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Antibacterial activity of *Pleurotus ostreatus* gemmotherapic extract

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Abstract The antibacterial activity of a gemmotherapic extract of *Pleurotus ostreatus* was investigated against 5 species of bacteria, three Gram-positive bacteria: *Bacillus subtilis, Bacillus cereus* var. *mycoides* and *Streptococcus faecalis* and two Gram-negative bacteria: *Pseudomonas aeruginosa* and *Serratia marcescens*, using the well diffusion assay. The extract was prepared from young parts of *P. ostreatus*, in according to the gemmotherapic principles. The result revealed that the extract had a significant inhibitory activity against *Bacillus subtilis* and *Bacillus cereus* var. *mycoide*s but moderate on the other species and minimal on *Serratia marcescens* at a concentration of 50mg/mL and 5 mg/mL. The results indicate that the gemmotherapic extracts can be a viable alternative to the modern extraction techniques.

Key words

antibacterial activity, *Pleurotus ostreatus*, gemmotherapic extract, well diffusion assay

Gemmotherapy or plant stem cell therapy as it is known today uses a wide variety of embryonic plant parts, collected in the spring at a critical stage in the plants growth when much of the plants energy is directed to the growing areas. Gemmotherapy is an important subsection of phytotherapy.

Gemmotherapic extracts are known for their higher content in active compounds [16]. They are prepared according to gemmotherapic principles from the French Pharmacopoeia, [as cited in 11], which consists in maceration of plant buds with equal thirds of water, alcohol and glycerin.

In recent years, there have been a revival of natural, plant based antimicrobial agents. This trend is the consequence of the limited effectiveness of synthetic products to fight against newer, drug resistant bacteria. For this purpose, the antimicrobial properties of many plant compounds from a wide variety of plant species have been assessed [7, 14].

Many species of macrofungi have long been used as food and as traditional medicines around the world since ancient times, especially in Asia.

Many Basidiomycetes mushrooms contain biologically active polysaccharides [20], some of which exhibiting haematological, antiviral, antitumour, antibiotic, antibacterial, and immunomodulating activities.

The genus Pleurotus from the family Pleurotaceae is a cosmopolitan group, including several cultivated species such as *P. pulmonarius, P. cornucopiae, P. sajor-caju, P. eryngii, P. cystidiosus*, and *P.ostreatus* [8]. To date approximately 70 species of Pleurotus have been recorded. Many of these species exhibit antimicrobial properties [3]. The oyster mushroom (*Pleurotus pulmonarius*) is commercially important in the world mushroom market. Fungi of the Pleurotus genus have an important place among the commercially employed basidiomycetes because they have gastronomic, nutritional and medicinal properties and can be easily cultivated on a large range of substrates.

The *P. ostreatus* is credited to be the third largest macrofungus cultivated for food and industrial purposes worldwide.

The effects of extracts from Pleurotus species against some pathogenic organisms have been widely reported by researchers.

At the present, there are scarce reports on the antimicrobial properties of *P. ostreatus* coupled with inadequate data on its phytochemistry. It is likely that knowledge of the phytoconsitituents of *P. ostreatus* would provide an insight into its biological functions beyond nutrition.

In this study, we explored a new approach by testing a gemmotherapic extract from young parts of *P. ostreatus* based on the principle that gemmotherapic extracts have a more intense inhibitory activity compared with the traditional extracts [4].

Although there are information regarding the antimicrobial properties of *P. ostreatus* crude extracts, in this study, we will test and compare the effects of gemmotherapic extracts, and to see if the principle, applied so far to green plants, can also be applied to mushrooms and fungi.

Materials and Methods

Plant material collection

Strains of *P. ostreatus* were cultivated in a greenhouse at the University of Agricultural Sciences in Timisoara. Young parts were collected from very young mushrooms and put immediately in ethanol of 96% concentration.

Preparation of gemmotherapic extracts

The solutions were made with equal thirds of alcohol, glycerol and distilled water. The fresh buds were collected, cleaned, washed with distilled water and then put in the solution for extraction. The process of extraction took place for a week in a dark place at 10^0C, using an orbital shaker. The extract was then filtered, concentrated by using a rotavap and weighted. The dry material was diluted for the tests and filtered through a sterile membrane filter. Two concentrations were tested: one of 50 mg/mL and the second, of 1:10 dilution factor solution.

