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Antimicrobial Activity of Functionalized Micellar Structures with Bioactive Substances from Mango Peels

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Micellar structures from medicinal mushrooms are self-growing, fibrous, polymeric structures that can be successfully functionalized with various bioactive substances. Moreover, micellar structures can successfully mimic the extracellular matrix of human body tissues. Therefore, they can be used as novel biomaterials in tissue engineering and wound healing. On the other hand, fruit by-products, such as mango peels, are mainly discarded but represent a renewable source from which products with exceptional added value can be obtained. The aim of our study was to determine the antimicrobial properties of functionalized micellar structures obtained from medicinal mushrooms such as *Ganoderma lucidum* and *Pleurotus ostreatus*. The adsorption method was used to functionalize the micellar structures with natural mango peel extract (MPE) obtained by an ultrasound-assisted extraction method. In addition, the antimicrobial activity of the functionalized micellar structures was validated using a disk diffusion method.
The results of our study show a successful functionalization of micellar structures with natural MPE with a loading efficiency of up to 50-60% and a release rate of 73-96%. Moreover, the antimicrobial properties of the micellar structures from the two selected medicinal mushrooms were successfully determined against the tested pathogenic microorganisms. Therefore, mycelium-based functional materials represent promising bio-composite materials, especially for biomedical applications.

1. Introduction

The micellar structures of fungi are one of Earth's largest groups of organisms. They consist mainly of cellulose, chitin, and various proteins (Manan et al., 2021). The micellar structures as biological materials have many advantages, including adaptability to different growing conditions, biodegradability, and low production costs (Majib et al., 2023). Their fibrous structure makes them promising for various biological applications (Antinori et al., 2021). A significant advantage of micellar membranes over bacterial cellulose membranes is the final purification process, as this step only requires heat treatment of the film (Haneef et al., 2017).
The mycelial membranes obtained from medicinal mushrooms are self-growing, fibrous polymer composites with suitable properties. They can successfully mimic the extracellular matrix of human body tissue (Alaneme et al., 2023). Additional added value results from their functionalization with various therapeutic agents, whose use in tissue engineering is particularly promising. Therefore, functionalized micellar structures represent potential bioscaffolds (Khamrai et al., 2018). A film of mycelial structures can successfully achieve the desired properties of coatings or wound healing patches, which include protection of the wound by limiting the loss of body fluid, reduction of water loss from the patches, compatibility with tissue, and high mechanical strength (Ruggeri et al., 2023; Verma et al., 2023).
Micellar structures can be functionalized with various extracts, bioactive substances, antibiotics, therapeutic enzymes, and other components (Haneef et al., 2017). Functionalized mycelial films containing the bioactive ingredient curcumin, for example, showed good antimicrobial activity, representing an important added value for various biomedical applications, especially in tissue engineering (Khamrai et al., 2018). In addition to biomedical applications, functionalized mycelium-based materials are also promising in cosmetics, packaging, and construction (Majib et al., 2023; Manan et al., 2021; Sivaprasad et al., 2021).

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Micellar structures obtained from therapeutic mushrooms such as *Pleurotus ostreatus* and *Ganoderma lucidum* are biocompatible and biodegradable, making them promising biocomposites for use in tissue engineering and wound healing (Antinori et al., 2021). Few studies have been conducted on their production and characterization. At the same time, functionalization with natural extracts has not yet been observed, representing the development of potential therapeutic materials with high added value.

Mango (*Mangifera indica* L.) is one of the most important and popular tropical fruits with a high nutritional, phytochemical, and medicinal value (Espinosa-Espinosa et al., 2022). However, inedible mango parts account for up to 35-55% of the total fruit weight and are largely discarded (García-Mahecha et al., 2023; Oliver-Simancas et al., 2020). Mango peels are an important source of biologically active compounds with many beneficial health effects, including exceptional antimicrobial potential (Coelho et al., 2019; Kim et al., 2021). Therefore, a new approach to their further use is urgently needed to reduce their accumulation and the resulting environmental pollution and create the possibility of producing high-value-added products such as functionalized mycelial membranes.

Therefore, in our study, micellar membranes from medicinal mushrooms *G. lucidum* and *P. ostreatus* were synthesized through cultivation in a liquid culture medium. The adsorption method was used to functionalize the micellar structures with natural mango peel extract (MPE) obtained by an ultrasound-assisted extraction method (UAE). The antimicrobial activity of the functionalized micellar structures was validated using a modified disk diffusion method. The antimicrobial potential was tested against the growth of various pathogenic microorganisms such as Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram-positive bacterial species (*Bacillus cereus*, *Staphylococcus aureus*). The adsorption efficiency and *in vitro* release study of the incorporated MPE were also investigated. Figure 1 represents the experimental design of the study.

