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Chemical characterization and biological properties of oyster and shiitake mushrooms extracts and their liposomal formulations

Caglar Macit [a], Ozan Emre Eyupoglu [b], Meltem Macit [c], Gokhan Zengin [d,*]

[a] Istanbul Medipol University, School of Pharmacy, Department of Pharmacology, Goztepe District, Ataturk Street, No:40, 34810, Beykoz, Istanbul, Turkey
[b] Istanbul Medipol University, School of Pharmacy, Department of Biochemistry, Goztepe District, Ataturk Street, No:40, 34810, Beykoz, İstanbul, Turkey
[c] Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Inönü District, Kayisdagi Street, 34755, Atasehir, Istanbul, Turkey
[d] Selçuk University, Faculty of Science, Department of Biology, Selcuklu, Konya, Turkey

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ABSTRACT

In recent years, the production of liposomal formulations using mushroom extracts has gained increasing interest to increase the bioavailability of bioactive compounds in the nutraceutical field. Based on this information, we aimed to determine the antioxidant capacity (by DPPH, ABTS, CUPRAC and FRAP assays) and enzyme inhibition (against cholinesterase, amylase, glucosidase and tyrosinase) activities of alone and liposomal formulations of *Pleurotus ostreatus* (OYE) or *Lentinus edodes* (SHE) in different extracts solvents which were methanol (MeOH), aqua (Aq), methanol/aqua (MeOH/Aq). The extracts and formulations were chemically characterized using HPLC-DAD. The mean diameter of SL:SHE and SL:OYE (in MeOH/Aq) extended in range between 60 and 165 nm. The entrapment yields of SL:SHE and SL:OYE (in MeOH/Aq) were 63.8% ± 3.7% and 71.2% ± 2.8%, respectively. HPLC-DAD analysis revealed that ferulic and cinnamic acids were main components in the liposomal formulations. Liposomal formulations (in MeOH) showed higher antioxidant activity in the FRAP and CUPRAC assays. SL:SHE (in Aq) showed effective enzyme inhibition activity on acetylcholinesterase, tyrosinase, amylase and glucosidase enzymes. Based on our findings, the liposomal formulations can be a valuable strategy in preparing functional applications with shiitake and oyster mushrooms.

1. Introduction

Recently, nutrient-rich medicinal plants are widely used for healthy life (Brewer, 2011). Mushrooms are one of the most frequently consumed these medicinal and functional foods in many cultures around the world due to their taste, mild aroma and antioxidant effects. For this reason, they are often preferred in low-calorie, low-fat, salt-free and hypolipidemic diets (Khatua et al., 2013). Numerous studies have found that consuming mushrooms reduces the risk of many diseases (Uffelman et al., 2023). In particular, non-toxic edible mushrooms such as oyster (*Pleurotus ostreatus*) and shiitake (*Lentinus edodes*) mushrooms also contain a large amount of bioactive compounds, including many mycochemicals, polysaccharides, selenium, various vitamins and crucial phenolic antioxidant components of such as cinnamic acid and ferulic acid. However, when the edible mushrooms are cooked, their activities decrease (Agrawal et al., 2010; Dicks & Ellinger, 2020; Spim et al., 2021; Zhang et al., 2022). In order to prevent activity reduction, some methods are used.

One of the most efficient methods is lipid-based drug delivery systems, which also increase the bioavailability of edible mushrooms. The suitability of delivery systems consisting of various lipids, surfactants and lecithin leads to a greater sinking effect (Martins et al., 2020; Tugba Degirmencioglu et al., 2019). This method is preferred to eliminate various toxicities of fungi and increase their stability, bioavailability, effectiveness and biocompatibility (Omran & Baek, 2021).

It is well known that the antioxidant content of natural substances can easily disappear. To eliminate the antioxidant activity reduction problem, the industry has been sought new methods like preparation of liposomal formulations, which allows controlled release of the drug, extending of storage time and preventing of reduced bioavailability (Elhusseiny et al., 2021; Macit et al., 2021). Thus, researchers are often working to demonstrate the antioxidant effect of natural foods such as mushrooms and prepare them in suitable formulation for human use. In literature, however there are studies comparing the antioxidant effects

* Corresponding author.
E-mail addresses: cmacit@medipol.edu.tr (C. Macit), oeeyupoglu@medipol.edu.tr (O.E. Eyupoglu), meltem.macit@yeditepe.edu.tr (M. Macit), gokhanzengin@selcuk.edu.tr (G. Zengin).

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and evaluating of enzyme inhibition effects of the extracts of mushroom species (Bakir et al., 2018; Bassi et al., 2024; Cao et al., 2020; Diallo et al., 2020; Nnemolisa et al., 2024), there are almost no studies comparing the antioxidant effects of their liposomal formulations.

