

ANTIFUNGAL ACTIVITY OF *ALLIUM FISTULOSUM* L.

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Abstract: A hydroalcoholic extract of *Allium fistulosum* was tested for *in vitro* germination and growth of phytopathogenic fungi (*Apergillus niger*, *Botrytis cinerea*, *Botrytis paeoniae*, *Penicillium gladioli*, and *Sclerotinia sclerotiorum*) on Czapek-agar nutritive medium, compared to Fluconazole and allicin. The minimum inhibitory concentration (MIC) of the plant extract varied between 80 μl/ml and 100 μl/ml, according to the fungal species.

Keywords: agar-dilution assay, allicin, *Allium fistulosum*, antifungal activity, Fluconazole, *in vitro*, MIC, phytopathogenic fungi, plant extract, repercolation

Introduction

Romania has 21 wild and 3 cultivated *Allium* species. One of the cultivated species is *A. fistulosum* L. It is a perennial species, has alimentary importance and is originated from Eastern Asia. It is not known as a wild species [4, 24].

Allium fistulosum (Welsh onion) is an Eastern species, unknown in a natural state. It is being cultivated for its leaves which are fresh consumed all over the year, still green over the winter.

Literature data regarding the phytotherapeutic properties of *Allium* species are focused mainly on *Allium cepa* and *A. sativum* plants [5, 12, 22].

Allium sativum plant extract has antihypertensive effect [2, 27], antihelminthic and antiparasitic effect [9], antidiabetic effect [27], hepatoprotective effect [25], antifungal effect against some *Candida albicans* isolates [15], and inhibitory effect against some *Staphylococcus aureus* isolates [8].

Biologically active compounds (allicin, alliin, E/Z-ajoene) isolated from plant extracts of *Allium sativum*, *A. cepa* and *A. ampeloprasum* have antibacterial and antifungal activity against some *Candida* isolates [10]. Antiinfective action of *A. sativum* plant extract may be used to cure the mycoses caused by *Candida albicans* [13], or by other *Candida* species [23].

Aqueous extracts from *Allium cepa* and *A. sativum* have antifungal action against *Candida albicans* and other *Candida* isolates, against *Malassezia furfur* isolates and dermatophyte species [7]. *A. cepa* plant extract also have antifungal action against *Tricophyton rubrum* and *Tricophyton mentagrophytes* species [6].

Other studies showed that crude extracts (water, ethanol, chloroform) of *Allium sativum* had antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, at minimum bacterial concentrations (MBC) depending on the species and on the type of plant extract [1].

The present study aims at evaluating the antifungal activity of *Allium fistulosum* plant extract against some phytopathogenic fungal species (*Apergillus niger*, *Botrytis cinerea*, *B. paeoniae*, *Penicillium gladioli*, *Sclerotinia sclerotiorum*).

Materials and Methods

Plant material of *Allium fistulosum* L. (**Liliaceae**) was obtained from the Agrobotanical Garden of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania. A voucher specimen (CL 659761) is deposited at the Herbarium of “A. Borza” Botanical Garden, “Babeș-Bolyai” University of Cluj-Napoca, Romania.

The plant extract was obtained from *A. fistulosum* leaves, stems and flowers (herba) in 70% ethanol. The used method was Squibb's repercolation technique, according to Romanian Farmacopoeia [11].

The fungal species were isolated from the attacked plants, as follows: *Aspergillus niger* from onion bulbs, *Botrytis cinerea* from rose flowers, *B. paeoniae* from paeony flowers, *Penicillium gladioli* from gladiolus corms and *Sclerotinia sclerotiorum* from carrot roots. These species were cultivated on Czapek-agar nutritive medium (BD Difco, Budapest, Hungary) by inoculation into the central point and incubation at 22°C for 5 days, and pure colonies were obtained.

