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Antioxidant and antibacterial studies on different extracts of *Ornithogalum cuspidatum* Bertol from Iran

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ABSTRACT: *Ornithogalum cuspidatum* is an Iranian species of the genus *Ornithogalum* L. (family: Liliaceae). The species of *Ornithogalum* are used widely in Iranian traditional medicine for treatment of inflammatory and respiratory disease. The purpose of the present study is to evaluate antioxidant and antimicrobial activities of n-hexane, ethyl acetate, methanol and aqueous extracts of *O. cuspidatum* leaves and to determine their relationship with the phenolic composition. The antioxidant activities of different extracts of *O. cuspidatum* were evaluated with DPPH radical-scavenging activity. The amounts of total phenolics were also determined spectrophotometerically. Antimicrobial activities of different extracts were examined against five Gram-positive and four Gram-negative bacteria. Methanol extract of *O. cuspidatum* showed the strongest antioxidant activity (IC_{50} = 35.7 µg/ml) and the highest total phenolic content (94.1 mg GA/g extract). n-hexane, methanol and ethyl acetate extracts demonstrated antibacterial effect against *Bacillus cereus* strain in concentration of 7.5 mg/ml.

Keywords: *Ornithogalum cuspidatum*, Extract, Antioxidant, Antimicrobial, Liliaceae, Medicinal plant.

INTRODUCTION

Free radicals or reactive oxygen species (ROS) such as superoxide, singlet oxygen, hydroxyl radicals, peroxyl, and peroxynitrite can damage the body by cellular or oxidative stress. This leads to the development of diseases like cancer, diabetes, cardiovascular, and cirrhosis. Free radicals generated in the body can be removed by its own natural antioxidant defense systems that include glutathione peroxidase, catalase, superoxide dismutase, etc. Endogenous antioxidant defense are not completely efficient. Therefore, natural and dietary antioxidants are required to reduce the effect of oxidative stress due to excessive free radicals occurring in our system (Badiee *et al*., 2015; Mohammadi *et al*., 2012). Antibacterial compounds such as antibiotics had been available for decades. Nowadays multi-drug resistant (MDR) human pathogens are considered among the most important health threatening problems worldwide (Poole, 2005). Application of new natural antibacterial such as plant extracts has been recently gained increasing attention (Brown and Wright, 2005; Dastan *et al*., 2014).

Ornithogalum cuspidatum is an Iranian species of Liliaceae family which is frequently used as a spice. Diverse species of this family is distributed in Asia, Europe, and Africa with different climatic conditions. The species of *Ornithogalum* are used widely in Iranian traditional medicine for treatment of inflammatory and respiratory disease (Asadi *et al*., 2014). Several species of genus *Ornithogalum* showed anti-tumour, cytotoxic, anti-bacterial and antioxidant activities (Chen *et al*., 2010; Delazar *et al*., 2010; Ebrahimzadeh *et al*., 2010; Kuroda *et al*., 1997; Makasci *et al*., 2010).

The chemical components of the extracts and essential oils allowed their use in traditional medicine and as food preservatives. Recently, there has been a growing interest in substances exhibiting antioxidant and antimicrobial properties that are provided to human as nutraceutics and pharmaceutics. It been well-known that plant extracts and essential oils have antioxidant and antimicrobial activity effects (Ghahremani-majd *et al*., 2012; Mumivand *et al*., 2010; Pezhmanmehr *et al*., 2009). The aim of the present study was to investigate the antimicrobial and antioxidant capacities of n-hexane, ethyl acetate, methanol and aqueous extracts of *O. cuspidatum* leaves.

MATERIALS AND METHODS

A. Chemicals

Butylated hydroxyl toluene (BHT), 2,2-diphenyl,1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), and gallic acid were obtained from Sigma Chemical Co. All other chemicals were of analytical grade.

B. Plant material

O. cuspidatum plants were collected from Mariwan, Kurdistan province, Iran, in May 2014. They were identified and authenticated and a voucher specimen (MPH-1066) was deposited at the Herbarium of the Institute of Forests and Rangelands Researches, Sanadaj-Iran.

C. Preparation of the extracts

The dried and powdered aerial parts of the plant (300 g) were powdered and extracted successively with n-hexane, ethyl acetate, methanol and aqueous (3 × 1 L, rt for 24 h). The extracts were filtered using Whatman filter paper (no. 1) and then concentrated in vacuum at 40°C using a rotary evaporator. The residues obtained were stored in a freezer until further tests.

D. Measurement of free radical-scavenging activity (DPPH assay)

The capacity of *O. cuspidatum* extracts to scavenge $DPPH^{\cdot}$ were determined according to the technique reported by (Esmaeili *et al*., 2010). The absorbance of the reaction mixture at 517nm was measured. BHT was used for comparison. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample (extract solution) concentrations.

E. Determination of total phenolics

The total phenolics content (TPC) of the plant extract was determined according to the Folin-Ciocalteu procedure (Slinkard and Singleton, 1977). Total phenols content was expressed as mg gallic acid equivalents per g plant extract (mg (GAE)/g).

F. Antimicrobial activity

The extracts of *O. cuspidatum* were tested individually against a range of 9 microorganisms, including *Bacillus pumilus* (PTCC 1274), *Klebsiella pneumoniae* (ATCC 10031), *Bacillus subtilis* (ATCC 465), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (PTCC 1015), *Enterococcus faecalis* (ATCC 29737) and *Pseudomonas aeruginosa* (ATCC 85327). The antimicrobial activity of extracts was determined by the disk diffusion method using Mueller-Hinton Agar plates with determination of inhibition zones. Also The MIC values were determined by the broth microdilution assay (Eftekhar *et al*., 2009).

