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Research Article

Assessment of antifungal activity of some boletes mushrooms found in Himalayan range of Pakistan against some fungi

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Abstract

Ectomycorrhizal mushrooms belonging to Boletales (Basidiomycota) viz. *Boletus edulis* Bull, *Hortiboletus rubellus* Krombh and *Suillus sibiricus* Singer were selected to evaluate their antifungal potential against some filamentous fungi viz. *Aspergillus terreus* Thom, *Aspergillus niger* Tiegh and *Rhizopus stolonifer* Vuill. Extracts of these selected mushrooms were prepared in methanol and their various concentrations (0%, 1%, 1.5%, 2% and 3%) were tested against selected pathogenic fungi. *B. edulis* showed maximum decline (51-53%) in the biomass of *R. stolonifer*, *S. Sibiricus* showed 40-45% decline in biomass of *A. terreus*, while all the concentrations of *H. rubellus* showed 50-55% decline in the biomass of *A. niger*. Present work revealed that selected indigenous mushrooms have strong antifungal potential against different pathogenic fungi. So, these mushrooms can be used as source material for fungal control methods.

Keywords: Ascomycetes; Boletales; Ectomycorrhizal fungi

Introduction

Fungi consist of a large group of eukaryotic organisms comprising more than 1,500,000 species **[1]**. Most species are usually used as a source of food whereas some fungi are mostly pathogenic to different organisms including plants **[2]** i.e., *Aspergillus terreus* causes damage to wheat and ryegrass as well as foliar blight of potatoes; *Rhizopus stolonifer* is saprophytic to bread **[3]**; *A. terreus* and *A. niger* are destroying over 125 million tons of rice, wheat, potato, maize and soya bean every year **[4]**. *A. niger* can also cause black mold on plants **[5]**.

Contrastingly, macromycetes fungi have known antitumor, antifungal, antibacterial activities due to their chemical composition **[6]**. They constitute ectomycorrhizal association with the roots of higher plants. *Boletus edulis*, *Hortiboletus rubellus* and *Suillus sibiricus* included in order Boletales are ectomycorrhizal, medicinal fungi. *B. edulis* is well known edible mushroom and in many parts of the world it is used as an important food source **[7]**. Their chemical composition makes them an antifungal agent. These mushrooms were collected from moist temperate Himalayan range of Pakistan and identified by morphological and molecular methods by first author **[8]** and for present research work these are selected to evaluate their antifungal potency against some pathogenic micromycetes.

Materials and methods

Selection of mushrooms

Ectomycorrhizal fungi (mushrooms) *Hortiboletus rubellus*, *Boletus edulis* and *Suillus sibiricus* used in the present work were identified by morphological and molecular methods by first author [8]. So, for the further progress these mushrooms were selected to evaluate their antifungal potential against *Aspergillus terreus*, *Aspergillus niger* and *Rhizopus stolonifer*.

Isolation and characterization of pathogenic fungi

Pure cultures of *A. terreus*, *A. niger* and *R. stolonifer* were prepared on 2% Malt extract agar (MEA) medium. These pure cultures were stored at 4 °C for further use [9]. In macroscopic characterization of fungal colonies, forms, colour, appearance on medium, margins, growth rate were observed while for microscopic identification slides were prepared by using Mezler's reagent and were observed under compound microscope to visualize their hyphae, conidiophores, conidia, sporangiophore and spores.

Antifungal bioassays

Antifungal bioassay was conducted by following the protocol set by [10]. Two grams material of tested mushrooms were soaked in 20 ml of methanol separately for 7 days at room temperature and then filtered through an autoclaved muslin cloth. The filtered solutions were allowed to evaporate and after evaporation 0.16g gummy mass of *H. rubellus*, *B. edulis* and *S. sibiricus* were obtained. 20% stock solutions were prepared by adding 0.1 ml of distilled water in the respective gummy mass of test mushrooms. These stock extracts were stored at 4^0C.