With this information in mind, to provide new insights and valuable candidates for health-promoting applications, we aimed to evaluate the antioxidant properties (free radical scavenging and reducing power) and enzyme (cholinesterase, tyrosinase, amylase, and glucosidase) inhibitory effects of various extracts of shiitake and oyster mushrooms, as well as their liposomal formulations. Furthermore, all extracts and formulations were chemically characterized using the HPLC-DAD system. The results obtained can serve as a novel scientific basis for further applications utilizing these mushroom species.

2. Material and methods

2.1. Materials

The mushrooms were obtained by traditional market (Istanbul, Turkey). Ferric chloride hexahydrate ($FeCl_2$ $6H_2O$), 6-hydroxy-2,5, 7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-S-triazine (TPTZ), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS*+), soy lecithin (SL), methanol (MeOH), butyl hydroxy toluene (BHT) and bis (neocuproine) copper (II) chelate were provided Sigma-Aldrich (Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation of mushroom extracts and their liposomal forms

Soy lecithin (SL) was mixed with distilled water by high-speed homogenizer (Homogenizer Bika) for 15 min at 1500 rpm (Hafner et al., 2011; Koynova & Tihova, 2010). The sonication method was then applied on this mixture. This method was applied with minor modification of studies of Macit et al. (2023).

The methanolic (MeOH), aqueous (Aq) and methanolic/aqueous (MeOH/Aq) extracts were performed almost same following methods. Shiitake and oyster mushrooms were dried in an oven at 45 °C for 24 h (Yim et al., 2013). Both of them were crushed into powder to obtain fine particles. The method was performed as similar as Macit et al. study with minor modifications (Macit et al., 2021). Meanwhile, the aqueous and MeOH/Aq (80/20%) extracts of mushrooms were prepared alike MeOH extract; instead of methanol.

The shiitake extract (SHE) and oyster mushroom extract (OYE) were incorporated in SL dispersions. Therefore, the freeze-dried extracts of mushrooms (200 mg) was incorporated in freeze dried soy lecithin (50 mg) dispersion by probe sonicator (Bandelin 2070) in 15 min, with an ultrasonic power of 70 W and a frequency of 10 kHz with ice bath and these were lyophilized. Alone extracts and their liposomal forms were stored at +4 °C till their characterization analysis, antioxidant capacity assays, HPLC analysis and enzyme inhibition studies.

2.2.2. Characterization of all mushroom extracts

Alone and liposomal formulations of mushroom extracts were redispersed with distilled water and poly dispersity index (PDI), physical appearance and zeta potential were measured by dynamic light scattering (DLS) Malvern Zetasizer (Malvern brand ZS 501).

The obtained SHE, OYE and liposomal SL:SHE and SL:OYE (in MeOH/Aq) were characterized for mean particle size and zeta potential (Rouser et al., 1970).

2.2.3. Transmission electron microscopy

The surface morphology and shape of SHE, OYE, SL:SHE and SL:OYE formulations were observed by transmission electron microscope (TEM; Thermo Fischer microscope). When TEM observation was performed, 1 mL of formulation was placed on 300 mesh copper grids and wait to stand for 15 min after which any excess fluid was removed to filter paper. Then, the sample was put in liquid nitrogen to freeze rapid. Before observation, 1 drop of 1% osmium tetrachloride was applied for fixation and wait to dry again for 5 min. Then, this was freeze-dried at -55 °C again.

The loading efficiency (LE) of liposomal formulations was measured by using UV–Vis spectrophotometer. The loading efficiency (LE) means to determine the amount of mushroom extracts in liposomal forms (n = 10) by using UV–Vis spectrophotometer. Then, to calculate the loading efficiency (LE) using the formula:

$$\%\text{loading efficiency} = \frac{\text{amount of measured of test drug (mg)}}{\text{amount of standard drug (mg)}} \text{x100}$$

2.3. Antioxidant capacity assays

Antioxidant capacity assays of oyster and shiitake mushrooms extracts (in methanolic, aqueous and methanolic/aqueous) and their liposomal forms were done. These assays were 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) Radical Scavenging, 2,2′-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS+) assays, Ferric Reducing Antioxidant Power (FRAP) and Cupric Ion Reducing Antioxidant Capacity (CUPRAC).

2.3.1. DPPH radical scavenging activity assay

The free DPPH radical scavenging capacities of all extracts (OYE and SHE) and their liposomal forms (SL:OYE and SL:SHE) in MeOH, Aq, and MeOH/Aq were determined according to the method developed by Brand-Williams et al. with minor modifications (Brand-Williams et al., 1995). The tested samples (1–0.0625 mg/mL) were dissolved in 100 μL of methanol and mixed with 100 μL of DPPH solution in a 96-well microplate. The mixture was kept in complete darkness and incubated at room temperature for 30 min. The absorption was measured at 517 nm using a microplate reader (Multiskan FC, Thermo, Waltham, MA, USA). In current study, butylhydroxytoluene (BHT) was used as reference substance.

