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Study on Antibacterial Effect of Some *Allium* Species from Hamedan-Iran

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ABSTRACT

Garlic and onion belonging to family Alliaceae are well known for having antibacterial effects. The antibacterial effects of six native *Allium* spp. were examined. Aqueous extracts were prepared from different parts of plants and six different bacterial isolates comprising of both Gram-negative and Gram-positive organisms were used. All extracts exhibited antimicrobial activities at a concentration 10 mg mL^{-1}. The highest zones of inhibition were exhibited by *Allium atroviolaceum* extract ranging from 6.4 to 42.6 mm. Inhibition zones of the other species were: *A. eriophyllum* 3.5 - 45 mm, *A. scabriscapum* 0.8 - 25.3 mm, *A. stamineum* 7.2 - 39.7 mm, *A. iranicum* 0 - 30 mm and *A. shelkovnikovii* 0-27.8 mm. In bulbous plants, the bulb extracts had stronger antimicrobial activity and in others, flower extracts were more effective. Among the tested organisms *Klebsilla pneamoniae* was the most resistant and *Bacillus subtilis* and *B. cereus* were more sensitive than others. Minimum inhibitory concentration (MIC) in *A. atroviolaceum* was nearest to that of Streptomycine, while MIC of *A. shelkovnikovii* was farther from it.

Key Words: Antibacterial activity; *Alliaceae*; Extracts

INTRODUCTION

Bacterial resistance is a growing-problem worldwide (Cohen, 2002; WHO, 2001). One of the measures to combat the increasing rate of resistance in the long run is to have continuous investigation for new, safe and effective antimicrobials as alternative agents to substitute with non-effective ones. Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Different extracts from traditional medicinal plants were tested and some natural products were approved as new antibacterial drugs. However, there is still an urgent need to identify novel substances active against pathogens with higher resistance (Rieco, 1989; Cragg *et al*., 1997; Malika *et al*., 2004). Lot of works reports antibacterial and phytochemical constituents of medicinal plants and their use for the treatment of microbial infections (both topical & systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. During the last ten years the pace of development of new antibacterial drugs has slowed down, while the prevalence of resistance (especially multiple) has increased astronomically (Hugo & Russell, 1984). Literature reports and ethno-botanical records suggest that plants are the sleeping giants of pharmaceutical industry (Hostettmann & Hamburger, 1991) and provide natural source of antimicrobial drugs that provides novel compounds that may be employed in controlling some infections globally.

Allium is the largest and important representative genus of the Alliaceae family and comprises 450 species, widely distributed in the northern hemisphere (Lonzotti, 2006). These species are characterized by a specific flavor and are used for cooking (Tada *et al*., 1988). In addition to their nutritional effects, the antibacterial and antifungal activities against the variety of Gram-negative and Gram-positive were and continue to be extensively investigated (Whitemore & Naidu, 2000). Garlic (*Allium sativum)* has traditional dietary and medicinal applications as an anti-infective agent (Lawson, 1991; Lawson, 1998). *In vitro* evidence of the antimicrobial activity of fresh and freeze-dried garlic extracts against many bacteria (Cavallito & Bailly, 1944; Rees *et al.,* 1993; Ross *et al.,* 2001; Onyeagba *et al*., 2004; Alves De Moura *et al*., 2005; Shalaby *et al.,* 2006), fungi (Adetumbi *et al.,* 1986) and viruses (Weber *et al.,* 1992) supports these applications.

Garlic contains pharmaceutically interesting compound that are mainly sulfur-based (Tada *et al,* 1988). Han *et al.* (1995) reported that the antibiotic activity of 1 mg of allicin, which is a (+)-s-methyl-L-cystein sulfoxide, was equated to that of 15 IU of penicillin. Recent investigations have also shown an inhibitory effect by aqueous extracts on numerous bacterial and fungal species (Sivam *et al*., 1997; Phay *et al*, 1999; Hsieh *et al*., 2001; Ward *et al*., 2002).

However, *Allium* genus contains several species of which *Allium sativum* is only one. In contrast to *A. sativum*, there is not any report about antibacterial ability of other species of *Allium*. The aim of this investigation was to study the effect of aqueous extracts of some other native *Allium* species on bacterial test organisms.

MATERIALS AND METHODS

Collection of plant material. *Allium* species examined in this study were collected from different region of Hamedan-Iran, in May to July 2006 and determined their scientific names in the herbarium. Names and used parts of species are: *A. atroviolaceum* Boiss. (bulbs, leaves, flowers); *A. eriophyllum* var *laceratum* Boiss and NÖe. (bulbs, leaves, flowers); *A. scabriscapum* Boiss and Ky. (rhizomes, leaves, flowers); *A. stamineum* Boiss. (bulbs, flowers); *A. iranicum* Wendelbo. (bulbs, flowers) and *A. shelkovnikovii* Grossh.(bulbs, flowers).

