Journal of Medicinal Plants Research Vol. 5(29), pp. 6544-6549, 9 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.818

Full Length Research Paper

Allicin and alliin content and antifungal activity of *Allium senescens* L. ssp. *montanum* (F. W. Schmidt) Holub ethanol extract

Marcel Pârvu[1], Alina Elena Pârvu[2]*, Laurian Vlase[3], Oana Roşca-Casian[4], Ovidiu Pârvu[5] and Mihai Puşcaş[4]

[1]Department of Biology, Faculty of Biology and Geology, Babeş-Bolyai University, Romania.
[2]Department of Pathophysiology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Romania.
[3]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Romania.
[4]A. Borza Botanical Garden, Babeş-Bolyai University, Cluj-Napoca, Romania.
[5]Faculty of Mathematics and Computer Science, Babeş-Bolyai University, Cluj-Napoca, Romania.

Accepted 10 October, 2011

***Allium senescens* ssp. *montanum* ethanol extract analysis by liquid chromatography coupled with mass spectrometry detection (LC/MS) revealed 58.37 µg alliin/ml extract and 0.919 mg allicin/ml plant extract. The plant extract had antifungal effects against all tested species such as *Aspergillus niger, Botrytis cinerea, B. paeoniae, Fusarium oxysporum* f. sp. *tulipae*, and *Penicillium gladioli*, with minimum inhibitory concentration value (MIC) varying from 100 to 160 µl/ml.**

Key words: Allicin, alliin, *Allium senescens* ssp. *montanum*, antifungal activity, liquid chromatography coupled with mass spectrometry detection (LC/MS).

INTRODUCTION

Fungal infections cause significant morbidity and mortality. Occupational exposure to organic and indoor dust are important routes of exposure to phytopathogenic fungi (such as *Alternaria* sp., *Aspergillus* sp., *Cladosporium cladosporioides*, *Fusarium* sp., *Penicillium* sp., *Botrytis cinerea,* etc.) and their metabolites (Piecková and Wilkins, 2004; Góra et al., 2009). According to allergen databases, 189 fungal species are suspected to produce allergens, the most common causes of fungal sensitization in populations being *Aspergillus*, *Alternaria,* and *Penicillium* (Bowyer et al., 2006). In the vegetable processing facilities, employees are exposed to micromycetes, that develop on vegetables (*Alternaria*, *Botrytis*, *Penicillium*, *Colletotrichum* and *Fusarium*), the secondary toxic metabolites they produce representing a risk for developing respiratory diseases (Góra et al., 2009). There are several synthetic or natural product-based drugs available for the treatment of fungal infections. Due to their lack of efficiency and their toxicity, fungal infections are often difficult to eradicate (Mokoka et al., 2010). The scientists enhanced their search for new antifungal agents, taking into consideration medicinal plants.

Generally, there are more than 300 *Allium* sp., some of them being recently described (Brullo et al., 2003; Ledezma and Apitz-Castro, 2006; Brullo et al., 2009). Research regarding the phytotherapeutic properties of *Allium* sp. showed that *Allium sativum, Allium porrum* (Fattorusso et al., 1999), *Allium ascalonicum* (Mahmoudabadi and Nasery, 2009), *Allium cepa* (Shams-Ghahfarokhi et al., 2006), *Allium fistulosum* (Pârvu et al., 2009), *Allium minutiflorum* (Barile et al., 2007), *Allium neapolitanum* (O'Donnell and Gibbons, 2007), *Allium*

*Corresponding author. E-mail: parvualinaelena@yahoo.com. Tel: +40364112682. Fax: +40364815349.

Abbreviations: LC/MS, Liquid chromatography coupled with mass spectrometry detection; **MIC,** minimum inhibitory concentration value; **HPLC,** high performance liquid chromatography; **P,** percentage; **ANOVA,** analysis of variance.

obliquum (Pârvu et al., 2010a) and *Allium ursinum* (Pârvu et al., 2011) have antibacterial and antifungal effects. The ethnobotanical data from Romania mention 32 wild and cultivated species of *Allium* L. (Ciocârlan, 2009). One of these is *Allium senescens* L. ssp. *montanum* (F. W. Schmidt) Holub, a wild species that occurs in the mountain area of Central and Submediteranean Europe (Ciocârlan, 2009). Although, no specific medicinal uses have been mentioned for *A. senescens* ssp. *montanum,* it is considered healthy, because when added to the diet on regular basis, it decreases blood cholesterol levels, acts as a tonic on the digestive system and tonifies the circulatory system (Fern, 1996). The juice of the plant is used as a moth repellent and the whole plant is said to repel insects and moles (Riotte, 1998).

