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Antioxidant and Antimicrobial Activity Evaluation and Essential Oil Analysis of *Artemisia aucheri* Boiss. From Iran

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ABSTRACT

Artemisia, represented by more than 34 species in Iran, is one of the main medicinal plants used worldwide in traditional medicine. Members of this genus have been reported as antiviral, anti-stimulants, antidepressant and stimulant bile flow. This plant used as a flavoring in the preparation of foods and alcoholic beverages and also for therapeutic purposes. There is, no published report related to antioxidant activity of essential oils from A. aucheri; however, the limited information on the volatile chemistry of this genus is thought to be due to low amount of essential oils being obtained. GC and GC/MS analysis identified 15 compounds representing 98.9% of the oil. The main components comprising 64% of the oil were lavandulyl acetate (25.7%), geranyl acetate (12.2%), trans- β -ocimene (8.3%), P-cymene (6.8%) and γ -terpinene (5.7%). The samples were screened for their antioxidant activities using 2.2-diphenyl-1-picrylhydrazyl (DPPH) and β -carotene/linoleic acid assay methods. None of the plant samples showed appreciable antioxidant activity in DPPH test. However, methanol extract exhibited considerable linoleic acid oxidation inhibition (75.63%) in the β -carotene/linoleic acid test, a value near to that of butylated hydroxytoluene (BHT, 98.15%). Total phenolic content of the plant extract as gallic acid equivalent was 6.9 µg mg⁻¹. The essential oil exhibited strong antimicrobial activity against all but one of the test microorganisms while the plant extract only inhibited two of them weakly.

Key words: Artemisia aucheri, essential oil, gc/ms analysis, antioxidant activity, total phenolic, antimicrobial activity

INTRODUCTION

The genus Artemisia (family, Asteraceae) consists of grassy aromatic permanent plants growing mostly in Mediterranean, Turkey and Iran mountain regions (Ghahreman, 1999; Omidbaigi, 2005). This family has about 300 genus and 3000 species and its plants are chiefly used as edible vegetables and also as sources of volatile oils and drugs (Mujeeb-ur-Rahman and Gul, 2002). These plants are known to accumulate flavonoids mainly in the form of flavones and flavonols (Gebhardt et al., 2005). Asteraceae family includes some well known medicinal and toxic plants as well as common food plants such as carrot, celery and parsley and some of the most bioactive polyacetylenes are also found in them (Christensen and Brandt, 2006). Of about 34 species of

genus Artemisia growing in Asia, eleven are found in Iran and five are endemic (Rechinger, 1987). Artemisia aucheri Boiss. is one of this endemic plant and its highly flavored leaves are frequently used in jams and pickles in the south-west of Iran (Batooli, 2000). Its dried fruits are also added to yoghurt and other dairy products as carminative and flavoring agent (Batooli, 2002). A literature survey revealed no reports on the chemical composition, antioxidant and antimicrobial activity of the essential oil and extract of this plant, thus, this is the first such study on it.

This work presents (1) The chemical composition of the essential oil from aerial parts of $A.\ aucheri$, (2) $In\ vitro$ antioxidant activity of the plant essential oil and methanol extract using two complementary assays: DPPH and β -carotene/linoleic acid tests, (3) Total phenolic contents of the plant extract and (4) Antimicrobial activity of the plant essential oil and methanol extract. Plant volatile and fixed (nonvolatile) secondary metabolites have wide applications in dietary regimens, food flavoring and preservation, folk medicine and fragrance industry (Kalemba and Kunicka, 2003; Huang et al., 2005).

Their application as dietary regimens and preservative is mainly due to their antioxidant and antimicrobial potentials. Antioxidants can inhibit or delay the oxidation of oxidizable substrates and it appears to be very important in the prevention of oxidative stress which is suggested as the leading cause of many oxidation related diseases (Halliwell *et al.*, 1992). Recently and mainly due to undesirable side effects such as toxicity and carcinogenicity of synthetic additives, interest has considerably increased for finding naturally occurring antioxidant and antimicrobial compounds suitable for use in food and/or medicine (Scalbert *et al.*, 2005). In this regard, a growing rate of research was conducted on many plant species in order to find new natural bioactive compounds in them.