2.3.2. ABTS activity assay

The antioxidant activity assay was determined based on the antioxidant ability to inhibit the 2,2′-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid or ABTS free radical (ABTS+) and compare the results with a reference standard (BHT) (Re et al., 1999).

2.3.3. FRAP activity assay

FRAP assay was done by following the method described by Benzie and Strain (1996). A 50 μL of the extracts (from 2 mg/mL stock solution) was pipetted into the plates. Then, 150 μL of FRAP reagent was added directly to each well. Trolox was used as positive control while methanol as negative control of the experiment. Then, 96-microwell plate was incubated at 37 °C for 30 min before measuring the absorbance. After 30 min incubated in the incubator, the absorbance of the extracts was measured using Multiscan Spectrum (Thermo Scientific) at a wavelength of 595 nm.

2.3.4. CUPRAC activity assay

Cupric ion reducing antioxidant capacity (CUPRAC) was performed according to the method described by Özyürek et al. (2011). The trolox equivalent antioxidant capacity (TEAC) was calculated. The absorbance of the color was measured at 450 nm. The results obtained from triplicate analysis were expressed for each sample TEAC μmol Trolox/g.

2.4. High performance liquid chromatography (HPLC) analysis

Chromatographic conditions for HPLC (Agilent 1100 Series) analysis of mushrooms included C-18 (250 × 4.6 mm; 5 μm) column (Purosphere Star RP-18 encapped guard column) with a column temperature of 30 °C, λ 238 nm (maximum absorption for phenolic contents), isocratic

pump flow and UV–Diode array detectors (DAD) detection. Primarily, last concentrations were adjusted to 0.5 mg/mL with methanol before analysis. Then, flow rate was maintained at 1.5 mL/min during analysis (15 min). All solutions were filtered through 0.45 μm cellulose membrane (Isolab) and 20 μL was injected. All solvents used in the HPLC analysis were purchased from Merck. Acetonitrile and 0.1 % phosphoric acid (65:35, v:v) prepared in HPLC grade ultra-pure water was used as a mobile phase to carry out HPLC analysis of the samples. Degassing of the solvents was also done. Reaction coil, which made of polytetrafluoroethylene (PTFE) tubing (0.20 mm i.d.) was used (Piecha et al., 2009). In this study, parameters related to detection and verification limits (limit of detection (LOD) and limit of quantification (LOQ), were determined as 3 times and 10 times the mean ± standard deviation (SD) of the HPLC system peak noise, respectively (Armbruster & Pry, 2008). All data obtained from ChemStation statistical program version 4.3 (Agilent) detection limit analysis were presented as mean ± SD. $p < 0.05$ was regarded as statistically significant.

2.5. Enzyme inhibition test

The assessment of extracts' inhibition of cholinesterase, tyrosinase, amylase and glucosidase were performed in accordance with the protocol described in the study by Zengin (2016). The inhibition percentage was calculated for each enzyme.

2.6. Statistical analysis

All obtained data was analyzed by GraphPad Prism v. 5.04 program (GraphPad Software Inc., La Jolla, CA). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by *post-hoc* Tukey analysis. To compare two different formulations, student t-test was used. $p < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. Results of characterization of liposomal shiitake and oyster mushroom extracts

Liposomal forms are preferred to deliver substances including food sciences due to enhance the solubility, bioavailability, uptake and stability (Wang et al., 2014). They can be also used to improve the absorption of insoluble active components in vegetables.

At current study, the final dry mushroom extracts (in MeOH) and liposomal forms of them were characterized. The size distribution of liposomal forms of mushroom extracts revealed the average diameter of lied from 60 to 165 nm as seen in Table 1 and in supplementary materials.

Zeta potential as also measured by DLS method at room temperature. The results were different among the formulations lie between −13 and −35 mV (see Supplementary Fig. S1). SL:OYE in MeOH/Aq had the best zeta-potential value (−34.9 ± 5.96) (Table 1).

It was supposed that PDI value showed the homogeneity of particle size of formulations and it was also prompted as stable. In literature, up to 0.5 value was considered as a narrow PDI value (Rouser et al., 1970). In our study, while the PDI value of SL:OYE was obtained around 0.3, and for SL:SHE, almost 0.5, as seen in Table 1. In addition, the value of poly dispersity index (PDI) showed that the size distribution of SL:OYE was more homogenous than size of SL:SHE.

Table 1
Characterization of mushroom extract and their liposomal forms.