Various concentrations viz. 1%, 1.5%, 2%, 3% were made for each mushroom extract by adding different quantities of stock solutions in flasks. Control treatments were without any mushroom extract. Each concentration was replicated three times and endowed with Chloromycetin capsule @ 50 mg/100 ml of medium to avoid bacterial contamination. 5 mm disc of each test fungus, from the base cultures, was placed in the center of 12 flasks having the mushroom extract *H. rubellus*, *B. edulis* and *S. sibiricus*. Three replicates were made for each treatment. All these plates were incubated at 25^0C for one week. After 7 days fungal growth was measured by filtering the solution of each concentration through pre weighed Whatsman no.1 filter paper. The test fungal biomass was allowed to dry in electric oven. Percentage growth inhibition of the test fungal biomass was measured by using the formula:

$$\text{Growth inhibition (\%)} = \frac{\text{growth in control - growth in treatment}}{\text{Growth in control}}$$

Results

Macroscopic and microscopic characterization of test fungi

Aspergillus niger

Colony colour: white to yellow with black conidia, Texture: velvety, Growth pattern: regular, Margins: entire, Odour: Pungent, Hyphae thick walled, septate and branched, Conidiophore wide with long stipe, smooth walled and colorless to light brown, Conidia varied in size globose in shape, brown in colour.

Aspergillus terreus

Colony color: Brown, Growth pattern: Regular, Margins: smooth, Texture: rough, conidiophore thin and postrate, columella spherical, conidia various in number.

Rhizopus stolonifer

Colony colour: black, Growth rate: rapid, Growth pattern: irregular, Texture: velvety, Odour: pungent, Margins: irregular, stolons prostate and thick walled, hyaline became brown towards nodes. Rhizoids long and brown, Columella brown, spherical, Sporangia spherical in appearance and black, Sporangiophore arise singly from nodes of stolons, Brown, smooth walled and non-septate.

Antifungal activity of Methanolic extracts of Macromycetes against test fungus

The effect of different concentrations of methanolic *H. rubellus*, *B. edulis* and *S. sibiricus* crude extract was inspected against *A. niger*, *R. stolonifer* and *A. terreus*. Out of five concentrations of methanolic extracts of *H. rubellus* i.e. 0%, 1%, 1.5%, 2%, 3% (Figure 1), the highest percentage showed best result in inhibition growth of *A. niger* up to 55% more than the control treatment. *B. edulis* found effective as antifungal agent against *R. stolonifer* and a 53% decline (Figure 2) in biomass of test fungus was recorded against different concentrations of applied extract. Results obtained by the extract of *S. sibiricus* against *A. terreus* retarded the test fungal biomass up to 54% (Figure 3). All the results were recorded after 7 days incubation period.

Discussion

In present study, the methanolic extracts of different concentrations (0%, 1%, 1.5%, 2%, 3%) of *H. rubellus*, *S. sibricus* and *B. edulis* were used against the target fungi *A. niger*, *A. terreus* and *R. stolonifer*. All selected mushrooms showed best results in separate pathogenic fungi. All the extract of these mushrooms exhibited strong antifungal activity against test pathogenic fungi. Previously, Ferreira *et al.* [11] evaluated the antifungal property of two wild edible mushroom species from the North East of Portugal, *Lactarius deliciosus* and *Tricholoma portentosum*. Iwalokun *et al*. [12] studied the antimicrobial potential of two organic extracts of *Pleurotus ostreatus* and suggested that *P. ostreatus* possesses antimicrobial and antioxidant potentials and reason of these activities is attributed due to the presence of terpenoids, tannins and steroidal glycosides. Ectomycorrhizal mushrooms possess antiallergic, anticarcinogenic, antibacterial, anticoagulant, antifungal, antihypertensive, anti-inflammatory, antinociceptive, antioxidant, antipyretic, antivenom, antiviral properties. Alves *et al.* [13] suggested that the antifungal activity of mushroom extracts might be due to the presence of sesquiterpene, steroids, organic acids, acylcyclopentenediones and quinoline compounds.