MATERIALS AND METHODS

Plant materials: The aerial parts (leaves and flowers/inflorescences) of *A. aucheri* were collected during the flowering period in October 2010 from Qamsar, around Kashan (Isfahan province, Iran) at an altitude of ca. 2650 m. The voucher specimen of the plant was deposited in the herbarium of Essential Oils Research Institute, University of Kashan, Iran.

Solvents and chemicals: 2,2-Diphenyl-1-picrylhydrazyl (DPPH), β-carotene, linoleic acid, 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BHT) and gallic acid were procured from Sigma-Aldrich Chemie (Steinheim, Germany). Analytical grade methanol, ethanol and dimethylsulfoxide (DMSO), HPLC grade chloroform, standard Folin-Ciocalteu's phenol reagent, anhydrous sodium sulfate, sodium carbonate, Tween 40 and all cultures media were obtained from Merck (Darmstadt, Germany). Ultra pure water was used for the experiments.

Preparation of the extracts

Isolation of the essential oil: The air-dried and ground aerial parts (50 g) of *A. aucheri* were subjected to hydrodistillation for 3.5 h using a Clevenger-type apparatus (Anonymous, 1996). After decanting and drying over anhydrous sodium sulfate, yellow colored oil was recovered and stored at low temperature (4°C) until analysis.

Preparation of the methanol extract: A portion (50 g) of air-dried and ground plant material was Soxhlet-extracted, with 500 mL methanol for 8 h at a temperature not exceeding the boiling point of the solvent (Sokmen *et al.*, 1999). The extract was concentrated using a rotary evaporator (Buchi, Flawil, Switzerland) at a maximum temperature of 45°C and dried extract (yield 9.04 g, 18.08% w/w) was stored in refrigerator before further analysis.

Chromatographic analysis

Gas chromatography (GC) analysis: Oil obtained from aerial parts of A. aucheri was analyzed using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS 5% phenylmethylsiloxane capillary column (30 m×0.25 mm, 0.25 μm film thickness; Restek, Bellefonte, PA) equipped with an FID detector. Oven temperatures were programmed as follows: 50°C (2 min), 50-130°C (5°C min⁻¹), 130°C (2 min) and 130-200°C (3°C min⁻¹). Injector and detector temperatures were set at 220 and 290°C, respectively. Helium was used as carrier gas at a flow rate of 1 mL min⁻¹ and diluted samples (1/1000 in n-pentane, v/v) of 1.0 μL were injected manually in the splitless mode. Peaks area percents were used for obtaining quantitative data.

Gas chromatography/mass spectrometry (GC/MS) analysis: GC/MS analysis of the oil was carried out on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m×0.25 mm, 0.25 µm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (Ionization energy: 70 eV) operating under the same conditions as described above. Retention indices were calculated for all components using a homologous series of n-alkanes injected in conditions equal to sample one. Identification of components of essential oil was based on Retention Indices (RI) relative to n-alkanes and computer matching with the Wiley 275. L and Wiley 7n.L libraries, as well as comparisons of the fragmentation pattern of the mass spectra with data published in the literature (Adams, 2001).

Antioxidant activity

DPPH assay: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay usually involves hydrogen atom transfer reaction but, based on kinetic data, an electron transfer mechanism has also been suggested for this assay (Huang et al., 2005; Foti et al., 2004). Radical-scavenging activity (RSA) of the plant essential oil and extracts was determined using a published DPPH radical scavenging activity assay method (Sarker et al., 2006) with minor modifications. Briefly, stock solutions (10 mg mL⁻¹ each) of the essential oil, extract and the synthetic standard antioxidant BHT were prepared in methanol. Dilutions are made to obtain concentrations ranging from 2 to 5×10⁻¹⁰ mg mL⁻¹. Diluted solutions (1 mL each) were mixed with 1 mL of a freshly prepared 80 μg mL⁻¹ DPPH methanol solution and allowed to stand for 30 min in the dark at room temperature for any reaction to take place. Ultraviolet (UV) absorbencies of these solutions were recorded on a spectrometer (Cintra 6, GBC, Australia) at 517 nm using a blank containing the same concentration of oil or extract or BHT without DPPH. Inhibition of free radical DPPH in percent (I%) was calculated as follow:

$$I\% = rac{A_{ ext{blank}} - A_{ ext{sample}}}{A_{ ext{blank}}} imes 100$$

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. The sample concentration providing 50% inhibition (IC₅₀) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC₅₀ values were reported as Means±SD of triplicates.

