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Antifungal activity of *Antrodia cinnamomea* extracts: Efficacy & resistance

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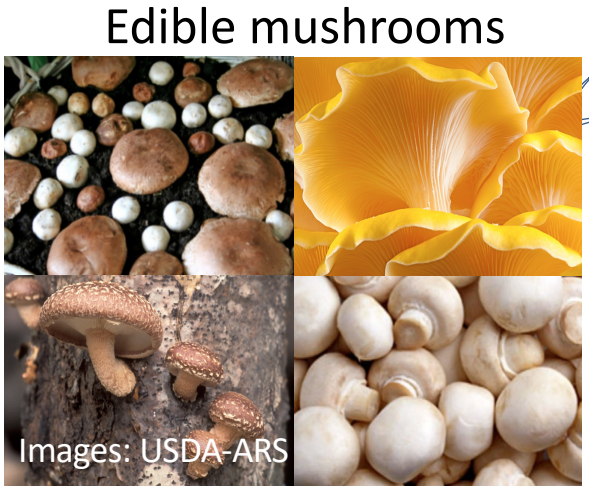
Graphical Abstract



ABSTRACT:

- We investigated **antifungal activities** of the water or methanol extracts of the **medicinal mushrooms** *Antrodia cinnamomea*, *Agaricus blazei* and *Ganoderma* spp. against yeast (*Candida albicans*) and filamentous (*Aspergillus fumigatus*) fungal pathogens.
- In the zone of inhibition bioassay, only the **methanol extracts of *A. cinnamomea* (AcM)** exhibited potent antifungal activity against ***C. albicans*** and ***A. fumigatus***.
- **Risk assessment** identified that two ***Penicillium expansum* antioxidant mutants** tolerant to the conventional antifungal agent, fludioxonil, also presented **tolerance to AcM**.
- Results indicated AcM antifungal action is mediated via the **normal antioxidant signaling system** in fungi, where the antioxidant mutants escape the toxicity triggered by AcM.
- In a **benzoic analog** bioassay, *P. exansum* mutants showed similar type of tolerance to the benzo derivative, thus indicating natural ingredients in AcM, such as **benzoics**, could **negatively affect** the efficacy of AcM when antioxidant mutants are targeted.
- Collectively, AcM could be developed as an effective antifungal agent; **caution** should be exercised in the use of AcM as an antifungal so as not to trigger resistance of antioxidant mutants to the treatment.

Keywords: antifungal; antioxidant mutant; *Antrodia cinnamomea*; medicinal mushrooms; resistance

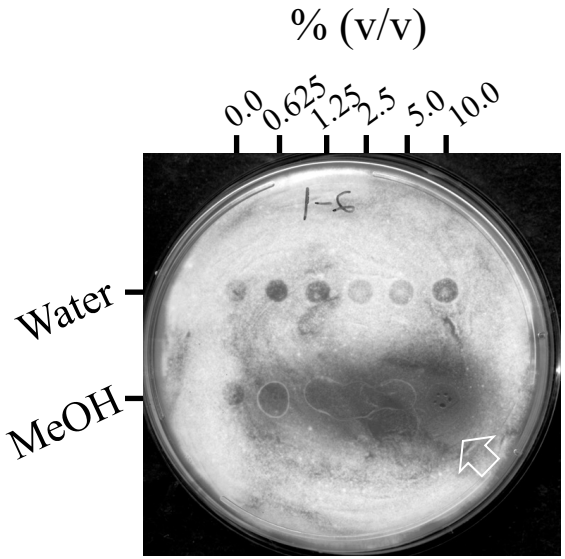
Antifungal activity of *Antrodia cinnamomea* methanol extracts (AcM)