The first aim of the study was to assess the *in vitro* antifungal efficiency of the *A. senescens* ssp. *montanum* plant extract on some fungal species. Because the allicin found in *Allium* plant extracts is an important antifungal agent the second aim of the study was to perform a quantitative analysis of allicin and its precursor alliin, from *A. senescens* ssp. *montanum* ethanol extract.

MATERIALS AND METHODS

Plant material

A. senescens ssp. *montanum* was cultivated in the Botanical Garden of Babeş-Bolyai University, Cluj-Napoca (Romania), from seeds collected in Piatra Craiului Mountains, Romania. The plant was identified at the Department of Botany, by Dr. Mihai Puşcaş and a voucher specimen (CL 661010) was deposited at the Babeş-Bolyai University Herbarium.

Preparation of alcoholic plant extract

Raw *A. senescens* ssp. *montanum* herba (leaves, stems and flowers fragments of 0.5 to 1 cm) was used for extraction with 50% ethanol (Merck, Bucuresti, Romania) in Mycology Laboratory of Babeş-Bolyai University, Cluj-Napoca, Romania, by modified Squibb's repercolation method (Ionescu-Stoian and Savopol, 1977). Briefly, three successive applications of the same menstruum were repercolated to the plant material. In each percolator, plant material (150 g in the first, 90 g in the second, 60 g in the third percolator) was moistened with the menstruum, macerated for two days and then percolated at a rate of about 4 to 6 drops per minute for each 100 g of raw material. The first percolated fractions from each percolator were saved and the next fractions were poured in the next percolator. Then, saved fractions (60 ml from the first one, 90 ml from the second one and 150 ml from the third one) were mixed and the resulting extract was 1:1 (w:v) (Pârvu et al., 2010a).

Quantitative analysis of alliin and allicin

The analysis of alliin and allicin from *A. senescens* ssp. *montanum* extract was performed using a newly developed LC/MS (Vlase et al., 2010). Briefly, an Agilent 1100 series high performance liquid chromatography (HPLC) system was used (Agilent Technologies, Darmstadt, Germany), coupled with an Agilent Ion Trap SL mass spectrometer equiped with an electrospray ion source. The mass spectrometer operated in positive multiple reaction monitoring mode, using nitrogen as nebulising and dry gas.

The chromatographic separation of alliin was made using a Zorbax SB-C18 100 mm x 3.0 mm i.d., 3.5 µm column (Agilent Technologies, Darmstadt, Germany). The mobile phase consisted in 100% ammonium acetate, 1 mM in water, isocratic elution, flow 1 ml/min. The nebuliser was set at 70 psi, the dry gas flow was 12 L/min at 350°C temperature. The mass spectrometer was set to record the transition m/z 178 > m/z 88, which is specific to alliin (Sigma-Aldrich NV/SA, Bornem, Belgium). The retention time of alliin in the above described conditions was 0.64 min.

The chromatographic separation of allicin was made in a Synergi Polar column, 100 mm x 2.0 mm i.d., 4 µm (Phenomenex, SUA). The mobile phase consisted in 100% aqueous ammonium acetate 1 mM, isocratic elution, at a flow 0.6 ml/min. A 1 mM aqueous silver nitrate solution was added to post-column using a mixing tee with a flow rate of 10 µl/min. The nebuliser was set at 60 psi, and the dry gas flow was 12 L/min at 350°C temperature. The mass spectrometer was set to record the transition m/z (449+451)>m/z (269; 271; 287; 289), which is specific to the allicin-silver adduct. The retention time of allicin in the above described conditions was 0.9 min.

Preparation of fungal colonies

A. niger Tiegh. isolated from *A. cepa* L. bulbs, *B. cinerea* Pers. isolated from rosa flowers, *Botrytis paeoniae* Oudem. isolated from *Paeonia officinalis* L. flowers, *F. oxysporum* f. sp. *tulipae* W.C. Snyder and H.N. Hansen isolated from *Tulipa gesneriana* L. flowers, *Penicillium gladioli* Machacek isolated from *Gladiolus* x *hybridus* C. Morr. corms were included in this study. Fungal colonies were obtained from the collection of the Mycology Laboratory, Babeş-Bolyai University Cluj-Napoca, and were grown in Petri dishes containing Czapek-agar medium (BD Difco, Budapest, Hungary), following inoculation into the central point and incubation at 22°C for 5 days.