Mushroom formulations	Particle size (nm ±SD)	PDI	Zeta potential (mV ±SD)
SHE	147.3 ± 7.93	0.471	−13.9 ± 6.72
OYE	60.7 ± 9.82	0.283	−30.3 ± 8.61
SL:SHE	164.4 ± 71.49	0.492	−17.9 ± 4.69
SL:OYE	65.44 ± 23.93	0.316	−34.9 ± 5.96

SHE, shiitake mushroom extract in MeOH/Aq; OYE, oyster mushroom extract MeOH/Aq; SL:SHE, Liposomal forms of shiitake mushroom extracts MeOH/Aq; SL:OYE, Liposomal forms of oyster mushroom extract MeOH/Aq; PDI, polydispersity index; SD, standart deviation (mean ± SD, n = 3).

According to the results of TEM analysis, while the alone SHE and OYE were not observed homogeneous, liposomal forms of the SHE and OYE showed homogeneity Supplementary Fig. S2. The entrapment efficiency of SL:SHE and SL:OYE (in MeOH/Aq) were found 63.8% ± 3.7% and 71.2% ± 2.8%, respectively.

The shape and surface morphology of mushroom extracts and their liposomal forms by TEM analysis is shown in Supplementary Fig. S3. TEM analysis showed the uniform particle size of liposomal forms and spherical shape. The particle size results were confirmed by TEM analysis.

3.2. Antioxidant capacity study results

Mushrooms have a great deal of beneficial active antioxidant compounds. Kozarski et al. stated that carotenoids, polyphenols, vitamins, polysaccharides, and minerals were the main source of antioxidant effect (Kozarski et al., 2015). The major phenolic components that have antioxidant effect in mushrooms are ferulic, cinnamic, chlorogenic and protocatechuic acid (Reis et al., 2012).

In this study, the antioxidant activity of alone and liposomal formulations of mushroom extracts were determined with DPPH, ABTS, FRAP and CUPRAC. In these tests, high FRAP and CUPRAC activity values and low DPPH and ABTS activity values supported each other and demonstrated the increased antioxidant power of mushroom extracts.

3.2.1. Results of DPPH radical scavenging activity

It has been well known that DPPH radical scavenging activity is a model analysis in order to determine the antioxidant activity of compounds. As IC_{50} concentration value decreases, antioxidant activity increases (Gulcin, 2020).

In current study, SHE and OYE formulations (in MeOH/Aq) had the highest IC_{50} of reducing powers as seen in at the left (0.01 (mg/mL)/g) and right (0.70 (mg/mL)/g) graphs in Fig. 1, respectively ($p < 0.05$). Moreover, there were significances between SL:SHE and SL:OYE (in MeOH/Aq).

3.2.2. Results of ABTS assay

The results showed that SHE (in MeOH/Aq) in the left graph and OYE (in MeOH/Aq) in the right, formulations had the highest IC_{50} value of BHT, as shown in Fig. 1. Accordingly, it was found that the IC_{50} values of alone formulations of two mushrooms (in MeOH/Aq) exerted significantly higher antioxidant effect than other formulations. When consider the ABTS value of liposomal formulations, while it was determined 0.59 (mg/mL)/g for shiitake mushroom, it was found 1.02 (mg/mL)/g for oyster mushroom (in MeOH/Aq). This is thought to be related to the solubility of the phenolic compounds in the compared mushroom with different polarities in the methanolic or aqueous phase.

Antioxidant phenolic components of liposomal forms released slowly by the controlled manner in ABTS and DDPH assays. Therefore, the antioxidant activity of liposomal forms was lower than those of alone extracts.

3.2.3. Results of FRAP (Ferric Reducing Antioxidant Power) activity

The FRAP assay of SHE, OYE and their liposomal forms (in MeOH, Aq and MeOH/Aq) were studied and the findings were given as the average Trolox equivalent (TEAC) in triplicates (Benzie & Strain, 1996) (Fig. 1). The results demonstrated that the antioxidant activity of SL:SHE and SL:OYE had highest activity when compared to SHE and OYE. The results showed statistically significance that the FRAP values of SL:SHE and SL:OYE (in MeOH) were 493 M Trolox/g and 671 M Trolox/g, shown in Fig. 1 ($p < 0.05$), respectively.