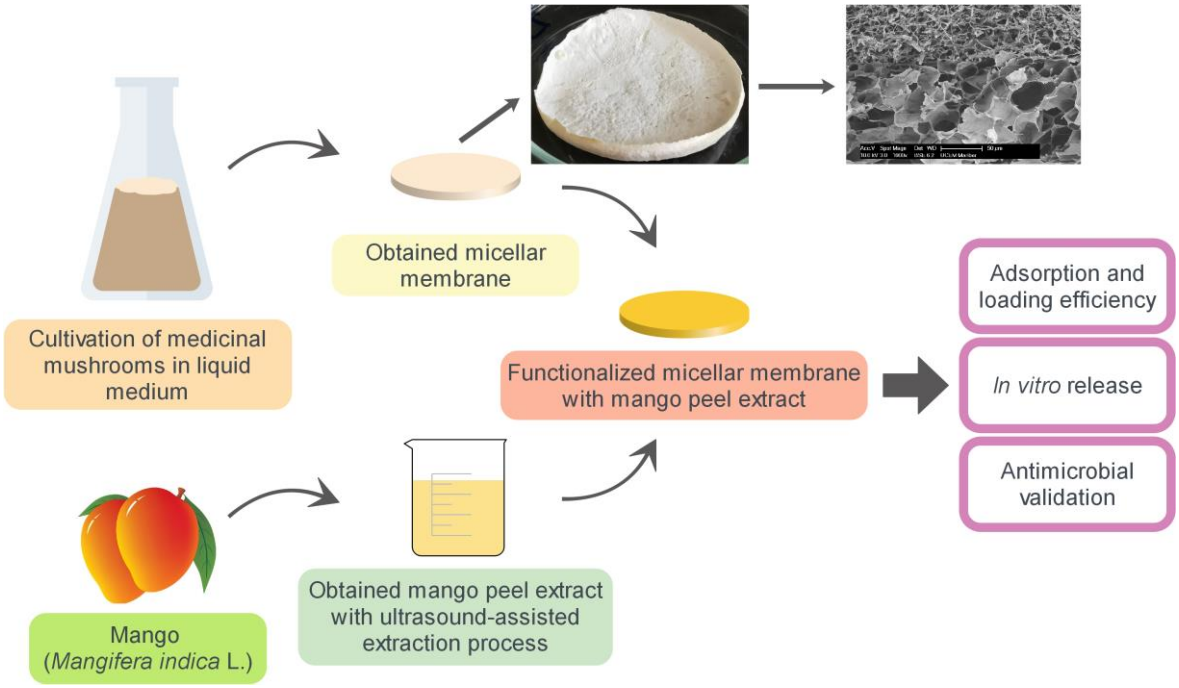

Figure 1: Experimental design of the study.

2. Materials and methods

2.1 Chemicals and reagents

The following chemicals: malt extract, potato dextrose broth, potato dextrose agar, and tryptic soy broth were obtained from Fluka, Buchs, Switzerland. Ethanol (EtOH, ≥99.5%), hydrochloric acid (HCl, 37.0%), magnesium sulphate heptahydrate ($MgSO_4 \cdot 7\ H_2O$), meat extract, meat peptone, potassium dihydrogen phosphate (KH_2PO_4) and sodium chloride (NaCl) were obtained from Merck, Darmstadt, Germany. D-(+)-glucose anhydrous was purchased from Kemika, Zagreb, Croatia. Agar, yeast extract, peptone from soybean, sodium hydroxide (NaOH, ≥95.0%), and starch were purchased from Sigma-Aldrich, St. Louis, USA.

2.2 Microorganisms

Selected bacterial species (*Bacillus cereus* (DSM 345), *Escherichia coli* (DSM 498), *Pseudomonas aeruginosa* (DSM 1128), and *Staphylococcus aureus* (DSM 346) were purchased from DSMZ-German Collection of Microorganisms and Cell Cultures GmbH from Berlin, Germany.

2.3 Preparation of plant material

Washed, fully ripe mango fruits were peeled with a fruit peeler. The resulting peels were air-dried at room temperature without pre-treatment and protected from direct sunlight. The completely dried peels were then ground to a uniform size.

2.4 Ultrasound-assisted extraction process

UAE was performed with EtOH as an extraction solvent. Dried mango peels were mixed with EtOH and sonicated at 20 °C and 40 kHz in an ultrasonic bath. The solution was filtered through a filter flask and a Buechner funnel to remove solid, insoluble particles. EtOH was evaporated from the resulting filtrate using a rotary evaporator. The resulting extract was stored in a freezer at -20 °C until further use.

2.5 Preparation of mycelial membranes

The fungal culture of *P. ostreatus* or *G. lucidum* was transferred to the liquid growth medium in the Erlenmeyer flasks and incubated at 27 °C under static conditions. After completion of growth, the mycelial membranes were removed from the medium and purified by washing with deionized water. The mycelial membranes were freeze-dried and then sterilized under UV light.