Assay of antifungal activity

The antifungal activity of the *A. senescens* ssp. *montanum* extract expressed as MIC was determined *in vitro* by the agar-dilution assay (Bhandari et al., 2000). The diameter of fungal growth was measured and expressed as percentage (P) of mycelial growth inhibition of four replicates using the formula: $P = (C-T) \times 100/C$, where C is the diameter of the control colony (nutritive medium and 50% EtOH) and T is the diameter of the treated colony (Nidiry and Babu, 2005). *A. senescens* ssp. *montanum* extract effect was compared to the antimycotic drug fluconazole (2 mg ml-1) (Krka, Novo Mesto, Slovenia) and allicin (Allimed liquid, Allicin International ltd., UK). Allicin tested doses corresponded to the allicin concentration from the *A. senescens* ssp. *montanum* extract, resulting from the allicin analysis.

Statistical analysis

The results for each group were expressed as mean ± standard deviation. Data were evaluated by analysis of variance (ANOVA). Statistical differences were considered significant at the $p<0.05$ level. The correlation analysis was performed by the Pearson test.

RESULTS

Quantitative analysis of alliin and allicin

A. senescens ssp. *montanum* extract analysis by LC/MS

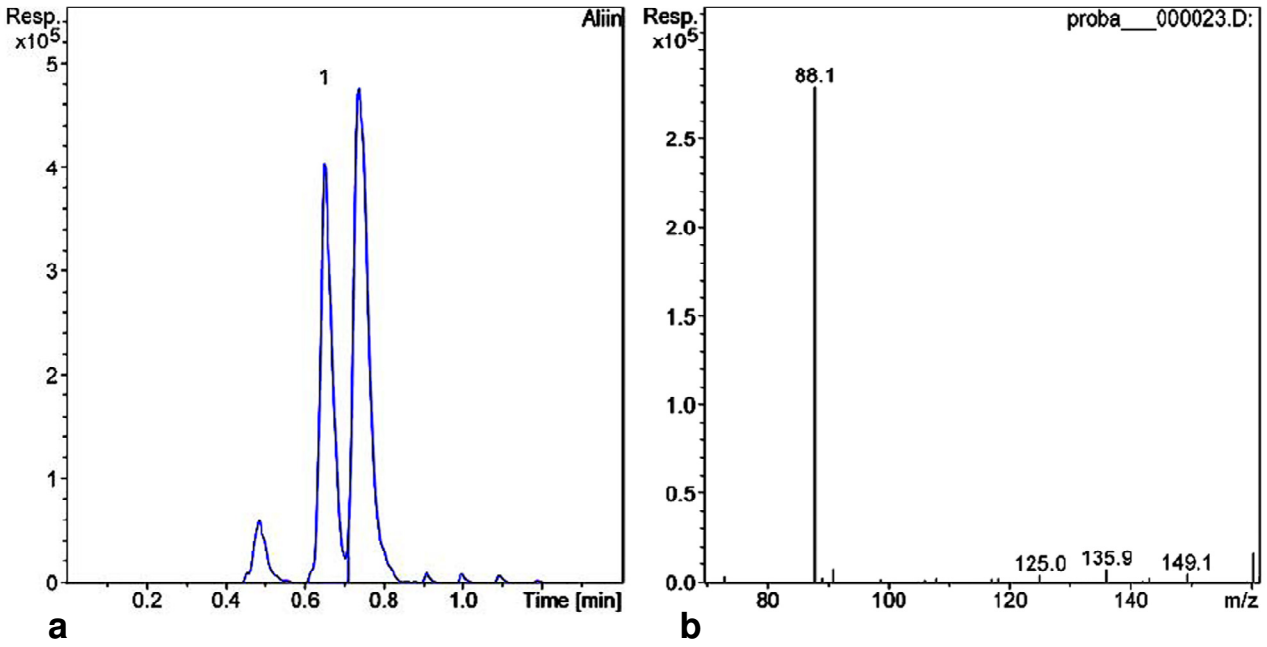

Figure 1. The chromatogram (a) and LC/MS (b) of alliin from *A. senescens* ssp. *montanum* extract.