The results of present study showed a maximum inhibition of test fungi (*A. niger* and *R. stolonifer*), when tested against boletes (*H. rubellus* and *B. edulis*). As previously, Elsayed *et al.* [14] reported the different types of bioactive metabolites and their relevant procedures, as well as the different mechanisms of action of mushroom compounds as potent anti-fungal and anti-inflammatory agents.

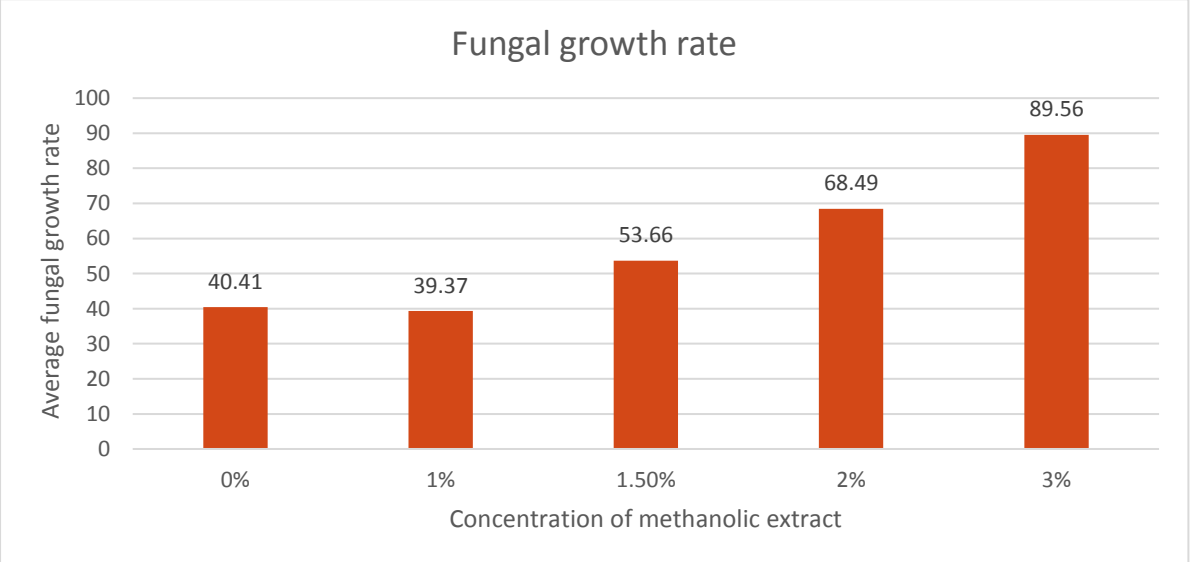

Figure 1. Antifungal activity of *Hortiboletus rubellus* against *Aspergillus niger*.