β-Carotene/linoleic acid bleaching assay: In this assay, antioxidant activity was determined by measuring the inhibition of volatile organic compounds and conjugated diene hydroperoxides arising from linoleic acid oxidation. The method described by Miraliakbari and Shahidi (2008) was used with slight modifications. A stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 mL chloroform, 25 μL of linoleic, acid and 200 mg Tween 40. The chloroform was evaporated under vacuum and 100 mL of oxygenated distilled water was then added to the residue. The samples (2 g L⁻¹) were dissolved in DMSO and 350 μL of each sample solution was added to 2.5 mL of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50°C for 2 h, together with two blanks, one contained the antioxidant BHT as a positive control and the other contained the same volume of DMSO instead of the extracts. The test tube with BHT maintained its yellow colour during the incubation period. The absorbencies were measured at 470 nm on an ultraviolet spectrometer (Cintra 6, GBC, Australia). Antioxidant activities (inhibition percentage, I%) of the samples were calculated using the following equation:

$$I\% = \frac{A_{-\beta \text{carotene after 2 h assay}}}{A_{\text{initial}_{\theta}\text{-carotene}}} \times 100$$

where, $A_{-\beta \text{carotene after 2 h assay}}$ is the absorbance of - β carotene after 2 h assay remaining in the samples and $A_{\text{initial }\beta\text{-carotene}}$ is the absorbance of β -carotene at the beginning of the experiments. All tests were carried out in triplicate and inhibition percentages were reported as Means±SD of triplicates.

Assay for total phenolics: Total phenolic constituents of methanol extract of A. aucheri was determined by literature methods involving Folin-ciocalteu reagent and gallic acid standard (Slinkard and Singleton, 1977). A solution of the extract (0.1 mL) containing 1000 μg of the extract was taken in a volumetric flask, 46 mL of distilled water and 1 mL Folin-Ciocalteu reagent were added and the flask was thoroughly shaken. After 3 min, 3 mL of 2% Na₂CO₃ solution was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbencies were measured at 760 nm. The same procedure was repeated for all the standard gallic acid solutions (0-1000 mg 0.1 mL⁻¹) and a standard curve obtained with the following equation:

Absorbance =
$$0.0012 \times \text{gallic}$$
 acid (µg)+ 0.0033

Total phenols of the extract, as gallic acid equivalent, was determined by using the absorbance of the extract measured at 760 nm as input to the standard curve and the equation. Test was carried out in triplicate and gallic acid equivalent value was reported as Mean±SD of triplicate.

Antimicrobial activity

Microbial strains: The essential oil and methanol extract of A. aucheri were individually tested against a panel of 11 microorganisms. Following microbial strains were provided by Iranian Research Organization for Science and Technology (IROST) and used in this research: Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 10536), Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 29737), Klebsiella pneumoniae (ATCC 10031), Staphylococcus epidermidis (ATCC 12228), Shigella dysenteriae (PTCC 1188), Proteus vulgaris (PTCC 1182), Salmonella paratyphi-A serotype (ATCC 5702), Candida albicans (ATCC 10231) and Aspergillus niger (ATCC 16404). Bacterial strains were cultured overnight at 37°C in Nutrient Agar (NA) and fungi were cultured overnight at 30°C in Sabouraud Dextrose Agar (SDA).