Test organism. Standard strains *Shigella flexinix* (PTCC 1234), *Klebsiella pneamoniae* (PTCC 1053), *Bacillus subtilis* (PTCC 1365), *B. cereus* (PTCC 1247), *Staphylococcus aureus* (Lio) and *Escherichia coli* (PTCC 1330) were used as test organisms: Microorganisms to be used were obtained from Persian Type Culture Collection (PTCC), Tehran, Iran, sub-cultured on nutrient broth and nutrient agar (Oxiol Ltd.), while diagnostic sensitivity test agar (DST) Hinton Agar (Merk, Darmstadt, Germany) was used in antibiotic sensitivity testing.

Preparation of plant extracts. Plant material including bulbs, leaves, flowers and rhizomes were removed from *Allium* species. under investigation. All samples were collected from original locations and dried in darkness. The air-dried and finely ground samples were extracted (Lee *et al*., 2004). A 5 g dry weight sample of different parts of each sample was washed, mined and added adequate amount of water to a concentration of 12.5% (w/v), respectively, then ground in a blender. The products were squeezed through gauze cloth to remove the larger particles and the extracts were passed through a 0.2 µm filter (Millipore, Spa, Italy). The procedures of extraction and filtration were operated at room temperature and then the sterilized filtrates were stored at 4°C and used in antibacterial assay.

Antibacterial assays. Antibacterial activity of extracts was evaluated by diffusion test (Kim *et al.,* 1995). A 100 µL of diluted bacterial suspension (5×10^6 cfu mL^{-1}) of test bacterial strains was spread on the surface of Hinton agar (Merk, Darmstadt, Germany). Then sterile blank paper disc (Padtan Teb Co, diameter 6.4 mm) containing 10 mg mL^{-1} of extracts was applied onto the surface of Hinton Agar. For negative control, disks were impregnated with sterile water. For positive control different antibiotic disks were used. Plates were incubated at 37°C for 48 h and diameters inhibition zones (mm) were determined.

Minimum inhibitory concentration. Minimum inhibitory concentration (MIC) of extracts was determined using two-fold dilutions method (Russel & Furr, 1977; Chehregani *et al.,* 2007). It ranged from 0.33 to 12.5 mg mL^{-1} in DM solvent as tested on above-mentioned bacterial species and described above. For determination of MIC, the plant organs having the highest antibacterial effect in each case were employed.

RESULTS AND DISCUSION

All *Allium* tissue extracts possessed antimicrobial activity against some or all of the tested organisms at a concentration of 10 mg mL^{-1} (Table I). Results showed that the highest antibacterial activity of *A. atroviolaceun* was against *B. subtilis* with diameter of inhibition zone (DIZ) 42.6 mm and the lowest was against *K. pneamoniae* (DIZ = 3.8). The extracts that were prepared from bulbs of this species were more effective as antibacterial effects against all tested organisms. The lowest antibacterial effects were seen for the extracts from leaves of *A. atroviolaceun* plants. The highest antibacterial activity of *A. eriophyllum* var. *laceratum* was against *B. subtilis* with DIZ = 36.8 mm and *Staphylococcus aureus* DIZ = 30 mm. The later one (*S. aureus*) was extensively studied and its sensitivity to plant extract is reported widely (Park *et al.,* 2002).

The extract prepared from flowers of *A. scabriscapum* had antibacterial property against all tested bacteria. The highest effects were against *B. cereus* (DIZ = 25 mm) and *Escherchia coli* (DIZ = 25.3 mm). The extract prepared from leaves of this species had the lowest antibacterial effect against all tested organisms; in addition the leaf extracts could not inhibit growth of *K. pneamoniae. A. stamineum* had antibacterial effect against tested organisms. Their highest effect was against *B. cereus* with diameter of inhibition zone 39.7 and the lowest one against *K. pneamoniae.* Results indicated that bulbs extract had the highest and leaf extract had the lowest effects. The extracts from *A. iranicum* and *A. shelkovnikovii* had antibacterial effects against *S. flexinix, B. subtilis, B. cereus, Staphylococcus aureus* and *Escherchia coli* but were not effective against *K. pneamoniae.* In both cases the extracts from flowers were more effective than bulb extract.

Our results showed that all tested plants had antibacterial activity against tested bacteria. Many prior reports showed that garlic and onions have considerable antibacterial and antifungal effects (Tsao &Yin, 2001; Benkeblia, 2004; Lee *et al*., 2004). Since the studied plants have edible parts, our finding is important regarding using them as edible medicinal plants. In most of the plants, bulbous extracts were more effective regarding antibacterial effects. It seems that this ability is due to having Allicine (Cavallito & Bailly, 1944) and *Allium* species have been reported to accumulate the higher concentration of Allicine in their bulbs than other organs. According to our results (Table I) in *A. iranicum* and *A. shelkovnikovii* plants, flower extracts were more effective than other parts. It seems that in these plants antibacterial agent is tannins (not Allicine) that were accumulated in the flowers and not in bulbs (Hsieh *et al.*, 2001).