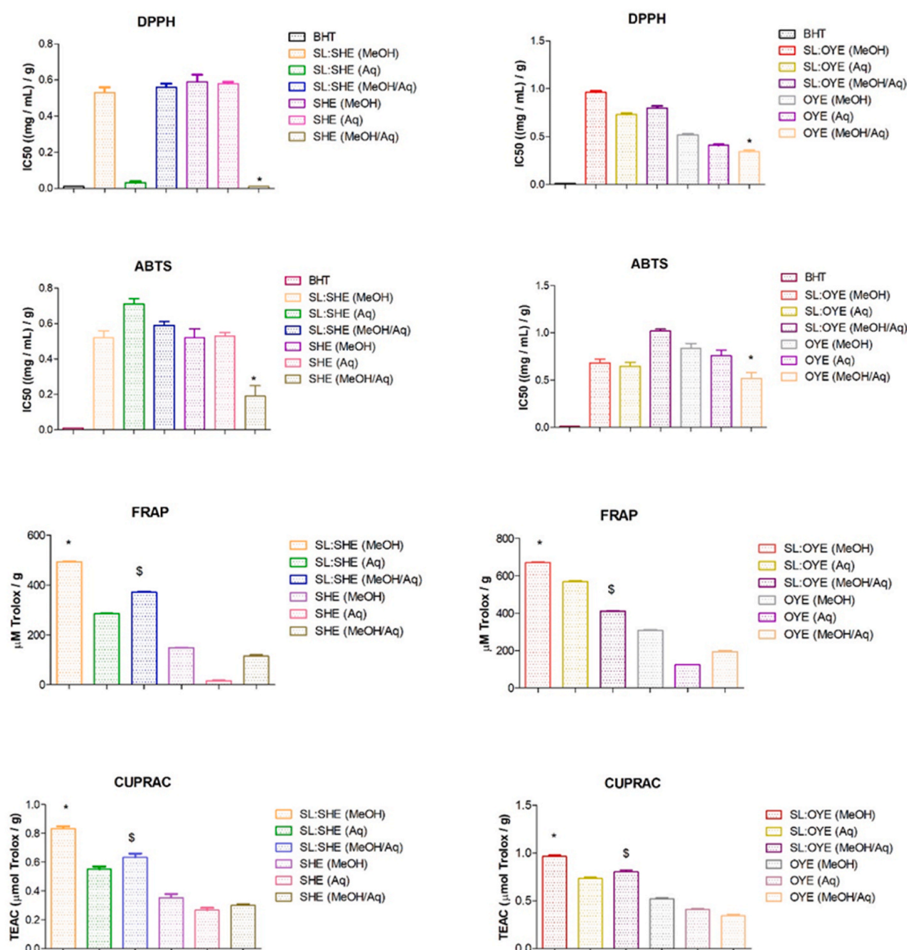

Fig. 1. Antioxidant properties of the extracts and liposomal formulations. (IC_{50} (mg/mL) values for DPPH and ABTS; μmol Trolox/g for FRAP and CUPRAC). *: $p < 0.05$ compared to all formulations, $: $p < 0.05$ when compared to alone extracts (mean ± SD, n = 3).

While SL:SHE (in MeOH) had the highest antioxidant capacity, the value for SL:SHE (in MeOH/Aq) was significantly different when compared to the other alone extracts. The same findings were observed for OYE and SL:OYE (in MeOH/Aq). The antioxidant capacity of SL:OYE (in MeOH/Aq) was significantly different than alone OYE (in all solvents) formulations despite the highest FRAP value of SL:OYE (in MeOH).

3.2.4. *Results of CUPRAC assay*

The CUPRAC assay of three different extracts of SHE, OYE and their liposomal forms of were performed. The results showed exhibited that SL:SHE and SL:OYE (in MeOH) had highest activity than other formulations (Fig. 1). CUPRAC values of SL:SHE and SL:OYE (in MeOH) were determined to be 0.85 mol Trolox/g and 0.95 mol Trolox/g, respectively ($p < 0.05$).

Although SL:SHE (in MeOH) had the highest antioxidant capacity, SL:SHE (in MeOH/Aq) was significantly different when compared to alone SHE like OYE and their liposomal forms.

The reason why the highest values of CUPRAC and FRAP (in MeOH) are observed in liposomal formulations is that they chelate with Fe and Cu.

These results could be related to the fact that liposomal formulations had higher intercellular permeability than normal formulations, so they spread more easily regardless of polarity. This property of liposomal formulations ensures easy release of phenolic compounds that have antioxidant effects into mushrooms (Cheung et al., 2003).

In a previous study, OYE (in MeOH) demonstrated the highest DPPH scavenging activity than OYE (in Aq) (Brand-Williams et al., 1995). In another study, results exhibited that methanolic extract of shiitake mushrooms had low DPPH scavenging activity, which was not same but similar with the results of our study (Re et al., 1999). Similar to the previous antioxidant studies, the ABTS result was approximately the same for SHE (in MeOH/Aq), while a worse result was found for OYE (Elhusseiny et al., 2021).

In a study that examined whether storage conditions affected the CUPRAC values of shiitake mushrooms, results showed that the highest CUPRAC values in samples stored at ambient temperature were significantly different compared to the control group (Wen et al., 2020).

In another study, Jiang et al. reported similar results about antioxidant effect shiitake mushroom (Jiang et al., 2010). Brazkova et al. (2022) found that the fruiting body of the oyster mushroom has higher antioxidant activity. Consistent with the results of previous studies, the current study showed that both mushrooms have higher CUPRAC values, meaning they have antioxidant activity.

