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ANTIFUNGAL ACTIVITY OF *LAETIPORUS SULPHUREUS* MUSHROOM EXTRACT

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Abstract: The hydroalcoholic extract of *Laetiporus sulphureus* mushroom had antifungal action against *in vitro* germination and growth of *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *tulipae*, *Penicillium gladioli* and *Sclerotinia sclerotiorum* fungi, on Czapek-agar nutritive medium. The minimum inhibitory concentration of *Laetiporus sulphureus* varied between 100 µl/ml and 160 µl/ml, according to the fungal species and was compared to the action of the antimycotic fluconazole.

Keywords: *Laetiporus sulphureus*, inhibitory action, phytopathogenic fungi, hydroalcoholic extract, MIC (minimum inhibitory concentration), fluconazole, repercolation, agar-dilution method, mycelial growth inhibition, antifungal activity

Introduction

Laetiporus sulphureus (Bull.) Murrill causes an intense brown rot in the heartwood of standing trees. The mycelium is clearly long-lived, with fresh crops of basidiocarps produced annually over several years in the living tree, and later from the dead trunk. *Laetiporus* is considered to be one of the main causes of the hollowing of old oak trees in parks [30].

Studies centred on the investigation of *L. sulphureus* ethanol extracts for antioxidant and antimicrobial activities have found positive correlations between total phenolic content in the mushroom extracts and their antioxidant activities. Investigations have also shown narrow anti-bacterial activity against Gram-negative bacteria and have demonstrated that fungus extracts strongly inhibited the growth of the Gram-positive bacteria. Additionally, the crude extract exhibited high anticandidal activity on *Candida albicans* [26]. For instance, a cyclo-depsipeptide, beauvericin, presenting antimicrobial activity, was isolated from *L. sulphureus*. Zjawiony also reported that compounds such as egonol, demethoxyegonol and egonol glucoside, all isolated from *L. sulphureus* var. *miniatus*, exhibited low cytotoxic activity [32].

Further analyses carried out on *L. sulphureus* and its hydro-alcoholic extracts showed their particular capacity to inhibit HIV-1 reverse transcriptase by 90.1% [14]. Extracellular polysaccharides (EPS) in submerged culture exhibited stimulatory effects on insulinoma cell (RINm5F) proliferation and insulin secretion [7].

Studies revealed that acetyl eburicoic acid (a lanostanoid triterpene from *L. sulphureus*) was a potent apoptosis inducer. Apoptosis was accompanied by both the activation of caspase-3 and the fragmentation of poly (ADP-ribose) polymerase-1 and was also associated with an early release of cytochrome c from the mitochondria [13]. Moreover, antithrombin substances and protein-polysaccharide fraction implying antitumor and imunostimulating activity were noticed in the composition of this fungus [10, 18].

Aspergillus niger is a fungus, the most common species of the genus *Aspergillus*, which causes a disease called black mould on certain fruits and vegetables such as grapes, onions and

peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments [24].

Botrytis cinerea is an important polyphagous fungus that causes grey mould in vegetables, ornamentals, fruits and even some field crops. At the same time, infection with this fungus can be particularly damaging to fruits and flowers held in storage [1].

Fusarium oxysporum f. sp. *tulipae* is a serious disease of tulips and occurs both in the field and in the glasshouse. Primary symptoms during flowering (October through November) include basal stem rot, yellowing and wilting of shoots, and bulb rot [3].

Penicillium gladioli attacks bruised and wounded gladiolus corms in storage. At low temperatures, an abundant blue-green mold grows over the lesions. Numerous egg-shaped, tan to cream-coloured sclerotia may be found embedded in the rotted corm tissue [25].

Sclerotinia sclerotiorum is among the most non-specific, omnivorous, and successful plant pathogens. Hosts include: cabbage, common bean, citrus, celery, coriander, melon, squash, soybean, and tomato. *S. sclerotiorum* is geographically cosmopolitan and has a broad ecological distribution, though it is most common in temperate regions. It was originally believed to occur only in cool, moist areas, but is now known to occur in hot, dry areas as well [21].

The phytopathogenic fungi were used to emphasize the antifungal action of mushrooms and/or plant extracts. Thus, *Botrytis cinerea*, *Fusarium oxysporum*, *Physalospora pyricola* isolates [28] were used in the studies with mushroom extracts.

The crude extracts obtained from the leaves of *Pistacia vera*, *P. terebinthus* and *P. lentiscus* were tested for antifungal activities against three pathogenic agricultural fungi, *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium sambucinum* [12].