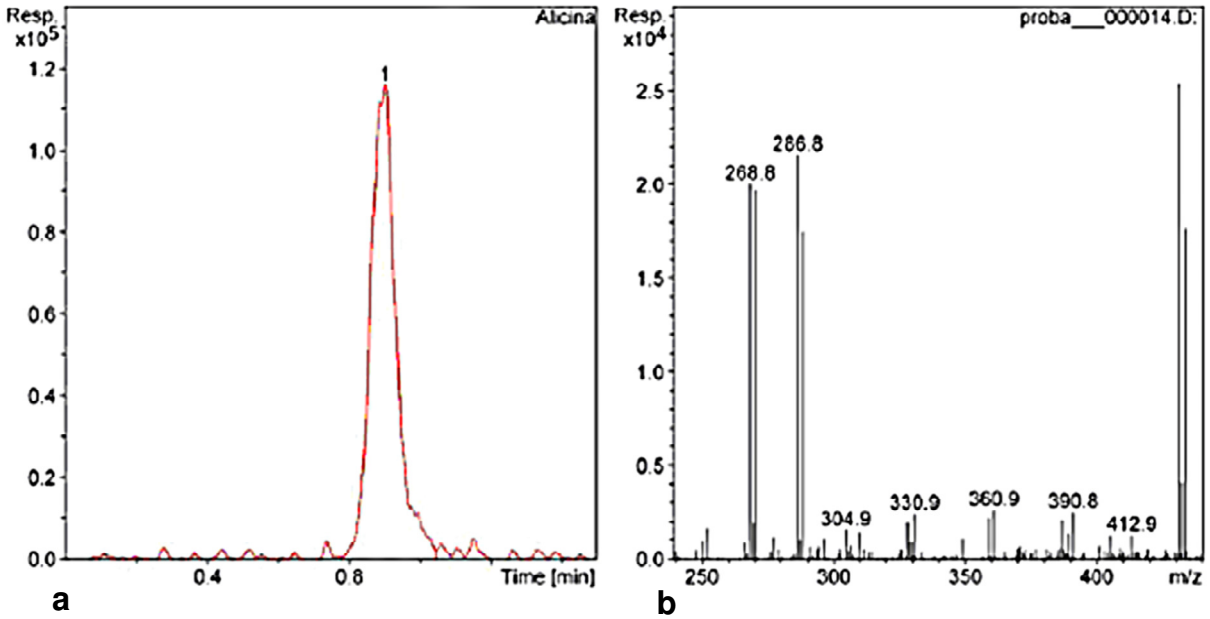

Figure 2. The chromatogram (a) and LC/MS (b) of allicin from *A. senescens* ssp. *montanum* extract.

revealed 58.37 µg alliin/ml extract (Figure 1) and 0.919 mg allicin/ml plant extract (Figure 2).

Antifungal activity

As shown in Table 1, the MIC of *A. senescens* ssp. *montanum* plant extract was 140 µl/ml for *A. niger,* 120 µl/ml for *B. cinerea*, 100 µl/ml for *B. paeoniae*, 140 µl/ml for *F. oxysporum* f. sp. *tulipae,* and 160 µl/ml for *P. gladioli*. Fluconazole MIC was 100 µl/ml for *F. oxysporum* f. sp. *tulipae*, 120 µl/ml for *B. cinerea* and *B. paeoniae*, and 300 µl/ml for *A. niger* and *P. gladioli* (Table 1). Standard allicin MIC was 100 µl/ml for *A. niger*, 120 µl/ml for *B. paeoniae* and *P. gladioli*, 140 µl/ml for *B. cinerea*, and 160 µl/ml for *F. oxysporum* f. sp. *tulipae* (Table 1).

Table 1. Antifungal activity of *Allium senescens* ssp. *montanum* extract on *in vitro* germination and growth of some phytopathogenic fungi.