3.3. *HPLC analysis result*

Fourteen different phenolic compounds (gallic acid, homogentisic acid, protocatechuic acid, catechin, chlorogenic acid, vanillin, naringin, myricetin, resveratrol, quercetin, caffeic acid, cinnamic acid, syringic acid and ferulic acid) were determined in different concentrations in MeOH/Aq mushroom extracts. Significant contributions had been noted for their ferulic acid, cinnamic acid and other important phenolic contents (Tables 2 and 3 and Supplementary Tables S1–S2).

Phenolic compounds in liposomal formulations of mushrooms extracts were more than the alone extracts (in Supplementary

Table 2
Phenolic contents detection and amount limits of the peaks defined at 238 nm for SL:OYE (in MeOH/Aq).

Component name	RT (min.)	Concentration (ng/mL) (%)	LOD (ng/mL)	LOQ (ng/mL)
Gallic acid	3.2	5 (0.5%)	0.3 ± 0.01	0.9 ± 0.01
Homogentisic acid	4.5	6.3 (0.63%)	1.4 ± 0.02	4.2 ± 0.03
Protocatechuic acid	5.3	4.6 (0.46%)	1.0 ± 0.01	3.0 ± 0.02
Catechin	6.1	3.7 (0.37%)	0.7 ± 0.01	2.1 ± 0.02
Chlorogenic acid	7.2	7 (0.7%)	1.7 ± 0.02[a]	5.1 ± 0.03
Vanillin	8.4	8.2 (0.82%)	2.4 ± 0.02[b]	7.2 ± 0.03
Naringin	9.3	6.4 (0.64%)	1.6 ± 0.02	4.8 ± 0.03
Myricetin	10.1	4 (0.4%)	0.9 ± 0.01	2.7 ± 0.02
Resveratrol	11.2	7.3 (0.73%)	1.6 ± 0.02[c]	4.8 ± 0.03
Quercetin	12.2	6 (0.6%)	1.3 ± 0.02[d]	3.9 ± 0.01
Caffeic acid	13.3	4.3 (0.43%)	1.1 ± 0.01	3.3 ± 0.02
Cinnamic acid	14.4	10.2 (1.02%)	4.2 ± 0.03[e]	12.6 ± 0.04
Syringic acid	14.8	7.3 (0.72%)	1.4 ± 0.03	4.2 ± 0.03
Ferulic acid	15	15.3 (1.53%)	5.2 ± 0.03f	15.6 ± 0.05

SD: Average Standard Deviation, 95% confidence interval; critical ratio: $p < 0.05$; RT: Retention time; LOD: Limit of Detection; LOQ: Limit of Quantification (mean ± SD, n = 3).
[a,b,c,d,e,f]: Different letters in the same column correspond to significant differences by Tukey test.

Table 3
Phenolic contents detection and amount limits of the peaks defined at 238 nm for SL:SHE (in MeOH/Aq).

Component name	RT (min.)	Concentration (ng/mL) (%)	LOD (ng/mL)	LOQ (ng/mL)
Gallic acid	3.2	3.5 (0.35%)	0.2 ± 0.01[a]	0.6 ± 0.01
Homogentisic acid	4.5	5.3 (0.35%)	1.1 ± 0.02	3.3 ± 0.03
Protocatechuic acid	5.3	3.6 (0.36%)	0.6 ± 0.01[b]	1.8 ± 0.02
Catechin	6.1	2.7 (0.27%)	0.4 ± 0.01	1.2 ± 0.02
Chlorogenic acid	7.2	6.1 (0.61%)	1.4 ± 0.02[c]	4.2 ± 0.03
Vanillin	8.4	6.2 (0.62%)	2.2 ± 0.02	6.6 ± 0.03
Naringin	9.3	5.4 (0.54%)	1.5 ± 0.02[d]	4.5 ± 0.03
Myricetin	10.1	3 (0.3%)	0.7 ± 0.01[e]	2.1 ± 0.02
Resveratrol	11.2	6.3 (0.63%)	1.3 ± 0.02	3.9 ± 0.03
Quercetin	12.2	5 (0.5%)	1.2 ± 0.02[f]	3.6 ± 0.01
Caffeic acid	13.3	3.3 (0.33%)	1.0 ± 0.01	3.0 ± 0.02
Cinnamic acid	14.4	8.2 (0.82%)	4.1 ± 0.03	12.3 ± 0.04
Syringic acid	14.8	5.3 (0.53%)	1.3 ± 0.03	3.9 ± 0.03
Ferulic acid	15	11.3 (1.13%)	5.0 ± 0.03	15.0 ± 0.05

LOD: Limit of detection; LOQ: Limit of Quantitation; SD: Average Standard Deviation; CI: 95% confidence interval; Critical ratio: $p < 0.05$ (mean ± SD, n = 3).
[a,b,c,d,e,f]: Different letters in the same column correspond to significant differences by Tukey test.