Disc diffusion assay: Determination of antimicrobial activity of dried extract and essential oil of A. aucheri was accomplished by agar disc diffusion method (NCCLS, 1997). The dried plant extract was dissolved in dimethylsulfoxide (DMSO) to a final concentration of 30 mg mL⁻¹ and filtered by 0.45 μm Millipore filters for sterilization. Antimicrobial tests were carried out by the disc diffusion method reported by Murray et al. (1995) using 100 μL of suspension containing 10° CFU mL⁻¹ of bacteria, 10° CFU mL⁻¹ of yeast and 10⁴ spore mL⁻¹ of fungi spread on the nutrient agar (NA), Sabouraud Dextrose (SD) agar and Potato Dextrose (PD) agar mediums, respectively. The discs (6 mm in diameter) impregnated with 10 μL of the essential oil or the extract solution (300 μg disc⁻¹) and DMSO (as negative control) were placed on the inoculated agar. The inoculated plates were incubated for 24 h at 37°C for bacterial strains and 48 h and 72 h at 30°C for yeast and mold isolates, respectively. Gentamicin (10 μg disc⁻¹) and rifampin (5 μg disc⁻¹) were used as positive controls for bacteria and nystatin (100 IU) for fungi. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated twice.

Micro well dilution assay: Bacterial strains and yeast sensitive to the plant extract and essential oil in disc diffusion assay were studied for Their Minimal Inhibition Concentration (MIC) values using micro-well dilution assay method (Gulluce et al., 2004). The inocula of the microbial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The extract and essential oil of A. aucheri dissolved in 10% dimethylsulfoxide (DMSO) were first diluted to the highest concentration (5 mg mL⁻¹) to be tested and then serial two-fold dilutions were made in a concentration range from 0.078 to 5 mg mL⁻¹ in 10 mL sterile test tubes containing Brain Heart Infusion (BHI) broth for bacterial strains and Sabouraud Dextrose (SD) broth for yeast. The 96-well plates were prepared by dispensing 95 μL of the cultures media and 5 μL of the inoculum into each well. A 100 μL aliquot from the stock solutions of the plant extracts initially prepared at the concentration of 5 mg mL $^{-1}$ was added into the first wells. Then, 100 μ L from their serial dilutions was transferred into six consecutive wells. The last well containing $195 \mu L$ of the cultures media without the test materials and $5 \mu L$ of the inoculum on each strip was used as the negative control. The final volume in each well was 200 µL. Gentamicin and rifampin for bacteria and nystatin for yeast were used as standard drugs for positive control in conditions identical to tests materials. The plates were covered with sterile plate sealers. Contents of each well were mixed on plate shaker at 300 rpm for 20 sec and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by the presence of a white pellet on the well bottom and confirmed by plating 5 µL samples from clear wells on NA medium. The MIC value was defined as the lowest concentration of the plant extracts required for inhibiting the growth of microorganisms. All tests were repeated two times.

RESULTS AND DISCUSSION

Chemical composition of the essential oil: The plant essential oil was consisted mostly of monoterpenes with both non-oxygenated and oxygenated alkene structures. Lavandulyl acetate (25.7%), geranyl acetate (12.2%), trans- β -ocimene (8.3%), P-cymene (6.8%) and γ -terpinene (5.7%) were the main components comprising 64% of the oil.

Air dried aerial parts of the plant were subjected to hydrodistillation using a Clevenger apparatus and the yellow colored essential oil was obtained (yield 0.6% v/w). GC and GC/MS analysis resulted in the identification of 15 compounds representing 98.9% of the oil (Table 1). The reports on the chemical composition of the essential oils of the plants of genus *Artemisia* are scant

Table 1: Chemical composition of the essential oil of A. aucheri

Compounda	Composition (%)	RI^{b}	RI°	
P-Cymene	6.8	1017	1025	
Trans-β-Ocimene	8.3	1042	1050	
γ-terpinene	5.7	1.052	1060	
Terpinolene	2.9	1082	1089	
Linalool	5.5	1096	1097	
Isopentyl isovalerate	3.4	1104	1103	
(-)-Lavandulol	1.8	1164	1181	
Dodecane	1.2	1193	1200	
Octanol acetate	2.3	1206	1214	
(-)-Bornyl acetate	1.4	1281	1289	
Lavandulyl acetate	25.7	1290	1290	
Verbenol acetate	2.8	1315	1321	
Neryl acetate	6.6	1355	1362	
Geranyl acetate	12.2	1378	1381	
Neryl propanoate	2.0	1456	1455	
Total	98.9			