The aim of this study was to evaluate *Laetiporus sulphureus* mushroom extract effect on the germination and growth of *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *tulipae*, *Penicillium gladioli* and *Sclerotinia sclerotiorum* fungi on Czapek-agar nutritive medium.

We mention that these phytopathogenic fungi were also used by us to emphasize the anti-fungal action of other plant extracts: *Berberis vulgaris*, *Chelidonium majus*, *Solanum nigrum* [19, 20], etc.

Materials and Methods

Fruiting bodies of *Laetiporus sulphureus* were collected from an ash (*Fraxinus ornus*) tree, growing in "Alexandru Borza" Botanical Garden in Cluj-Napoca, during May 2008. The fungus was identified by Dr M. Pârvu, "Babeş-Bolyai" University of Cluj-Napoca.

A. niger was isolated from bulbs of *Allium cepa*, *B. cinerea* from flowers of *Rosa* spp., *F. oxysporum* f. sp. *tulipae* from bulbs of *Tulipa* spp., *P. gladioli* from corms of *Gladiolus* spp. and *S. sclerotiorum* from the root of carrot (*Daucus carota*). The isolated species were acquired from the Mycology Laboratory, "Babeş-Bolyai" University, Cluj-Napoca. Colonies were obtained in Petri dishes, on Czapek agar medium (BD Difco, Budapest, Hungary), by inoculation at the central point and incubation at 22ºC for 5 days.

Laetiporus sulphureus extract (1:1) was obtained from the fresh fragments (0.5-1 cm) of fruiting bodies of mushroom with 70% ethanol (Merck, Bucureşti, Romania), in the Mycology Laboratory of "Babeş-Bolyai" University by modified Squibb's repercolation method [9], and stored at low temperature.

Antifungal activity of *L. sulphureus*, expressed as minimum inhibitory concentration (MIC), was determined by agar-dilution method with the antimycotic drug fluconazole (2 mg/ml; Krka, Novo mesto, Slovenia). The percentage of mycelial growth inhibition (P) at each concentration, for every phytopathogenic fungus, was calculated by the formula $P=[C-T] \times 100/C$, where C represents the diameter of the control colony and T the diameter of the treated colony [16].

Results and Discussion

The research conducted on *A. niger* revealed that the inhibitory action of *L. sulphureus* extract was proportional to its concentration in the culture medium. Several experimental tests were done using concentrations varying from 40 to 140 µl/ml extract in culture medium. The mushroom extract had a significant inhibitory *in vitro* effect on the mycelial growth of *A. niger*, with MIC of 140 µl/ml, compared to the value of fluconazole (300 µl/ml) (Tab. 1).

Table 1: The action of *Laetiporus sulphureus* mushroom hydroalcoholic extract on *in vitro* germination and growth of phytopathogenic fungi

Phytopathogenic fungus	*L. sulphureus* extract (µl/ml)	Colony diameter[a] (mm)	P[b] (%)	Fluconazole (µl/ml)	Colony diameter[c] (mm)	P[d] (%)
Aspergillus niger	C	22	0	C	22	0
	40	18	18.18	100	11.66	47.27
	60	13	40.90	200	7.66	65.18
	80	10	54.54	250	4.33	80.31
	100	7	68.18	300	0	100
	120	4	81.81			
	140	0	100			
Botrytis cinerea	C	65	0	C	65	0
	20	62	4.61	20	40.33	37.95
	40	51	21.53	60	20	69.23
	60	30	53.84	100	5.33	91.80
	80	15	76.92	120	0	100
	100	0	100			
Fusarium oxysporum f. sp. *tulipae*	C	32	0	C	32	0
	40	26	18.75	20	20	37.50
	60	22	31.25	60	8	75
	80	18	43.75	80	2	93.75
	100	14	56.25	100	0	100
	120	10	68.75			
	140	5	84.37			
	160	0	100			
Sclerotinia sclerotiorum	C	64	0	C	64	0
	40	62	3.12	20	30	53.12
	60	50	21.87	40	15	76.56
	80	22	65.62	60	5	92.18
	100	5	92.18	80	0	100
	120	0	100			
Penicillium gladioli	C	15	0	C	15	0
	20	13	13.33	100	11	26.66
	40	10	33.33	120	11	26.66
	60	6	60	160	10	33.33
	80	3	80	200	10	33.33
	100	0	100			

Legend: [a]Mycelial growth of the phytopathogenic fungi at 5 days after inoculation, in presence of *L. sulphureus* extract.
[b]Mycelial growth inhibition in presence of *L. sulphureus* extract.
[c]Mycelial growth of the phytopathogenic fungi at 5 days after inoculation, in presence of fluconazole.
[d]Mycelial growth inhibition in presence of fluconazole.
C = 70% aq. EtOH; colony diameter is expressed as mean ± SE of four replicates.