Antrodia cinnamomea powder

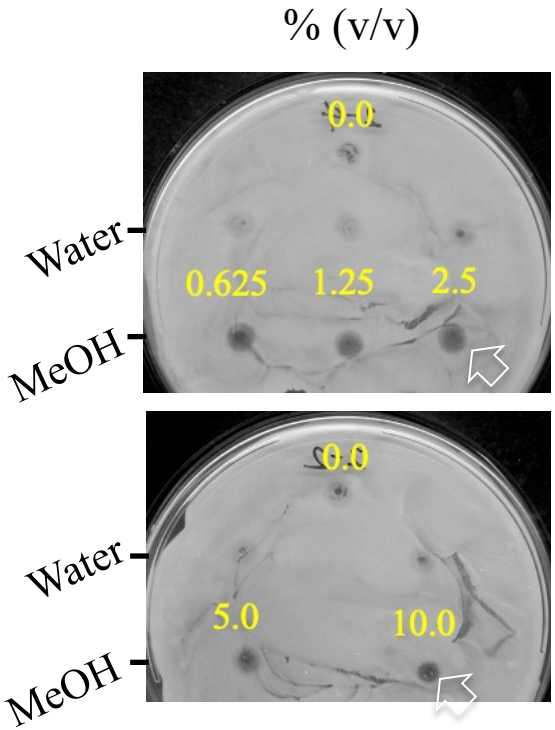
Water extract

Methanol extract

Testing antifungal activity in *Candida albicans* & *Aspergillus fumigatus*



A. fumigatus AF293



C. albicans ATCC10231

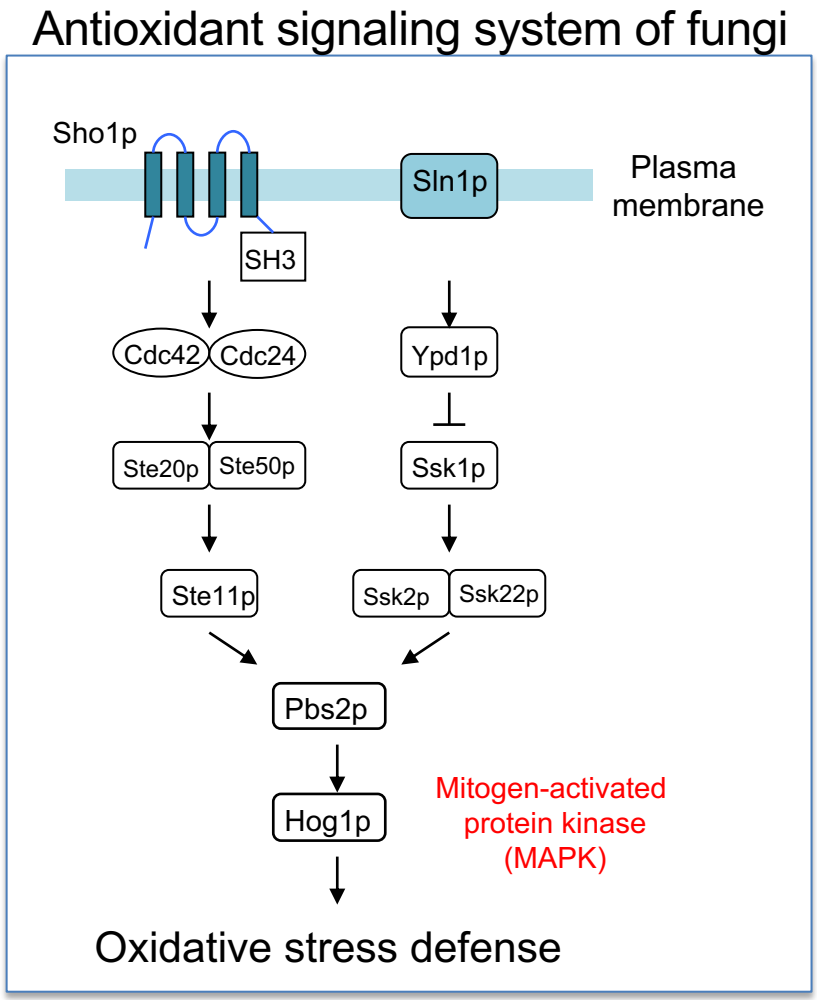
Antioxidant mutants of fungi showed tolerance to AcM