Microorganisms

The bacteria used in this experiment are three Gram-positive bacteria: *Bacillus subtilis*, *Bacillus cereus* var. *mycoides* and *Streptococcus faecalis* and two Gram-negative bacteria: *Pseudomonas aeruginosa* and *Serratia marcescens.* The bacterial cultures were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Broth (Merck).

Bacteria counting

The bacteria were counted using a Burker chamber. The values are shown in (Table 1).

Table 1

The Burker chamber count data

Bacteria species	Number of cells/ml
Pseudomonas aeruginosa	$1.6x10^8$
Serratia marcescens	$1.7x10^8$
Bacillus subtilis	$8x10^7$
Bacillus cereus var. *mycoides*	$15x10^5$
Streptococcus faecalis	$2.2x10^8$

Well diffusion assay and antibacterial activity

The antibacterial activity was determined using the hole in plate assay procedure [14]. All bacterial cultures were maintained on nutrient agar slants at temperature of 4^0C and sub cultured onto nutrient agar broth for 24 hours prior to testing. The pure cultures of the microorganisms were inoculated onto Muller-Hilton nutrient broth incubated at temperature of 37^0C for 24 hours. 25 ml of nutrient agar was poured into the 100 mm plate, with an even depth of 4 mm on a level surface shaken and allowed to cool.

The nutrient agar plates were seeded with 0.1 ml of standardized inoculums of each of the five test organisms. The inoculum was spread evenly over plate with a sterile glass spreader. Using a sterile cork-borer of 5 mm diameter, three holes were made into the Petri dishes seeded with bacterial culture. The bottoms of the holes were sealed with agar to avoid seepage. 50µl of extracts were introduced in the wells, using a micro liter syringe. Concentrations of 5 and 50 mg/mL extracts were reconstituted in distilled water and transferred into the wells. The plates were kept for 30 min at room temperature to allow diffusion of the extract, and then were incubated at temperature of 37^0C for 24 hours. After the incubation period, the zones of inhibition were measured using a digital caliper. In this study, the measurement is taken including the 5 mm diameter of the hole. The diameter of the zone of inhibition was measured at three different angles and the mean of those measurements was taken.

Antibacterial activity was recorded when the zone of inhibition was greater than 6 mm.

Studies were performed in triplicates and the mean value was calculated. A solution of only alcohol, glycerol and water in equal ratios was used as a negative reference.

Statistical analysis

Data were averages of three results ± Standard Deviations (SD) by using Microsoft Excel.

Results and Discussions

In the (Table 2) are presented the mean zone of inhibition measured after 24 hours and in the (Figure 1) is represented the graph with the measurements.

Table 2

Antimicrobial activity of *Pleurotus ostreatus* by well diffusion method after 24 hours

Bacteria/Zone of inhibition in mm*	50mg/ml	5mg/ml	Control
Bacillus subtilis	19.2±0.2	13.2±0.3	7.2±0.2
Bacillus cereus var. *mycoides*	17.5±0.3	10.6±0.2	6.1±0.2
Streptococcus faecalis	13.6±0.2	8.4±0.4	5.8±0.4
Pseudomonas aeruginosa	12.5±0.3	7.4±0.2	5.2±0.2
Serratia marcescens	11.4±0.4	6.8±0.2	5.2±0.2

. *Measurement taken including the 5 mm diameter of the hole

The extract inhibited the growth of all bacteria species, although the intensity of the inhibition varied, ranging from 11 mm to 19 mm.
The 1:10 dilution factor solution presents little or no visible inhibitory effect, most likely because the concentration here is too low, with the exception of *Bacillus subtilis*, as shown in the (Figure 2). All values were expressed as means ± standard error means.

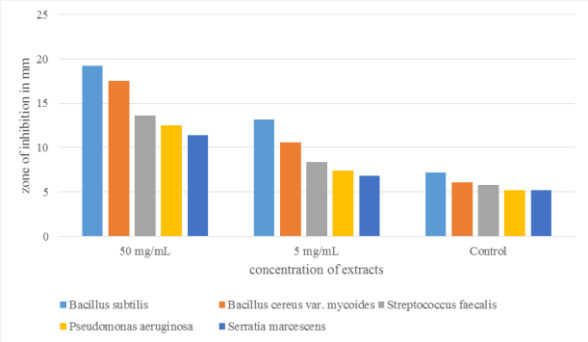

Fig. 1. Antimicrobial activity of *Pleurotus ostreatus* solutions and the control, zone of inhibition in mm