The antifungal activity, expressed as minimum inhibitory concentration (MIC), was determined by agar-dilution assay [3], and was compared to the control (nutritive medium and 70 % EtOH), an antimycotic drug (Fluconazole) and allicin (Allicin International LTD - Allimed Liquid), as standards. There were obtained three replicates for each considered concentration. The percentage of mycelial growth inhibition (P) was calculated for each concentration using the formula: $P = (C-T) \times 100/C$, where C is the diameter of the control colony and T is the diameter of the treated colony [16].

Results and Discussions

The antifungal activity of *A. fistulosum* plant extract is briefly presented in Table 1. The MIC of the plant extract depended on the fungal species. In the case of *Aspergillus niger*, the MIC was 100 µl/ml for the plant extract and allicin and 300 µl/ml for Fluconazole. In case of *Botrytis* species, the MIC was the same in case of *A. fistulosum* plant extract (80 µl/ml) and 120 µl/ml in case of Fluconazole. The allicin mostly inhibited *B. paeoniae* (120 µl/ml). The growth of *Penicillium gladioli* fungus was inhibited by *A. fistulosum* plant extract at a MIC of 100 µl/ml, compared to allicin (120 µl/ml). The antimycotic drug, Fluconazole, had no important antifungal activity against *P. gladioli*. At a concentration of 200 µl/ml, the colony's diameter was still 10 mm (Tab. 1). *A. fistulosum* plant extract proved to be very efficient against *Sclerotinia sclerotiorum* fungus, the same as Fluconazole (MIC = 80 µl/ml). Allicin had the same effect at a double concentration (MIC = 160 µl/ml) (Tab. 1).

Allium plants and extracts contain different chemical compounds. Allicin is the most important biologically active substance of *Allium sativum* crude extract [12, 25]. It is formed from its precursor, alliin, by the action of allinase enzyme. Allicin has antifungal, antibacterial, antiviral, antitumour, anticoagulation, antihypertensive, antiparasitic, hepatoprotective, etc., activity. Allicin is efficient against many fungal species, like *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Fusarium laceratum*, *Microsporium canis*, *Mucor racemosus*, *Penicillium* spp., *Rhizopus nigricans*, *Saccharomyces* spp., *Trichophyton granulosum*, etc. [12]. *Allium sativum* also has another biologically active compound, called ajoene, which is a secondary substance resulting from alliin decomposition. Besides these biologically active substances, a novel antifungal peptide was isolated from *Allium cepa* bulbs, called allicepin [26].

Another species with antimicrobial activity is *Allium ascalonicum*. It has antifungal action against *Candida albicans*, dermatophytes (*Microsporium gypseum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*), *Syncephalastrum* spp., *Aspergillus niger*, *Penicillium* spp., *Paecilomyces* spp., *Scopulariopsis* spp., *Cladosporium* spp., *Alternaria* spp., *Drechslera* spp. at MIC of 0.25 % [14].

Table 1. The action of *Allium fistulosum* plant extract on *in vitro* germination and growth of some phytopathogenic fungi

Fungal species	<i>Allium fistulosum</i> extract (µl/ml)	Colony's ^a diameter (mm)	P ^a (%)	Fluconazole (µl/ml)	Colony's ^b diameter (mm)	P ^b (%)	Allicin (µl/ml)	Colony's ^c diameter (mm)	P ^c (%)
<i>Aspergillus niger</i>	C	22	0	C	22	0	C	22	0
	30	20	9.09	100	11.66	47	20	20	9.09
	60	8	63.63	200	7.66	65.18	40	12	40
	80	3	86.36	250	4.33	80.31	60	8	63.63
	100	0	100	300	0	100	80	4	81.81
							100	0	100
<i>Botrytis cinerea</i>	C	65	0	C	65	0	C	65	0
	20	60	7.69	20	40.33	37.95	30	61	6.15
	40	32	50.76	60	20	69.23	60	44	32.30
	60	12	81.53	100	5.33	91.80	80	31	52.30
	80	0	100	120	0	100	100	19	70.76
							120	5	92.30
							140	0	100
<i>Botrytis paeoniae</i>	C	60	0	C	60	0	C	60	0
	20	55	8.33	20	50	16.66	40	58	3.33
	40	31	48.33	60	24	60	60	46	23.33
	60	10	83.33	100	5	91.66	80	28	53.33
	80	0	100	120	0	100	100	5	91.66
							120	0	100
<i>Penicillium gladioli</i>	C	15	0	C	15	0	C	15	0
	20	13	13.33	100	11	26.66	30	13	13.33
	40	10	33.33	120	11	26.66	60	10	33.33
	60	6	60	160	10	33.33	80	6	60
	80	3	80	200	10	33.33	100	3	80
	100	0	100				120	0	100
<i>Sclerotinia sclerotiorum</i>	C	64	0	C	64	0	C	64	0
	20	62	3.12	20	30	53.12	40	58	9.37
	40	32	50	40	15	76.56	60	48	25
	60	12	81.25	60	5	92.18	80	28	56.25
	80	0	100	80	0	100	100	5	92.18
							160	0	100