The minimum inhibitory concentration (MIC) of plant extract was also determined using the method of Russel and Furr (1977). Results indicated that the minimum inhibitory concentration (MIC) of plant extracts against the tested organism varied between 0. 313 and 12.50 mg mL^{-1}. The

Table I. Antibacterial activity of aqueous extracts of *Allium* plants against bacterial strains after 24 h. Concentration of all plant extracts were 10 mg mL^{-1}. Each data represented means ± S.D mm (n = 5). All data are significant (P ≤ 0.05). [a]Inhibition zone can not be detected

Bacterial strains	Plants and Parts	*Shigella flexinix*	*Klebsiella pneamoniae*	*Bacillus subtilis*	*B. Cereus*	*Staphylococcus aureus*	*Escherchia coli*
A. atroviolaceum	Flowers	12±0.4	8.5±0.8	36.2±1.9	15.0	15.6±1.0	23.5±1.4
	Bulbs	14±0.9	10.5±0.6	42.6±2.1	25.0	19.4±0.9	27.0±0.8
	leaves	9.6±0.5	3.8±0.5	21.6±1.2	8.7±0.9	7.2±0.0	5.7±0.6
A.eriophyllum var.	Flowers	6.4±0.4	11.7±0.8	36.8±1.4	26.3±0.8	30±2.6	28.7±1.6
laceratum	Bulbs	8.3±0.5	14.5±1.2	45±1.8	26.7±0.9	19.3±0.9	30.0±0.9
	leaves	3.5±0.4	6.8±0.5	18.6±0.5	15.0±0.0	12.3±1.2	6.4±0.0
A.scabriscapum	Flowers	9.7±0.9	5.4±0.5	18.3±0.9	25.0±0.8	13.7±0.9	25.3±0.7
	Rhizomes	3.4±0.5	2.8±0.5	15.6±0.5	17.0±0.8	11.0±0.8	17.0±0.8
	leaves	0.8±0.5	0.0[a]	5.6±0.4	12.3±0.5	5.5±0.3	9.2±o.5
A.stamineum	Flowers	7.2±0.6	4.4±0.4	16.7±0.6	39.7±1.3	24.8±1.4	18.3±0.6
	Bulbs	11.8±0.8	7.6±0.5	18.6±0.8	28.3±1.7	16.9±1.9	15.3±0.5
A. iranicum	Flower	5.3±0.4	3.9±0.3	9.7±0.5	28.3±1.4	6.3±0.5	16.7±0.0
	Bulbs	1.6±0.5	0.0[a]	13.7±0.5	30.0±0.7	11.5±0.9	16±0.8
A. shelkovnokovii	Flower	1.2±0.4	0.0[a]	3.2±0.4	27.8±1.6	5.0±0.6	13.0±0.6
	Bulbs	1.8±0.5	0.0[a]	5.4±1.6	23.0±1.4	8.9±0.5	13.7±0.5
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table II. The minimum inhibitory concentration (MIC) (mg mL^{-1}) of *Allium* plants extract and *Streptomycin* against the bacterial isolates

Bacterial strains Plants and Parts		*Shigella flexinix*	*Klebsiella pneamoniae*	*Bacillus subtilis*	*B. Cereus*	*Staphylococcus aureus*	*Escherchia coli*
A.atroviolaceun	Bulbs	1.25	2.50	0.313	1.25	0.625	2.50
A.eriophyllum var. laceratum	Bulbs	2.50	1.25	0.313	0.625	0.625	0.313
A.scabriscapum	Flowers	2.50	5.00	0.75	0.625	0.75	0.75
A.stamineum	Bulbs	10.00	12.50	2.50	0.50	1.25	1.25
A. iranicum	Flower	7.50	12.50	5.00	2.50	5.00	7.50
A. shelkovnokovii	Flower	10.00	-	7.50	2.50	5.00	5.00
Streptomycin		0.25	-	0.0625	0.0313	0.50	0.0

standard streptomycine had MIC values varying between 0.0313 mg mL^{-1} and 2.50 mg mL^{-1}. The results indicated that standard antibiotic streptomycine had the nearest activity to some plant extracts as shown in Table II. The lowest MIC (12.5 mg mL^{-1}) was detected for *A. iranicum* and *A. stamineum* against *K. pneumonia.* It seems that it is due to high resistance of the *K. pneumonia*. The lowest MICs were evaluated for *A. atroviolaceum* and *A. eriophyllum* (0.313 mg mL^{-1}) against *B. subtilis* and *Ecsherchia coli*. They seem as the excellent antibacterial extracts against *B. subtilis* and *Ecsherchia coli.* Also we can regard the plants that have MICs between 0.645 and 2.5 mg mL^{-1} as relatively good antibacterial agents (Akinpelu & Onakoya, 2006).

The species studied had different compounds and formulation. It is hoped that this study would lead to the establishment of some new and more potent antimicrobial drugs from natural origin and native plants.

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