^aCompounds listed in order of elution from HP-5 MS column, ^bRelative retention indices to C₈-C₂₄ n-alkanes on HP-5MS column, ^cLiterature retention indices

in the literature. Lavandulyl acetate and trans- β -ocimene were also found in the essential oil of $A.\ sieberi$ as major components (Rustaiyan et al., 1999) and geranyl acetate was reported as one of the main compounds in the oil of $A.\ melanolepis$ (Ashraf and Bhatty, 1978). P-Cymene, γ -terpinene and trans- β -ocimene were also found in considerable amounts in the oil of $A.\ kermanensis$ collected from the south east of Iran (Masoudi et al., 2002). Terpinolene and linalool were also found in both Masoudi et al. (2002) and our samples but in sub major quantities. Finally, caryophyllene oxide and β -pinene which were reported as major compounds in the essential oil of $A.\ aucheri$ (Masoudi et al., 2005) were not found in our sample at all.

Antioxidant activity: Antioxidant activity is a complex process usually occurring through several mechanisms. Due to its complexity, the evaluation of the antioxidant activity for pure compounds or extracts should be carried out by more than one test method (Aruoma, 2003). In this study, two classical antioxidant tests namely DPPH and β -carotene/linoleic acid tests were carried out alongside with Folin-ciocalteu test, which evaluate total phenolic content of the plant extract.

During 2,2-diphenyl-1-picrylhydrazyl (DPPH) test the capacity of the samples to donate hydrogen atom and/or electron to this blue/purple stable radical and converting it to yellow diphenylpicrylhydrazine molecule was measured (Tepe *et al.*, 2005). This reaction is used for measuring the ability of the extracts or pure molecules (such as BHT) to scavenge free radicals. The results of this test are presented in Table 2. Compared to synthetic standard antioxidant BHT (IC₅₀ = 19.52 μ g mL⁻¹), both essential oil and methanol extract of the plant were exhibited very weak radical scavenging activities, but the free radical scavenging activity of the methanol extract (IC₅₀ = 437.55 μ g mL⁻¹) was superior to that of the oil (less than 9% inhibition in concentrations up to 10 mg mL⁻¹). Due to the major contribution of phenolic compounds in antioxidant activity, low antioxidant activity of the plant extract may be a consequence of its low phenolic compounds content which was reflected in its Folin-Ciocalteu test result (7.5 μ g mg⁻¹, 0.75% w/w as gallic acid equivalent).

Table 2: Antioxidant activity of the essential oil and methanol extract of *A. aucheri* and BHT in DPPH free radical scavenging activity and β-carotene/linoleic acid bleaching assay methods

Sample	DPPH IC $_{50}$ ($\mu g~mL^{-1}$)	β-carotene/linoleic acid inhibition (%)			
Methanol extract	437.55±1.13	72.63±0.022			
Essential oil	$\mathrm{ND^a}$	9.44±0.010			
ВНТ	19.52±0.8	98.15±0.026			
Blank	NA	6.25±0.044			

^aLess than 9% inhibition for the essential oil concentration of up to 10 mg mL⁻¹, ND: Not determined, NA: Not applicable

Table 3: Antimicrobial activity of the essential oil and methanol extract of A. aucheri

					Antibiotics					
	Essential oil		Methanol extract		Rifampin		Gentamicin		Nystatin	
Test microorganisms	DD^{a}	MIC ^b	DD	MIC	DD	MIC	DD	MIC	DD	MIC
P. aeruginosa ATCC 27853	11	0.076	-	-	-	-	23	0.50	NA	NA
B. subtilis ATCC 6633	13	5.00	7	2.50	13	0.125	21	0.50	NA	NA
E. coli ATCC 10536	11	5.00	-	-	11	0.50	20	0.50	NA	NA
S. aureus ATCC 29737	19	5.00	-	-	10	0.50	21	0.50	NA	NA
K. pneumoniae ATCC 10031	13	5.00	-	-	7	0.50	22	0.25	NA	NA
S. epidermidis ATCC 12228	24	5.00	-	-	40	0.50	35	0.50	NA	NA
S. dysenteriae PTCC 1188	13	5.00	7	2.50	8	0.50	18	0.50	NA	NA
P. vulgaris PTCC 1182	11	5.00	-	-	10	0.50	23	0.50	NA	NA
S. paratyphi-A serotype ATCC 5702	10	5.00	-	-	-	-	21	0.50	NA	NA
C. albicans ATCC 10231	15	5.00	-	-	NA	NA	NA	NA	33	0.125
A. niger ATCC 16404	-	-	-	-	NA	NA	NA	NA	NT^c	Nt^{c}