Tables S1–S2). In this study, there was no difference in phenolic composition between OYE and SHE (in MeOH/Aq) ($p > 0.05$). Liposomal formulations of oyster and shiitake mushrooms extracts were determined particularly rich in ferulic and cinnamic acids compounds that contribute to the antioxidant activity of mushrooms as shown in Tables 2 and 3, respectively.

In HPLC study, only MeOH/Aq extracts were studied because they were necessary to change the polarity and draw it to the methanolic phase. For these small amount of water was used and collision was more effective on the surface of the capillary small volume. This showed that concentration gave more detection.

Similar to results of the current study, Gąsecka et al. reported parallel findings that the two mushrooms had high cinnamic and ferulic acid contents (Gąsecka et al., 2016). Beside these phenolic components, chlorogenic acid (Miao & Xiang, 2020), resveratrol (Sánchez, 2017), quercetin (Abdullah et al., 2012) and caffeic acid (Kozarski et al., 2015) also had antioxidant activity.

Furthermore, with increasing cell permeability in the liposomal formulation, the concentrations are quite good because the phenolic component is less retained in the silica column during HPLC analysis. Cinnamic and ferulic acids came to liposomal forms more easily due to the hydroxyl groups in their side chains, and their pore permeability became stronger. This situation increased the radical scavenging capacity and binding capacity of the liposomal forms in accordance with the antioxidant activities. As the molecular weight of liposomal form phenolic components increases, the detection limits improve significantly. Based on repeated solvent polarity changes, it can be concluded that particle flows and pressure changes within the silica HPLC column in liposomal formulations affect the adsorption of phenolic compounds.

Moreover, the findings of HPLC-DAD analysis clearly exhibited that cinnamic acid and ferulic acid are the basic and major antioxidant phenolic contents in both mushrooms. In a study to determine bioactive compounds and antioxidant capacity in shiitake mushroom, both phenolic contents were found to be significantly determine (Nam et al., 2021). Mwangi et al. stated that species of mushrooms influence the active compounds that contribute to the antioxidant activity of mushrooms (Mwangi et al., 2022). Both oyster and shiitake mushrooms were effective in inhibiting of OH, and DPPH radicals and chelating iron ions (Fontes Vieira et al., 2013; Mwangi et al., 2022).

3.4. Enzyme inhibitory effects

Enzymes are considered unique therapeutic tools for treating some health problems (Mohammed et al., 2020; Zengin et al., 2018). This fact makes them important targets in pharmaceutical applications. Inhibiting enzymes is thought to alleviate the symptoms of these diseases (Hennigan & Lynch, 2022). For example, inhibition of cholinesterase can increase acetylcholine levels in the synaptic cleft and help improve cognitive function in AD patients (Martins et al., 2023). Likewise, amylase and glucosidase are the main carbohydrate-hydrolyzing enzymes and can control blood sugar levels in diabetics following a high-carbohydrate diet (Kashtoh & Baek, 2023). Similar connections exist between tyrosinase and hyperpigmentation; lipase and obesity. For this purpose, several compounds have been chemically prepared as enzyme inhibitors. However, there are serious concerns about their long-term use. Therefore, the synthetic inhibitors need to be replaced with natural ones that are safe and effective (Li et al., 2023).

Given this information, we investigated the enzyme-inhibiting effects of mushrooms and their liposomal forms. The results are shown in Table 4. Regarding AChE inhibition, SHE extracts and their liposomal forms generally showed stronger ability than those of OYE (with the exception of SL: SHE (in MeOH)). Among the tested samples, the best

Table 4
Enzyme inhibition findings of extracts (%, SD).