2.6 Functionalization of mycelial membranes

Mycelial membranes were functionalized with MPE by an adsorption process. The membranes with a diameter of 10 mm were immersed at room temperature in 5 mL of MPE solution with a concentration of 14.5 mg/mL. The change in concentration of the MPE was determined using a UV-VIS spectrophotometer at a wavelength of 274 nm. The percentage of MPE loaded onto the mycelial membranes was calculated as adsorption efficiency (AE) using Equation 1 and as loading efficiency (LE) using Equation 2:

$$AE = \frac{m_{MPE}}{m_{total\,MPE}} \cdot 100\% \quad (1)$$

$$LE = \frac{m_{MPE}}{m_{membrane}} \cdot 100\% \quad (2)$$

Where is:
AE – adsorption efficiency of MPE on mycelial membranes (%),
LE – loading efficiency of MPE on mycelial membranes (%),
m_{MPE} – the amount of MPE loaded into mycelial membrane (mg),
$m_{total\,MPE}$ – the amount of MPE in feed solution (mg),
$m_{membrane}$ – the amount of the loaded dry mycelial membrane (mg).

2.7 *In vitro* release study of MPE from mycelial membranes

An *in vitro* release study of MPE from functionalized mycelial membranes at body temperature was performed. Functionalized mycelial membranes of 10 mm in diameter were immersed in PBS buffer at pH 7.4. This was followed by incubation at 37 °C with constant shaking at 100 rpm. The samples were analyzed spectrophotometrically. The cumulative release (%) of MPE from mycelial membranes was calculated using Equation 3.

$$CR = \frac{m_{MPE,t}}{m_{MPE}} \cdot 100\% \quad (3)$$

Where is:
CR – cumulative release of MPE (%),
$m_{MPE,t}$ – the amount of total MPE released from mycelial membrane at different time intervals (mg),
m_{MPE} – the amount of MPE loaded into mycelial membranes (mg).

2.8 Determination of antimicrobial activity of mycelial membranes

The antimicrobial properties of mycelial membranes functionalized with MPE were verified for the growth of selected microbial cells using a modified qualitative Kirby–Bauer disk diffusion method. The inoculum of each

microorganism tested (Gram-negative bacteria *E. coli* and *P. aeruginosa* and Gram-positive bacteria *S. aureus* and *B. cereus*) was plated in the optimal medium for its growth at a concentration of 1-3·10^6 CFU/mL and spread on the agar plates. Then native and functionalized mycelial membranes with a diameter of 10 mm were placed on the prepared agar plates. After 24 hours of incubation, the diameter of the inhibition zone was measured. All tests were performed in three replicates, and the results are given as mean values.

3. Results

The micellar structures were successfully synthesized from the two selected medicinal mushrooms *P. ostreatus* and *G. lucidum*. The mycelial membranes of *G. lucidum* were grown in malt extract medium and those of *P. ostreatus* in glucose medium. Complete growth of the mycelial membranes of *G. lucidum* and of *P. ostreatus* was achieved after 14 days and after 21 days at a constant temperature of 27 °C under static conditions. After freeze-drying the mycelial membranes, the mycelium was completely inactivated by sterilization under UV light. In general, the mycelial membranes of *G. lucidum* were slightly thicker than those of *P. ostreatus*. The average thickness of the membranes of *G. lucidum* was 1.0 mm and 0.7 mm for *P. ostreatus*. However, the membranes of *P. ostreatus* appeared to be fluffier and heavier. The average weight of the obtained membranes of *G. lucidum* was 0.6 g, and 0.7 g for *P. ostreatus*.

3.1 Adsorption efficiency of MPE on mycelial membranes

First, an extract of air-dried mango peels was prepared using a UAE with EtOH as the extraction solvent. The extraction efficiency was 12.8%.
The adsorption method was used for the successful functionalization of the micellar structures with the obtained MPE at a concentration of 14.5 mg/mL. The obtained results are presented in Table 1.

Table 1: Adsorption and loading efficiency of MPE on mycelial membranes.

Mycelial membrane origin	$m_{adsorbed\ MPE}$ (mg)	AE (%)	LE (%)
G. lucidum	14.1	32	50
P. ostreatus	14.7	34	60

The percentages of adsorption efficiency of MPE at a concentration of 14.5 mg/mL obtained are comparable for mycelial membranes obtained from both selected medicinal mushrooms. However, it was found that the membranes of *P. ostreatus* adsorbed the MPE slightly better than those of *G. lucidum*, as a higher adsorption efficiency (34%) and a higher loading efficiency (60%) were achieved with the membranes of *P. ostreatus*.