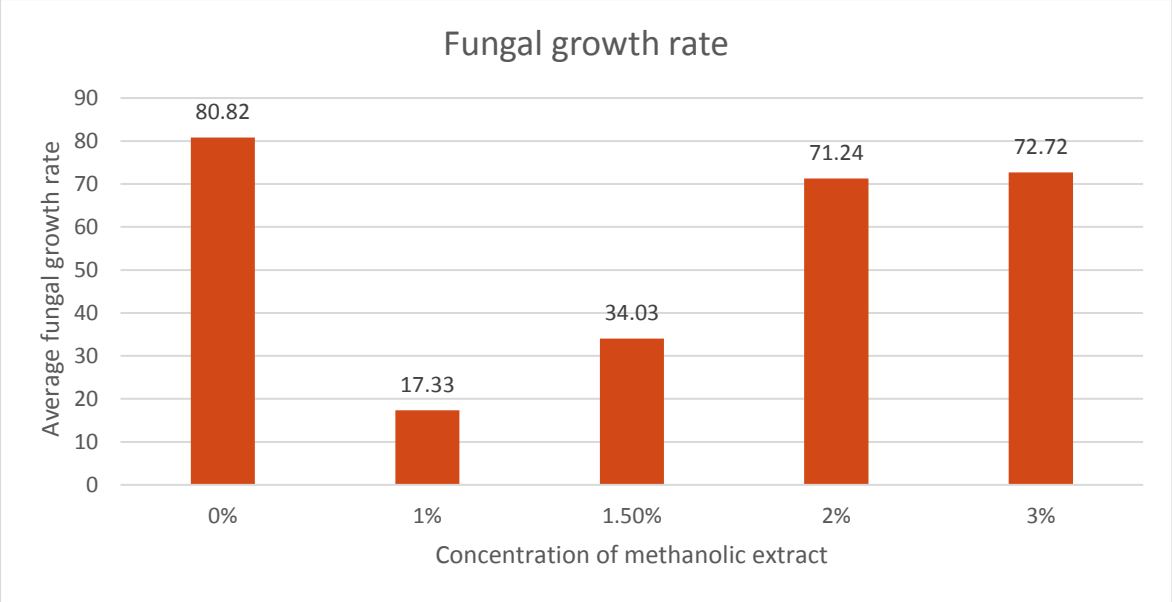

Figure 2. Antifungal activity of *Boletus edulis* against *Rhizopus stolonifer*

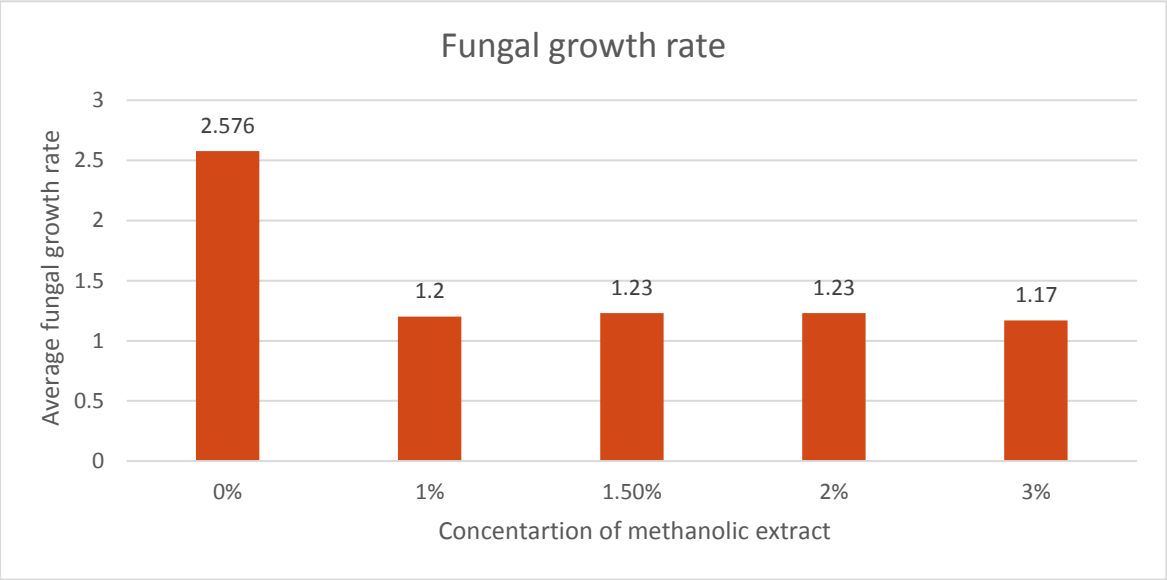

Figure 3. Antifungal activity of *Suillus sibiricus* against *Aspergillus terreus*

Conclusion

This study concludes that methanolic extracts of ectomycorrhizal fungi contain different antifungal substances that showed variable antifungal activity. The antifungal substances are low and high molecular weight compounds. All selected mushrooms have marked antifungal properties against phytopathogenic fungi.

Authors' contributions

Conceived and designed the experiments: S Sarwar & K Jabeen, Performed the experiments: F Batool & T Shafiq, Analyzed the data: S Sarwar & K Jabeen, Contributed materials/ analysis/ tools: AN Khalid, Wrote the paper: F Batool, S Sarwar, K Jabeen & T Shafiq.

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