Fungal species	*Allium senescens* ssp. *montanum* extract (µl/ml)	Colony [a] diameter (mm)	P [a] (%)	Flucona-zole (µl/ml)	Colony [b] diameter (mm)	P [b] (%)	Allicin (µl/ml)	Colony [c] diameter (mm)	P [c] (%)
A. niger	C	22	0	C	22	0	C	22	0
	60	20	9.09 ± 0.02	100	11.66	047 ±0.45	20	20	9.09 ±0.1
	80	12	45.45 ± 0.8	200	7.66	65.18 ± 0.62	40	12	40 ± 0.31
	100	6	72.72 ±0.57	250	4.33	80.31 ± 0.75	60	8	63.63 ± 0.54
	120	3	86.36 ± 0.82	300	0	100 ± 0.91	80	4	81.81 ± 0.68
	140	0	100 ±1.02				100	0	100 ±0.79
B. cinerea	C	65	0	C	65	0	C	65	0
	40	60	7.69 ±0.1	20	40.33	37.95 ± 0.32	30	61	6.15 ±0.06
	60	41	36.92±0.25	60	20	69.23 ± 0.52	60	44	32.30 ± 0.28
	80	19	70.76 ±0.78	100	5.33	91.80 ± 0.92	80	31	52.30 ± 0.52
	100	5	92.30 ± 0.89	120	0	100 ±0.87	100	19	70.76 ± 0.64
	120	0	100 ± 0.9				120	5	92.30±0.59
							140	0	100 ± 0.71
B. paeoniae	C	60	0	C	60	0	C	60	0
	30	57	5 ±0.02	20	50	16.66±0.14	40	58	3.33 ± 0.05
	50	28	53.33±0.61	60	24	60 ±0.48	60	46	23.33± 0.21
	60	17	71.66±0.59	100	5	91.66 ± 0.87	80	28	53.33±0.46
	80	4	93.33 ± 0.77	120	0	100 ± 0.92	100	5	91.66 ± 0.87
	100	0	100 ± 0.98				120	0	100 ± 0.9
F. oxysporum	C	32	0	C	32	0	C	32	0
f.sp. *tulipae*	30	29	9.37 ± 0.09	20	20	37.50 ± 0.22	40	30	6.25 ± 0.09
	60	24	25 ± 0.21	60	8	75 ± 0.63	80	24	25 ± 0.18
	80	19	40.62 ± 0.33	80	2	93.75 ±0.8	120	14	56.25 ± 0.54
	100	16	50 ± 0.51	100	0	100 ± 0.75	140	6	81.25 ± 0.74
	120	12	62.5 ± 0.57				160	0	100 ± 0.87
	140	3	90.62 ±0.76						
	160	0	100 ± 1.02						
P. gladioli	C	15	0	C	15	0	C	15	0
	60	12	20 ± 0.14	100	11	26.66 ± 0.19	30	13	13.33 ±0.12
	80	9	40± 0.21	160	10	26.66 ± 0.2	60	10	33.33 ± 0.31
	100	7	53.33±0.38	200	8	46.66 ± 0.31	80	6	60 ± 0.58
	120	4		250	5	66.66 ± 0.74	100	3	80±0.81
	140	2	73.33 ± 0.44	300	0	100 ± 0.08	120	0	100 ± 0.9
	160	0	86.66 ± 0.72						
			100 ± 0.85						

Legend: [a] = the effect of *A. senescens* ssp. *montanum* extract; [b] = the effect of Fluconazole; [c] = the effect of allicin; C = control (50% aq. EtOH); P = mycelial growth inhibition; Results are the mean of 4 experiments ± SEM. The same doses of *A. senescens* ssp. *montanum* extract, allicin and fluconazole were tested against all fungal species. The doses that related to the previous dose did not significantly reduce colony diameter were excluded from the table. The correlation coefficient between the concentration and antifungal effect of *A. senescens* ssp. *montanum* extract, allicin and fluconazole was $r^2 = 0.94 – 0.99$.

Compared to allicin, *A. senescens* sp. *montanum* plant extract inhibitory effect against *B. cinerea* and *B. paeoniae* was stronger, against *F. oxysporum* f. ssp. *tulipae* was equivalent, and against *A. niger* and *P. gladioli* was weaker (Table 1). Compared on a weight basis, the antifungal activity of the total *A. senescens* ssp. *montanum* plant extract was clearly stronger than that of fluconazole against *A. niger* and *P. gladioli* (Table 1).

DISCUSSION

Analysis by LC/MS showed that *A. senescens* ssp. *montanum* extract contains important amounts of allicin and alliin. In *Allium* plants and extracts different biologically active substances were determined, such as alliin and allicin (Duke et al., 2002; Josling, 2003), allicepin (Wang and Ng, 2003), E/Z-ajoene (Singh et al., 1990), saponins (Carotenuto et al., 1999; Barile et al., 2007), steroids (Ren et al., 2010), flavones (Huma et al., 2009), fistulosin (Phay et al., 1999), and polyphenolcarboxylic acids (Pârvu et al., 2010b). The quality and quantity of the biologically active compounds from *Allium* sp. significantly depend on the species (Fritsch and Keusgen, 2006; Vlase et al., 2010), plant organ (Stajner et al., 2008) and the harvest time (Schmitt et al., 2005). That is why the biologically active compounds have to be determined from each plant extract.