3.2 *In vitro* release study of incorporated MPE from mycelial membranes

The percentage of MPE released from the mycelial membranes of *G. lucidum* and *P. ostreatus* was determined after exposure to PBS for 24 and 96 hours at 37 °C and shaking at 100 rpm. Figure 2 represents the percentage of MPE released after 24 and 96 hours.

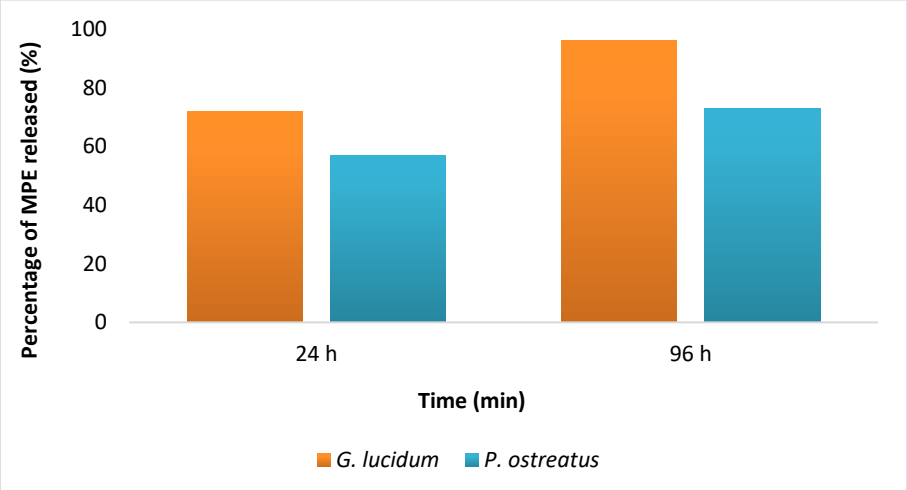

Figure 2: Percentage release of MPE from the mycelial membranes of G. lucidum and P. ostreatus after 24 h and 96 h at 37 °C.

After 24 h exposure of functionalized mycelial membranes to PBS at 37 °C, 72% and 57% of the MPE was released from *G. lucidum* and *P. ostreatus* micellar membranes, respectively. The release of the MPE increased with increasing time and reached 96% and 73% of the release from the membranes of *G. lucidum* and *P. ostreatus* after 96 h, respectively. No significant difference in MPE release from membranes after 96 h was observed.

3.3 Antimicrobial efficiency of functionalized mycelial membranes

The antimicrobial properties were validated using the modified qualitative disk diffusion method on nutrient agar plates, whereby the zone of inhibition was determined. The susceptibility of Gram-negative (*E. coli*, *P. aeruginosa*) and Gram-positive bacterial species (*S. aureus*, *B. cereus*) to the addition of MPE, functionalized mycelial membranes with MPE and native mycelial membranes was studied. Table 2 shows the measured diameters of the inhibition zone (in mm) of the individual bacterial species after exposure to the tested samples.

Table 2: Zone of inhibition obtained against the growth of selected bacterial species.

Sample	Zone of inhibition (mm)			
	Gram-negative bacteria		Gram-positive bacteria	
	E. coli	*P. aeruginosa*	*B. cereus*	*S. aureus*
G. lucidum native membrane	/	/	/	/
Functionalized *G. lucidum* membrane with MPE	16	13	16	13
P. ostreatus native membrane	/	/	/	/
Functionalized *P. ostreatus* membrane with MPE	12	11	12	13
MPE	17	15	18	14

The native mycelial membranes showed no inhibitory effect on bacterial growth. In contrast, the antimicrobial properties of the functionalized micellar structures from the selected medicinal mushrooms were successfully determined against the tested Gram-negative and Gram-positive bacterial species. *B. cereus* appeared most susceptible to the MPE while for the functionalized mycelial membranes the inhibition zone was equal to that for *E. coli*.

4. Conclusions

The synthesized mycelial membranes from two selected medicinal mushrooms *G. lucidum* and *P. ostreatus* were successfully functionalized with natural MPE. The loading efficiency was up to 50% and 60% for membranes from *G. lucidum* and *P. ostreatus*, respectively. In the *in vitro* release study, it was found that the majority of the MPE contained was successfully released from the mycelial membranes after 96 hours. In addition, the functionalized mycelial membranes also showed important inhibitory properties against the tested pathogenic bacterial species. Therefore, functionalized mycelium-based materials with natural extracts are promising biocomposites, especially for biomedical applications.

Nomenclature

AE – adsorption efficiency of MPE on mycelial membranes, %
CR – cumulative release of MPE, %
LE – loading efficiency of MPE on mycelial membranes, %
m_{MPE} – the amount of MPE loaded into mycelial membrane, mg
$m_{membrane}$ – the amount of the loaded dry mycelial membrane, mg
$m_{MPE,t}$ – the amount of total MPE released from mycelial membrane at different time intervals, mg
$m_{total\ MPE}$ – the amount of MPE in feed solution, mg

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