In the case of *Botrytis cinerea*, the level of the concentration varied between 20 and 100 µl/ml, and the result consisted of an inhibitory action of the *L. sulphureus* extract, proportional to its concentration in culture medium. The MIC occurred at a concentration of 100 µl/ml extract in culture medium in comparison with fluconazole, which had 120 µl/ml (Tab. 1).

As far as the results for the *Fusarium oxysporum* f. sp. *tulipae* and *Sclerotinia sclerotiorum* species are concerned, the registered MIC was higher compared to that of fluconazole. For *Fusarium oxysporum* f. sp. *tulipae*, the concentrations varied between 40 and 160 µl/ml with the MIC recorded at the value of 160 µl/ml extract in culture medium (Tab. 1). A similar result has been registered in the case of *Sclerotinia sclerotiorum*, where the MIC surpassed that of fluconazole, establishing a value of 120 µl/ml compared to 80 µl/ml in culture medium (Tab. 1).

The MIC obtained for *P. gladioli* reached 100 µl/ml extract in culture medium, a better result than that registered for fluconazole (Tab. 1).

Some literature data mention the fact that basidiocarp extract preparation from *Laetiporus sulphureus* has a relatively high antimicrobial activity against yeasts and bacteria [5]. Also, the ethanolic extract of *L. sulphureus* shows antimicrobial activity against *Yersinia enterocolitica* [26]. A few studies report that the bioactive compounds from *L. sulphureus* responsible for antioxidant and antimicrobial activities, are represented by phenols and flavonoids [2].

The remarkable biological activities of *L. sulphureus* preparations against major diseases [22] are well known. For example, there is evidence that the polysaccharides derived from the cell walls of *L. sulphureus* have antitumor effects. They prevent carcinogenesis, show direct anti-cancer effects, and prevent tumor metastasis. Such protein-bound polysaccharides are found on the market as anti-cancer drugs [32].

Other studies present the antibacterial and/or antifungal activity of different mushrooms, such as: *Agrocybe cylindracea* [15], *Stereum ostrea*, *Pycnoporus cinnabarinus*, *P. coccineus*, *Oudemansiella mucida*, *Cordyceps sobolifera*, *Armillaria mellea*, *Calvatia craniiformis*, *Dictyophora indusiata*, *Pholiota adiposa* [8], *Ganoderma lucidum* [17, 28], *Lentinula edodes* [6, 11], *Phellinus linteus* [23], *Pleurotus ostreatus* [4, 6], *Ganoderma colossum*, *G. resinaceum*, *G. lucidum* and *G. boninense* [17], *Russula delica* [31], *Morchella conica* [27], etc. Recent studies show the presence of different antifungal peptides and/or proteins, in mushrooms extracts, such are: agrocybin in *Agrocybe cylindracea* [15], pleurostrin in *Pleurotus ostreatus* [4], alveolarin in *Polyporus alveolaris* [29], ganodermin in *Ganoderma lucidum* [28], etc.

Conclusions

Our studies present new published data regarding the antifungal action of *Laetiporus sulphureus* mushroom extract against the phytopathogenic fungi *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *tulipae*, *Penicillium gladioli*, and *Sclerotinia sclerotiorum*. *L. sulphureus* extract has inhibitory action against the germination and growth of some common phytopathogenic fungi comparable to the antimycotic fluconazole and it will be used in further extensive studies.

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ACȚIUNEA ANTIFUNGICĂ A EXTRACTULUI DIN CIUPERCA *LAETIPORUS SULPHUREUS*

(Rezumat)

Extractul hidroalcoolic din ciuperca *Laetiporus sulphureus* a manifestat acțiune antifungică *in vitro* asupra creşterii şi germinării ciupercilor fitopatogene *Aspergillus niger, Botrytis cinerea*, *Fusarium oxysporum* f. sp. *tulipae*, *Penicillium gladioli* şi *Sclerotinia sclerotiorum*, pe mediul Czapek-agar. Concentrația minimă inhibitoare a extractului de *Laetiporus sulphureus* a variat între 100 µl/ml şi 160 µl/ml extract în mediul de cultură, în funcție de specia analizată şi a fost comparată cu acțiunea produsului antimicotic fluconazol.

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