Alliin is the precursor of allicin formed by the action of allinase enzyme. There is also a secondary substance resulting from alliin decomposition, called ajoene (Wang and Ng, 2003). The analysis of alliin from *A. senescens* ssp. *montanum* extract by LC/MS determined a smaller quantity than that from *A. obliquum* extract (Pârvu et al., 2010a).

Allicin has antibacterial (Cai et al., 2007; Josling, 2003), antiviral, antitumor, anticoagulant, antihypertensive, antiparasitic and hepatoprotective effects (Josling, 2003). It is also efficient against many fungal species, such as *Aspergillus flavus*, *A. niger*, *Candida albicans*, *Fusarium laceratum*, *Microsporum canis*, *Mucor racemosus*, *Penicillium* sp., *Rhizopus nigricans*, *Saccharomyces* sp., *Trichophyton granulosum* (Josling, 2003; Davis, 2005), *F. oxysporum* (Ogita et al., 2006), and other species (Davis, 2005; Khodavandi et al., 2010; Yamada and Azuma, 1977). That is why allicin content in *A. senescens* ssp. *montanum* extract is important for the antifungal activity.

In the present study, the total *A. senescens* ssp. *montanum* plant extract had antifungal effect against *A. niger, B. cinerea*, *B. paeoniae*, *F. oxysporum* f. sp. *tulipae,* and *P. gladioli*. The results are in accordance with other studies, which showed that antibacterial and antifungal effects of *Allium* plant extracts depend on the pathogenic species and on the type of plant extract (Abubakar, 2009; Pârvu et al., 2009, 2010a). The susceptibility of tested fungi to the extract of *A.* senescens ssp. *montanum* is noteworthy, since these fungi cause a broad spectrum of diseases.

Allicin proved to have important antifungal activity against the same fungi as *A. senescens* ssp. *montanum,* respectively *A. niger*, *B. cinerea, B. paeoniae, P. gladioli*, and *F. oxysporum* f. sp. *tulipae* (Table 1). It was observed that antibacterial and antifungal activities of allicin can be attributed to its interaction with the thiol group of proteins and amino acids and that, especially with the latter, allicin forms S-allyl derivatives. By these reactions SH-compounds inhibit the antibiotic properties of extract derived allicin and authentic allicin (Ogita et al., 2007). Another antifungal mechanism is the allicin-mediated lipoperoxide production in fungal plasma membrane with increased permeability (Horev-Azaria et al., 2009). These mechanisms may be involved in *A. senescens* ssp. *montanum* plant extract and allicin effects on tested fungi.

Against *F. oxysporum* f. sp. *tulipae*, *A. senescens* ssp. *montanum* plant extract was as efficient as allicin, suggesting that allicin may be the main antifungal compound. The reason why the antifungal activity of *A. senescens* ssp. *montanum* plant extract against *B. paeoniae* and *B. cinerea* is higher, and against *A. niger* and *P. gladioli* is lower, as compared to equivalent doses of allicin, is not yet fully explained. However, the extract contains other additional compounds that may influence the antifungal activity of allicin, in an additive or inhibitory manner (Pârvu et al., 2010b). Fluconazole is a reference antifungal drug. Knowing that tested fungi are involved in plant and human infections, it is important that the antifungal effect of the total *A. senescens* ssp. *montanum* plant extract against *A. niger* and *P. gladioli* was higher than that of fluconazole.

In conclusion, our present findings demonstrate that *A. senescens* ssp. *montanum* content in allicin is 0.919 mg/ml plant extract and in alliin is 58.37 µg/ml plant extract, and that the plant extract has antifungal properties against *A. niger*, *B. cinerea*, *B. paeoniae*, *F. oxysporum* f. sp. *tulipae*, and *P. gladioli*. The *in vitro* inhibitory action of the *A. senescens* ssp. *montanum* extract against the tested phytopathogenic fungi is higher, comparable or lower than that of allicin and fluconazole, depending on the species. These data may be useful in the development of new natural antifungal products in the near future.