From the measurements we obtained, it can be observed that *B. subtilis* and *Bacillus cereus* var. *mycoides* presented the highest sensitivity, being susceptible, the lowest being *Serratia marcescens*, which is resistant. Another observation that can be made is that Gram-positive bacteria are more susceptible to the extract than the Gram-negative bacteria. The diameter of the zones of inhibition approximately doubles in size at a tenfold concentration.
There are few reports available on possible use of mushrooms for the management of diseases. This can be due to a lack of information on the screening of mushrooms for their antimicrobial activity. Therefore, in the present investigation, *P. ostreatus* was evaluated for its antibacterial potential.
The present study has revealed the antimicrobial property of gemmotherapic extracts from fungi. Similar studies have evidenced that *P. ostreatus* has a broad spectrum of antimicrobial activity. By using solvents with different polarities [6], found that non-polar solvent extracts of *P. ostreatus* like petroleum ether had a stronger inhibition activity on both Gram-positive and Gram-negative bacteria but with varying degrees of intensity. These observations are in accordance with the findings of Nehra, [10] who further validated the antimicrobial potential of *P. ostreatus* and found that "organic solvents consistently displayed better antimicrobial activity than that of the aqueous extract" [10, p. 394]. From the polar solvents, the ethanol extract exhibits the highest activity as found by Vamanu [19], with a 20 mm inhibition area at 10 mg/mL for *E. coli*.
However, not all bacteria species are sensitive to *L. edodes* extracts, as found by Kuznetsov [8], who insensitivity of Bifidobacteria and Lactobacilli to these extracts.
The data in this study also confirms another supposition that as in the case of plant extracts, in the case of mushroom extracts, the Gram-positive bacteria are more susceptible to inhibition as compared to Gram-negative bacteria. This difference in the case of plant extracts was known from numerous previous reports [15, 18].

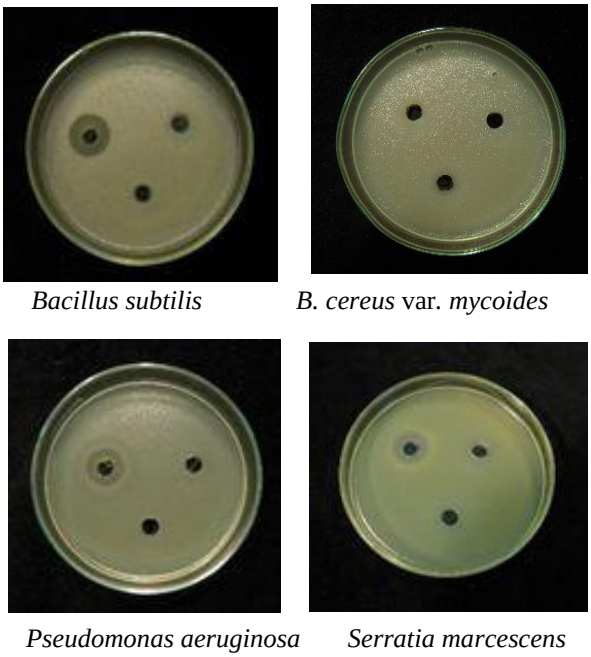

Fig. 2. The zones of inhibition for the extracts of M. charantia at 50 mg/ml (left), 1:10 dilution (right) and reference (bottom), for *Bacillus cereus* var. *mycoides*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Serratia marcescens.*

The use of antibiotics has reduced the incidence of infectious diseases but their extensive uses in therapy, has led to the appearance of drug-resistant bacteria [12], which is a major public health issue worldwide. For this purpose, numerous plant extracts were screened for antimicrobial properties that could protect people from microbial infections [9].
The mushroom extracts can also be used in combination with traditional antibiotics, the same way the plant extracts are used. In the literature, there are reports regarding the use of plant crude extracts [1, 2] in combination with fewer amounts of antibiotics for anti-bacterial activities, especially for antibiotic-resistant bacteria, compared to antibiotics alone [17].

Conclusions

Based on the results obtained from the present study, it can be concluded that the gemmotherapic principles can be successfully applied for mushroom extracts in the development of more potent and efficient antimicrobial agents.
Also, the gemmotherapic extracts obtained from *P. ostreatus* young parts, using the classic gemmotherapic principles, exhibit an intense antimicrobial activity.
Further investigation is needed in order to study the synergy of fractions from *P. ostreatus.*

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