 1. Antimicrobial activity of *Colchicum balansae* Planchon ethanol extract against the bacterial strains tested based on disc diffusion method.

Bacteria Species	E (mm)	A (mm)	N
Staphylococcus aureus ATCC 25923	10.00	30.00 (P) [a]	-
Staphylococcus epidermidis ATCC 12228	-	19.50 (VA) [b]	-
Enterococcus faecalis ATCC 29212	8.00	23.00 (VA)	-
Klebsiella pneumoniae ATCC 13883	8.00	29.00 (CAZ) [c]	-
Escherichia coli ATCC 25922	9.00	23.00 (AMC) [d]	-
Enterobacter cloacae ATCC 23355	9.00	34.00 (MEM) [e]	-
Serratia marcescens ATCC 8100	-	33.00 (MEM)	-
Proteus vulgaris ATCC 13315	-	40.00 (FEP) [f]	-
Pseudomonas aeruginosa ATCC 27853	-	30.00 (MEM)	-
Salmonella typhimurium ATCC 14028	-	25.00 (AMP) [g]	-

E: Extract, **A:** Antibiotic, **N:** Negative control; **a.** Penicillin G (10 units), **b.** Vancomycin (30 µg), **c.** Ceftazidime (30 µg), **d.** Amoxycillin / Clavulanic acid 2:1 (30 µg), **e.** Meropenem (10 µg), **f.** Cefepime (30 µg), **g.** Ampicillin (10 µg).

which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out and their activity was calculated by the percentage of DPPH scavenged.

Antimicrobial activity tests

Disc diffusion method

The standard disc diffusion method recommended by CLSI (CLSI 2006) was used to determine the antimicrobial properties of *Colchicum* ethanol extract. Bacteria species were first inoculated into Blood Agar (Merck KGaA, Darmstadt, Germany) and incubated over-night at 37°C and checked for purity. Then all bacteria suspensions were prepared in 0.9% NaCl solution as 0.5 McFarland (1x 10^8 cells per ml, BioMérieux, Marcy l'Etoile, France) standard density. Prepared bacteria suspensions were spread on Mueller Hinton Agar (Merck KGaA, Darmstadt, Germany) by sterile cotton swab. 20 µl of the ethanol extract was impregnated into standard empty antibiotic discs (6 mm in diameter), then they were placed on the plates one by one. Standard antibiotic discs, recommended by CLSI, that are suitable for microorganisms, were placed into the same plates as positive controls. An empty disc was used to test if it was sterile or not. Bacteria species were incubated at 37°C for 24 h. The diameters of the inhibition zones were calculated as millimetres. Each assay was performed three times.

RESULTS AND DISCUSSION

The results of total antioxidant activity and radical scavenging

A great number of plants have been studied with respect to their antioxidant activity. The highest antioxidant activitiy (64%) were observed on leaves extracts with ethanol of *Colchicum balansae,* also the lowest antioxidant activitiy was observed (14.5%) corms with extract in Benzine. The reason of the same plant's extracts showing different antioxidant activitiy can be polarities of the solvents (Figure 1).

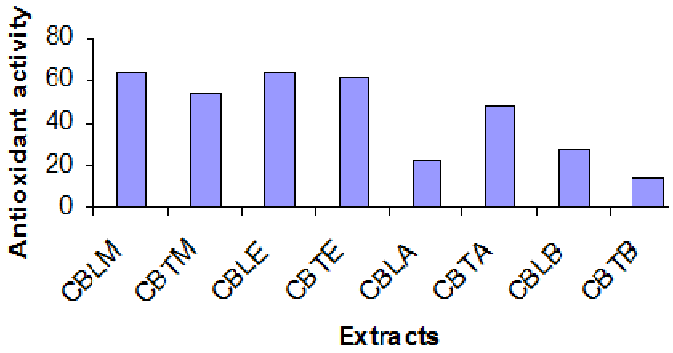

Figure 1. The antioxidant activities in the methanol, acetone, benzine and ethanol extracts; *Colchicum balansae* (CB), Tuber-methanol (CBTM), Tuber-ethanol (CBTE), Tuber-acetone (CBTA), Tuber-benzine (CBTB), Leaf-methanol (CBLM), Leaf-ethanol (CBLE), Leaf-acetone (CBLA) and Leaf-benzine (CBLB).

The highest free radical scavenging activity (68.35%) was recorded on *Colchicum balansae* leaves extracted with Benzine, following free radical scavenging activities are acetone (61.48%), methanol (58.67) and ethanol (54.74). On tubers the highest free radical scavenging activity is extracts with Benzine (61.28%), the lowest activity was observed with ethanol (20.48%) (Figure 2).

The results of antimicrobial activity

The antimicrobial activity of *Colchicum* L. ethanol extract was evaluated by the disc diffusion method against ten bacterial strains. The results presented in Table 1 showed that *Colchicum* ethanol extract had a weak inhibitory effect against tested bacteria. *S. aureus* ATCC 25923 was more sensitive against ethanol extract (10 mm inhibition zone). On the other hand, the ethanol extract of *Colchicum* plants showed no antimicrobial

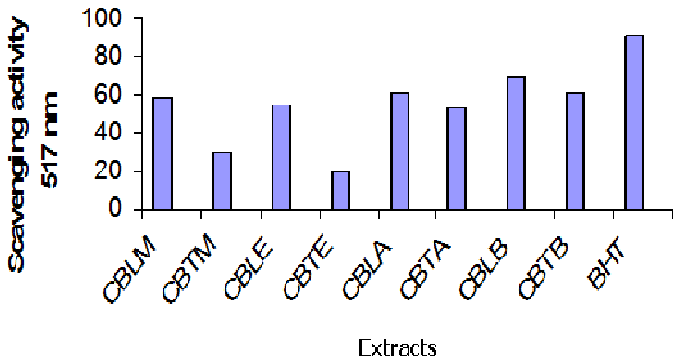

Figure 2. The free radical scavenging capacity of the extracts with methanol, ethanol, acetone and benzine through DPPH method; Extracts obtained from. *Colchicum balansae* (CB), Tuber-methanol (CBTM), Tuber-ethanol (CBTE), Tuber-acetone (CBTA), Tuber-benzine (CBTB), Leaf-methanol (CBLM), Leaf-ethanol (CBLE), Leaf-acetone (CBLA) and Leaf-benzine (CBLB), Butylated hydroxytoluene (BHT).

activity against *Staphylococcus epidermidis* ATCC 12228, *Serratia marcescens* ATCC 8100, *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028 strains. When comparing the antimicrobial activity of the control antibiotics, the ethanol extract exhibited lower antimicrobial activity (Table 1).

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