ACKNOWLEDGEMENTS

This work was supported by CNCSIS –UEFISCSU, project number PNII – IDEI code 2272/2008.

REFERENCES

Abubakar EM (2009). Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. J. Med. Plants Res., 3: 179-185.

Barile E, Bonanomi G, Antignani V, Zolfaghari B, Sajjadi SE, Scala F,

Lanzotti V (2007). Saponins from *Allium minutiflorum* with antifungal activity. Phytochemistry, 68: 596–603.
Bhandari DK, Nath G, Ray AB, Tewari PV (2000). Antimicrobial activity of crude extracts from *Berberis asiatica* stem bark. Pharm. Biol., 38: 254-257.
Bowyer P, Fraczek M, Denning DW (2006). Comparative genomics of fungal allergens and epitopes shows widespread distribution of closely related allergen and epitope orthologues. BMC Genomics, 7: 251-264.
Brullo S, Guglielmo A, Pavone P, Salmeri C, Terrasi M (2003). Three new species of *Allium* sect. Codonoprasum from Greece. Plant Biosyst., 137: 131– 140.
Brullo S, Pavone P, Salmeri C, Terrasi MC (2009). *Allium garganicum* (Alliaceae), a new species from Apulia (SE Italy). Plant Biosyst., 143: S78 – S84.
Cai Y, Wang R, Pei F, Liang BB (2007). Antibacterial activity of allicin alone and in combination with β-lactams against *Staphylococcus* sp. and *Pseudomonas aeruginosa.* J. Antibiotics, 60: 335-338.
Carotenuto A, Fattorusso E, Lanzotti V, Magno S (1999). Spirostanol saponins of *Allium porrum* L. Phytochemistry, 51: 1077-1082.
Ciocârlan V (2009). Illustrated Flora of Romania Pteridophyta et Spermatophyta.Bucharest: Ed Ceres.
Davis SR (2005). An overview of the antifungal properties of allicin and its breakdown products--the possibility of a safe and effective antifungal prophylactic. Mycoses, 2: 95-100.
Duke JA, Bogenschutz-Godwin MJ, duCellier J, Duke PA (2002). Handbook of Medicinal Herbs. London, New York and Washington DC: CRC Press, Boca Raton.
Fattorusso E, Lanzotti V, Taglialatela-Scafati O (1999). Antifungal N-feruloyl amides from roots of two *Allium* species. Plant Biosyst., 133: 199-203.
Fern K (1996). Plants for a future edible and useful plants for a healthier world, Hampshire, UK: Permanent Publications.
Fritsch RM, Keusgen M (2006). Occurrence and taxonomic significance of cysteine sulphoxides in the genus *Allium* L. (Alliaceae). Phytochemistry, 67: 1127-1135.
Góra A, Mackiewicz B, Krawczyk P, Golec M, Skórska C, Sitkowska J, Grażyna C, Larsson L, Jarosz M, Wójcik-Fatla A, Dutkiewicz J (2009). Occupational exposure to organic dust, microorganisms, endotoxin and peptidoglycan among plants processing workers in Poland. Ann. Agric. Environ. Med., 16: 143-150.
Horev-Azaria L, Eliav S, Izigov N, Pri-Chen S, Mirelman D, Miron T, Rabinkov A, Wilchek M, Jacob-Hirsch J, Amariglio N, Savion N (2009). Allicin up-regulates cellular glutathione level in vascular endothelial cells. Eur. J. Nutr., 48: 67-74.
Huma Z, Vian MA, Maingonnat JF, Chemat F (2009). Clean recovery of antioxidant flavonoids from onions: Optimising solvent free microwave extraction method. J. Chromatogr. A, 1216: 7700-7707.
Ionescu-Stoian P, Savopol E (1977). Pharmaceutical plant extracts. Bucharest: Publishing Medical.
Josling P (2003). Allicin the Heart of Garlic. NWI Publishing Callahan Florida.
Khodavandi A, Alizadeh F, Aala F, Sekawi Z, Chong PP (2010). *In Vitro* investigation of antifungal activity of allicin alone and in combination with azoles against *Candida* species. Mycopathologia, 169(4): 287-295.
Ledezma E, Apitz-Castro R (2006). Ajoene, the major active compound derived from garlic (Alliumsativum), a new antifungal agent. Rev. Iberoam Micol., 23: 75-80.