A dash (-) indicate no antimicrobial activity, ^aInhibition zone in diameter (mm) around the impregnated discs, ^bMinimal Inhibition concentrations (as mg mL⁻¹), ^cPlant samples were inactive against mold in disc diffusion test so that the MIC agar dilution test for nystatin was not carried out, NT: Not tested, NA: Not applicable

Potential ability of the antioxidants to delay lipid peroxidation by reacting with chain propagating peroxyl radicals faster than the reaction of these radicals with proteins or fatty acid side chains is usually evaluated by β -carotene/linoleic acid test (Sacchetti et al., 2005; Tepe et al., 2005; Trouillas et al., 2003). Methanol extract of the plant was showed an inhibition percentage (72.63%) comparable to that of synthetic standard BHT (98.15%, Table 2) in this test. According to our best knowledge, literature has no report on the antioxidant activity of the Artemisia genus plant species and this is the first one. Peroxy radicals usually initiate lipid peroxidation by the abstraction of easily removable hydrogen atoms such as allylic or benzylic hydrogens from the molecule under oxidation (Larson, 1997). Thus, the considerable antioxidant activity of the plant methanol extract in the β -carotene/linoleic acid test suggests the possible presences of secondary metabolites with allylic, benzylic and/or other readily abstractable hydrogens in its extract.

Antimicrobial activity: The antimicrobial activity of A. aucheri essential oil and methanol extract against a panel of 11 microorganisms was examined and their potencies were assessed both qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters and MIC values. The results are given in Table 3. The plant essential oil showed great antimicrobial activities against all but one of the tested microorganisms in the disc diffusion test but MIC values

obtained in the micro well dilution test for the oil were appreciable only for *P. aeruginosa*. The maximum inhibition zones and MIC values for microbial strains sensitive to the essential oil of the plant were in the range of 10-24 mm and 0.076-5 mg mL⁻¹, respectively. The methanol extract of the plant showed only weak antimicrobial activities against *B. subtilis* and *S. dysenteriae* in both disc diffusion and micro well dilution tests. Totally, the essential oil of the plant showed stronger and broader spectrum of antimicrobial activity than the methanol extract.

Thanks to their extra protective outer membrane, gram-negative bacteria are usually considerably more resistant to antibacterial agents than their gram-positive counterparts (Saier, 2009). In this regard, the essential oil of the plant was showed its best antibacterial activity in disc diffusion test on gram-positive bacteria (i.e. B. subtilis, S. aureus and S. epidermidis) tested. However, its inhibitory activity on gram-negative bacteria (especially on remarkably resistant P. aeruginosa bacterium) and yeast was also considerable. The only report in the literature on the antimicrobial activity of a plant from the genus Artemisia belongs to A. vulgaris and shows a pattern of antibacterial activity similar to our results for the essential oil of this plant (Masoudi et al., 2005). The literature reports moderate to high antibacterial activities for oxygenated monoterpenes (Berger, 2007) and the appreciable antimicrobial activity of the essential oil of A. aucheri may be attributed to the dominant presence of these compounds in this oil. However, due to the complexity of both essential oils compositions and bacterial skeletons characteristics, finding of the main factors affecting the antimicrobial activity in each case is usually difficult.

CONCLUSION

Growing tendency for replacing synthetic additives by natural ones has emerged great interest on the evaluation of antioxidant and antimicrobial properties of plants products in both academia and the food industry. In this respect, our study can be considered as the first report on the *in vitro* antioxidant and antimicrobial activity of the essential oil and methanol extract of A. aucheri. Appreciable antioxidant activity of the plant extract in the β -carotene/linoleic acid test and considerable antimicrobial activity of its essential oil encourages more elaborate investigations in this respect.

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