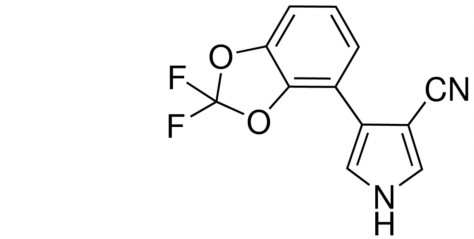
Table 1. Antifungal activity of AcM (% Radial growth compared to no treatment control)[1]

Extract, % (v/v) / Fungi	0.0	0.8 [2]	1.6 [2]
N. fischeri 96468	100±0%	15±2%	15±0%
A. flavus 3357	100±3%	48±5%	38±0%
A. parasiticus 5862	100±2%	54±0%	42±0%
P. expansum W1 (wild type)	100±0%	57±0%	36±11%
P. expansum FR2 (mutant)	100±15%	91±0% ($p < 0.5$)	82±5% ($p < 0.1$)
P. expansum W2 (wild type)	100±0%	43±16%	43±4%
P. expansum FR3 (mutant)	100±6%	70±0%	40±23% ($p < 0.05$)
Average	**100±4%**	**54±3%**	**42±6%**

[1] Potato Dextrose Agar (PDA; defined medium) assay; red characters: tolerant compared to the wild type.
[2] $p < 0.005$ except where noted.

Intact antioxidant signaling system is necessary for fludioxonil antifungal activity





Fludioxonil (antifungal agent)

MAPK mutant

Risk assessment tool

Resistance

Fungal pathogen control

Antioxidant mutants of fungi showed tolerance to AcM

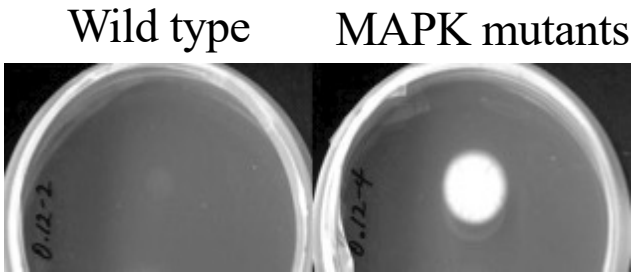
Table 2. Antifungal activity of AcM (% Radial growth compared to no treatment control)[1]

Extract, % (v/v) / Fungi	0.0	0.8 [2]	1.6 [2]
N. fischeri 96468	100±6%	45±9%	41±3%
A. flavus 3357	100±6%	66±11% ($p < 0.01$)	55±9%
A. parasiticus 5862	100±5%	58±10%	58±3%
P. expansum W1 (wild type)	100±10%	56±10%	43±8%
P. expansum FR2 (mutant)	100±17%	95±40% ($p = 0.8$)	100±28% ($p = 1$)
P. expansum W2 (wild type)	100±11%	53±6%	40±8%
P. expansum FR3 (mutant)	100±16%	154±40% ($p < 0.5$)	108±43% ($p < 1$)
Average	**100±10%**	**75±18%**	**64±15%**

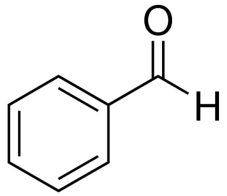
[1] Complex medium (1.5% select agar base) assay; red characters: tolerant compared to the wild type.
[2] $p < 0.005$ except where noted.

Fungal MAPK mutants are also tolerant to benzo analog (mushroom extracts)

(a)



(b)



Fludioxonil (antifungal agent), Benzoics (mushroom extracts)

MAPK mutant

Risk assessment tool

Resistance

Fungal pathogen control

Conclusions

- **Edible mushrooms** are rich sources of bioactive metabolites that also possess potent **antifungal activity**.
- Natural ingredients in mushroom extracts, such as **benzoic derivatives**, could negatively affect the fungal antioxidant signaling mutants.
- For example, *P. expansum* **mitogen-activated protein kinase (MAPK)** mutants exhibited tolerance to the benzoic analogs while the wild type strains remain susceptible to the molecule.
- Collectively, results proved the significance of **risk assessment** during the preclinical stage of antifungal development, thus circumventing the unfavorable downside of mushroom extracts.

Acknowledgments

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