Mahmoudabadi AZ, Nasery MKG (2009). Antifungal activity of shallot, *Allium ascalonicum* Linn. (Liliaceae)*, in vitro*. J. Med. Plants Res., 3: 450-453.
Mokoka TA, McGaw LJ, Eloff JN (2010). Antifungal efficacy of ten selected South African plant species against *Cryptococcus neoformans*. Pharm. Biol., 48: 397-404.
Nidiry ESJ, Babu CSB (2005). Antifungal activity of tuberose absolute and some of its constituents. Phytother. Res., 19: 447-449.
O'Donnell G, Gibbons S (2007). Antibacterial activity of two canthin-6-one alkaloids from *Allium neapolitanum*. Phytother. Res., 21: 653-657.
Ogita A, Fujita KI, Taniguchi M, Tanaka T (2006). Dependence of synergistic fungicidal activity of Cu^{2+} and allicin, an allyl sulfur compound from garlic, on selective accumulation of the ion in the plasma membrane fraction via allicin-mediated phospholipid peroxidation. Planta Med., 72: 875-880.
Ogita A, Nagao Y, Fujita K, Tanaka T (2007). Amplification of vacuole-targeting fungicidal activity of antibacterial antibiotic polymyxin B by allicin, an allyl sulfur compound from garlic. J. Antibiot. (Tokyo), 60: 511-518.
Pârvu M, Roşca-Casian O, Puşcaş M, Groza G (2009). Antifungal activity of *Allium fistulosum* L. Contrib. Bot., 44: 125-129.
Pârvu M, Pârvu AE, Roşca-Casian O, Vlase L, Groza G (2010a). Antifungal activity of *Allium obliquum*. J. Med. Plants Res., 4 (2): 138-141.
Pârvu M, Toiu A, Vlase L, Pârvu AE (2010b). Determination of some polyphenolic compounds from *Allium* species by HPLC-UV-MS'. Nat. Prod. Res., 24: 1318-1324.
Pârvu M, Pârvu AE, Vlase L, Roşca-Casian O, Pârvu O (2011). Antifungal properties of *Allium ursinum* L. ethanol extract. J. Med. Plants Res., 5(10): 2041-2046.
Phay N, Higashiyama T, Tsuji M, Matsuura H, Fukushi Y, Yokota A, Tomita F (1999). An antifungal compound from roots of Welsh onion. Phytochemistry, 52: 271-274.
Piecková E, Wilkins K (2004). Airway toxicity of house dust and its fungal composition, Ann. Agric. Environ. Med., 11: 67-73.
Ren G, Qiao HX, Yang J, Zhou CX (2010). Protective effects of steroids from *Allium chinense* against H2O2-induced oxidative stress in rat cardiac H9C2 cells. Phytother. Res., 24: 404-409.
Riotte L (1998). Roses love garlic. Companion Planting for Successful Gardening, North Adams, US: Storey Publishing.
Schmitt B, Schulz H, Storsberg J, Keusgen M (2005). Chemical characterization of *Allium ursinum* L. depending on harvesting time. J. Agric. Food Chem., 53: 7288-7294.
Shams-Ghahfarokhi M, Shokoohamiri MR, Amirrajab N, Moghadasi B, Ghajari A, Zeini F, Sadeghi G, Razzaghi-Abyaneh M (2006). *In vitro* antifungal activities of *Allium cepa*, *Allium sativum* and ketoconazole against some pathogenic yeasts and dermatophytes. Fitoterapia, 77: 321-323.
Singh UP, Pandey VN, Wagner KG, Singh KP (1990). Antifungal activity of ajoene, a constituent of garlic (*Allium sativum*). Can. J. Bot., 68: 1354-1356.
Stajner D, Popovic BM, Canadanovic-Brunet J, Stajner M (2008). Antioxidant and scavenger activities of *Allium ursinum*. Fitoterapia, 79(4): 303-305.
Vlase L, Pârvu M, Toiu A, Pârvu AE, Cobzac CS, Puşcaş M (2010). Rapid and Simple Analysis of Allicin in Alium Species by LC-CIS-MS/MS. Studia UBB. Chemia, 55(4): 297-304.
Wang HX, Ng TB (2003). Isolation of allicepin, a novel antifungal peptide from onion (*Allium cepa*) bulbs. J. Pept. Sci., 10: 173-177.
Yamada Y, Azuma K (1977). Evaluation of the *in vitro* antifungal activity of allicin.Antimicrob. Agents Chemother.,11(4): 743-749.