Samples	AChE inh.	BChE inh.	Tyrosinase inh.	Amylase inh.	Glucosidase inh.
SL:SHE (MeOH)	NA	NA	NA	12.40 ± 0.47	NA
SL:SHE (MeOH/Aq)	93.27 ± 0.34	52.04 ± 3.59	35.52 ± 0.89	13.12 ± 0.40	NA
SL:SHE (Aq)	88.45 ± 0.74	59.83 ± 0.96	32.58 ± 0.34	23.33 ± 0.47	11.03 ± 0.88
SL:OYE (MeOH)	59.66 ± 1.21	44.64 ± 8.28	20.86 ± 1.14	30.57 ± 0.73	NA
SL:OYE (MeOH/Aq)	67.50 ± 0.33	37.14 ± 4.17	43.86 ± 0.37	14.04 ± 0.30	NA
SL:OYE (Aq)	76.74 ± 0.94	51.82 ± 1.19	45.59 ± 1.33	43.44 ± 1.33	NA
SHE (MeOH)	96.82 ± 0.30	98.97 ± 0.27	NA	79.68 ± 0.42	97.86 ± 0.84
SHE (MeOH/Aq)	86.49 ± 0.52	47.70 ± 1.27	32.85 ± 1.54	12.75 ± 0.50	NA
SHE(Aq)	77.57 ± 0.28	48.33 ± 3.14	28.58 ± 0.25	13.22 ± 0.21	NA
OYE (MeOH)	72.20 ± 1.39	47.84 ± 0.74	26.06 ± 2.11	31.18 ± 0.32	NA
OYE (MeOH/Aq)	63.63 ± 1.04	38.13 ± 0.48	35.24 ± 0.47	18.11 ± 0.16	NA
OYE(Aq)	65.18 ± 0.63	41.30 ± 1.86	33.14 ± 1.06	28.71 ± 0.42	NA
SL	10.31 ± 1.14	NA	13.04 ± 1.63	7.79 ± 1.11	NA

AChE: Acetylcholinesterase; BChE: Butyrylcholinesterase; SD: Standard deviation; SL: Soy lecithin; SHE: Shiitake; OYE: Oyster; (MeOH): Methanolic extract; (Aq): Aqueous extract; (MeOH/Aq): Methanolic/Aqueous extract; NA: Not Active (mean ± SD, n = 3).

AChE inhibitory effect was found for SHE (in MeOH) at 96.82%. As for BChE inhibition, SHE (in MeOH) extract was found as the best inhibitory effect with 98.97%.

In terms of tyrosinase inhibitory activity, SL:OYE aqueous extract was rated best with 45.59%. SHE and SL:SHE (in MeOH) were not active on tyrosinase. In general, the formation of liposomal forms was positively influenced by the observed tyrosinase inhibitory effects. We observed different results regarding amylase and glucosidase inhibition. The best amylase inhibition was demonstrated by SHE (in MeOH) with 79.68%. Only SHE (in MeOH) and SL:SHE (in Aq) exhibited glucosidase inhibitory activity. When all enzyme inhibition results were evaluated together, it was seen that enzyme inhibition abilities were almost preserved with the formation of liposomal forms, and even the inhibition of some enzymes was positively affected by the formation. Clearly, various antioxidant phenolic contents (ferulic, cinnamic, caffeic and/or chlorogenic acid) in the extracts and their liposomal formulations can be shown as factors that increase enzyme inhibition (Drakontaeidi & Pontiki, 2024; Oboh et al., 2013; Zhou & Dong, 2023). Various reports have favorably mentioned the enzyme blocking abilities of *P. ostreatus* (Agunloye & Oboh, 2022; Ćilerdžić et al., 2019) and *L. edodes* (Alam et al., 2010; Sepčić et al., 2019; Yim et al., 2013). Only a few publications in the literature have reported the enzyme-inhibiting effect of liposomal forms (Karatoprak et al., 2020; Mancini et al., 2018; Therdphapiyanak et al., 2013). As a finding, the formation of liposomal forms can occur in a stearin region close to the active site of enzymes and therefore may be more effective compared to extracts (Sercombe et al., 2015).

4. Conclusion

The fence of the current study is to exhibit and highlight the antioxidant activities of liposomal formulations of *Pleurotus ostreatus* and *Lentinus edodes* in different extracts. Findings of this study demonstrated that liposomal formulations have higher antioxidant capacity. This results support that it is related to the high phenolic contents determined in SL: SHE and SL: OYE formulations. In addition, findings showed that extraction of SL:OYE pointed out the best antioxidant activity followed by SL:SHE formulation extract in methanol/aqueous. Although, the methanolic extract of shiitake mushroom contains the most active enzyme inhibitors, these inhibitors cannot have an inhibitory effect in liposomal form due to their controlled micelle binding in cellular membrane transitions. In oyster mushrooms, since the enzyme-inhibiting compounds are freely distributed in the cytosol, they are also active in liposomal form and subject to controlled release. The addition of aqueous forms to the methanolic structure creates hydroscopic pore openings in micellar form, thus supporting the passage of active compounds with inhibitory effect. In acetylcholine esterase inhibition, the lipidic structure of the choline compound supports the adhesion of inhibitory active components to the active site by harmonizing it with the liposomal structure. In summary, our results may be valuable for the development of health-promoting applications from shiitake and oyster mushrooms, for example functional ingredients in the nutraceutical field. However, further studies are required to understand their toxic potential *in vivo* cell models as well as bioavailability in gastrointestinal models.

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CRediT authorship contribution statement

Caglar Macit: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Ozan Emre Eyupoglu:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Meltem Macit:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Gokhan Zengin:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation.

Declaration of competing interest

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability

Data will be made available on request.

Acknowledgments

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.104737.

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