RESULTS

A. Antioxidant activity

Free radical scavenging capacities of different extracts of *O. cuspidatum* measured by DPPH assay are shown in Table 2. According to the results the highest scavenging activity was found for methanol extract (IC_{50} = 35.7 µg/ml), followed by ethyl acetate (IC_{50} = 40.2 µg/ml), aqueous (IC_{50} = 65.3 µg/ml), and n-hexane (IC_{50} = 121.1 µg/ml) extracts. The total phenolics of various extracts of the plant were measured using Folin-Ciocalteu's assay. The highest phenolic content was found for the ethyl acetate fraction of methanol extract (94.1 mg GAE/g sample f) and the lowest was found for the n-hexane extract (12.3 mg GAE/g sample f).

Table 1: Antioxidant activities and total phenolics content of the different extracts from *Ornithogalum cuspidatum* leaves.

Extracts	DPPH assay IC_{50}(µg/ml)	TPC mg gallic acid/g Sample
n-hexane extract	121.1±0.2	12.3±0.5
Ethyl acetate extract	40.2±0.1	78.4±1.2
Methanol extract	35.7±0.1	94.1±1.2
Aqueous extracts	65.3±0.2	55.4±0.6
BHT	26±0.1	

Values were the means of three replicates ± standard deviation.

B. Antimicrobial activity

The different extracts of *O. cuspidatum* were tested against five Gram-positive and four Gram-negative bacteria. The results indicated that the extracts had moderate to high inhibitory activity against the *Bacillus cereus*, and *Staphylococcus epidermidis* (Table 2). The most sensitive microorganism was *Bacillus cereus* with inhibition zones of 20 mm and MIC values of 7.5 mg ml^{-1} for n-hexane extract.

DISCUSSION

Recently, there has been a growing interest in natural product exhibiting antimicrobial and antioxidant properties that are provided to human as nutraceutics and pharmaceutics. The extracts of plant possess various biological activities such as antimicrobial, antioxidant, anti-inflammatory, antidiabetic, anticancer activities, etc. (Broadhurst *et al*., 2000; Miliauskas *et al*., 2004). The chemical components of the extracts allowed their use in traditional medicine and as food preservatives. The results showed that the extent of antioxidant activities of extracts is in accordance with the amounts of phenolics existing. It is widely accepted that the antioxidant activity of a plant extract is correlated to its phenolic content (Elmastas *et al*., 2006).

Table 2: *In vitro* antibacterial activities of the different extracts from *Ornithogalum cuspidatum* leaves.

				Microorganism					
Sample	***B.PU***	***B.sub***	***S.au***	***B.Ce***	***KL.b***	***E.NT***	***E. coli***	***St.Ep***	***PS***
n-hexane extract	11[a] (>15)[b]	10 (>15)	12(15)	20 (7.5)	9(-)	9 (-)	10(>15)	13 (15)	-
Ethyl acetate extract	14(15)	11 (>15)	11 (>15)	18 (7.5)	14 (15)	10 (>15)	11(>15)	10(>15)	-
Methnol extract	10 (>15)	13 (15)	13(15)	19 (7.5)	11 (15)	11 (15)	14 (15)	11(>15)	-
Aqueous extract	12(15)	12(>15)	11(15)	14(15)	14 (15)	10(>15)	10 (>15)	12(15)	-
Tetracycline[b]	nt	21 (3.2)	20 (3.2)	nt	nt	nt	-(nt)	34 (1.6)	t
Gentamicin[c]	nt	-(nt)	-(nt)	nt	nt	nt	23 (3.2)	-(nt)	nt
Ampicillin[d]	15(15)	14 (15)	13 (15)	nt	nt	nt	12 (15)	19(15)	nt

a: Zone of inhibition (in mm) includes diameter of the disc (6 mm), b: Minimum inhibitory concentration values as mg ml^{-1}, (–): Inactive, (7 – 13): moderately active, (> 14): highly active, nt: not tested

Afacan Makasci *et al*. investigated the antimicrobial and antioxidant activities of various extracts of *O. alpigenum* and found that the extracts of *Ornithogalum* (ethanol extract 11 ± 2 mm diameter) and (Acetone extract 8 ± 2 mm diameter) were effective on *Bacillus subtilis* ATCC 6633, (Benzene extract 9 ± 1 mm diameter) and (Acetone extract 10 ± 2 mm diameter) were effective on *Bacillus cereus* RSKK 86. Also free radical scavenging activity of the extracts using the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was also determined. The overall results showed that some extracts have values (90.9%>90.5% > 90.4% > 88.4%) very close to those of Butylated Hydroxytoluene (BHT) (90.0%) (Makasci *et al*., 2010).

Also the antioxidant and antimicrobial activities of different *Ornithogalum* species, including *O. narbonense* L., O. *Brachystachys* and *O. sintenisii* L. have been studied by different researchers (Ebrahimzadeh *et al*., 2010; Tabaraki *et al*., 2013; Zengin *et al*., 2015). Similar to the other species, the extracts of *O. cuspidatum* showed significant antioxidant and antimicrobial effect.

The high antioxidant activity and good antimicrobial inhibitory effect of the extracts of plant supports its potential as a prospective source of antimicrobial and antioxidant agent in food and pharmaceutical industries.

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