Legend: ^a = the effect of *A. fistulosum* extract; ^b = the effect of Fluconazole; ^c = the effect of allicin; C = control (70% aq. EtOH); P = mycelial growth inhibition

There were done several studies regarding the action of plant extracts against some phytopathogenic fungi. Such studies were those concerning the antifungal action of *Berberis vulgaris*, *Chelidonium majus*, and *Solanum nigrum* plant extracts against *in vitro* germination and growth of *Coniothyrium concentricum* fungus [18]. Others dealt with the antifungal action of *Berberis vulgaris*, *Solanum nigrum*, walnut peel extract, and a mixture of five plants extracts against *Fusarium oxysporum* phytopathogen [19]. Other results concerning the antifungal action of plant extracts were those obtained in testing *Aloë vera* against *Botrytis gladiolorum*, *Fusarium oxysporum* f.sp. *gladioli*, *Heterosporium pruneti*, and *Penicillium gladioli* [21] and *A. arborescens* against *Aspergillus niger*, *Botrytis cinerea*, *B. gladiolorum*, *Fusarium oxysporum* f. sp. *gladioli*, and *Penicillium gladioli* [20]. Plant extracts were also tested regarding the ultrastructural changes they cause in some fungal species, such as *Berberis vulgaris* against *Sclerotinia sclerotiorum* [17].

Conclusions

Allium fistulosum plant extract was active against the studied phytopathogenic fungi, and the inhibition degree depended on its concentration in the nutritive medium and the phytopathogenic fungus. The MIC of *A. fistulosum* plant extract varied between 80μl/ml – 100μl/ml, according to the species. The inhibitory activity of plant extract was more powerful than that of Fluconazole and allicin, respectively. The MIC for Fluconazole varied between 80μl/ml – 300μl/ml, and for allicin between 100μl/ml – 160μl/ml.

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ACȚIUNEA ANTIFUNGICĂ A EXTRACTULUI OBȚINUT DIN *ALLIUM FISTULOSUM* L.

(Rezumat)

Extractul hidroalcoolic de *Allium fistulosum* a fost testat asupra germinării și creșterii *in vitro* a unor ciuperci fitopatogene (*Apergillus niger*, *Botrytis cinerea*, *Botrytis paeoniae*, *Penicillium gladioli* și *Sclerotinia sclerotiorum*) pe mediul nutritiv Czapek-agar, comparativ cu produsul antimicotic Fluconazol și allicină (substanță etalon). Concentrația minimă inhibitoare (CMI) a extractului vegetal a variat între 80 μl/ml și 100 μl/ml, în raport de ciuperca fitopatogenă. Comparativ cu acesta, pentru produsul Fluconazol, CMI a variat între 80 μl/ml – 300 μl/ml, iar pentru allicină, între 100 μl/ml – 160 μl/ml. Rezultatele obținute completează datele din literatura de specialitate referitoare la acțiunea antifungică a speciilor de *Allium* și subliniază eficacitatea crescută a extractului vegetal comparativ cu allicina și produsul Fluconazol, în cazul unor specii.