Brazilian Journal of Microbiology (2022) 53:1187–1197
https://doi.org/10.1007/s42770-022-00745-x

BIOTECHNOLOGY AND INDUSTRIAL MICROBIOLOGY - RESEARCH PAPER

Phenolic compounds of *Phellinus* spp. with antibacterial and antiviral activities

Francisco Javier Vazquez-Armenta[1] · Juan Manuel Leyva[1,2] · Veronica Mata-Haro[1] · Gustavo A. Gonzalez-Aguilar[1] · Manuel R. Cruz-Valenzuela[1] · Martin Esqueda[1] · Aldo Gutierrez[1] · Filomena Nazzaro[3] · Florinda Fratianni[3] · Rigoberto Gaitán-Hernández[4] · J. Fernando Ayala-Zavala[1]

Received: 25 August 2021 / Accepted: 27 March 2022 / Published online: 5 April 2022

Abstract

Phellinus Quél is one of the largest genera of Hymenochaetaceae; it comprises about 220 species widely distributed on Earth. Most *Phellinus* species are lignicolous mushrooms that accumulate bioactive compounds. This research studied the phenolic composition of *Phellinus* spp. and their relationship with antibacterial and antiviral capacity. Phenolics were extracted from *Phellinus badius*, *P. fastuosus*, and *P. grenadensis*; their antiviral and antibacterial activities were evaluated against *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, and *Escherichia coli* O157: H7; and the bacteriophages MS2 and Φ-×174. Gallic acid, chlorogenic acid, caffeic acid, epicatechin, ferulic acid, catechin, 1,3-dicaffeoylquinic acid, *p*-coumaric acid, and rutin were found in different proportions among *Phellinus* spp. Total phenolic content ranged from 96 to 209 mg GAE/g, and total flavonoids from 10 to 27 QE/g. The minimum inhibitory concentrations of *P. badius*, *P. grenadensis*, and *P. fastuosus* against *E. coli* O157: H7 were 13, 20, and 27 mg/mL, against *S. enterica* were 20, 30, and 15 mg/mL, and against *L. monocytogenes* were 10, 15, and 25 mg/mL, respectively. The phenolic content was better correlated with the antibacterial effect against *E. coli* O157: H7 and *L. monocytogenes* ($r = 0.8–0.9$), but not against *S. enterica* ($r = 0.05$). The antiviral activity of the extracts (0.9 mg/mL) was 29 to 41% against MS2 and 27 to 38% for Φ-X174 virus ($r = 0.8–0.9$). In silico analysis showed binding energy values of − 7.9 and − 4.8 kcal/mol between the identified phenolic compounds and the M and G proteins of each virus. The antibacterial and antiviral properties of *Phellinus* species were correlated with the phenolic content.

Keywords Medicinal mushrooms · Viral agents · Pathogenic bacteria · Gallic acid

Introduction

Recently, the search for antiviral and antibacterial properties in fungi species has increased [1]. Fungi are organisms with higher biodiversity; more than 80,000 species have been identified only in the soil [2], and their bioactive properties have been recognized by traditional medicine. In addition, the content of active compounds in fungi is being used by the pharmaceutical, medical, agriculture, and food industries. Despite this, the proportion of studied bioactive metabolites from fungi and their properties is relatively low; therefore, these organisms remain an untapped resource with enormous industrial potential [1]. *Phellinus* Quél is one of the largest genera of Hymenochaetaceae, which is comprised of about 220 species. Most *Phellinus* macro-fungi are perennial lignicolous mushrooms widely distributed on Earth. Several species of *Phellinus* have pharmacologically important bioactive metabolites like polysaccharides, phenols, and flavonoids, which are traditionally used to treat different diseases such as cancer, diabetes, and hepatitis allergy inflammation [3]. Some *Phellinus* fungi are historically recorded as traditional medicines used to treat various diseases in

Responsible Editor: MelissaFontes Landell

✉ J. Fernando Ayala-Zavala
jayala@ciad.mx

[1] Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora CP. 83304, Mexico

[2] Tecnológico Nacional de México, Campus Valle del Yaqui, Bacum, Sonora CP. 85276, Mexico

[3] ISA CNR, Institute of Food Sciences, 83100 Avellino, Italy

[4] Instituto de Ecologia, A.C. Xalapa, Veracruz, CP. 91073, Mexico

eastern Asian countries, especially China, Japan, and Korea [4]. Polysaccharides and polyphenols are responsible for the therapeutic potential of *Phellinus* spp. An array of pharmacological activities was found in the mycelia, submerged culture, and fruiting bodies of *Phellinus* species, which includes antioxidant, anti-inflammatory, anti-diabetic, reduction in triglyceride absorption and obesity, system protection, on dermatological conditions like eczema, antimicrobial, and anticancer activity [5]. Phenolic compounds are also considered potent antioxidant, antimicrobial, and antiviral agents, among other properties [6].

Phellinus species have been studied as a source of bioactive compounds with antioxidant and antibacterial properties [7]. For example, *P. merrillii* (Murrill) Rivarden, *P. fastuosus* (Lév.) S. Ahmad, *P. grenadensis* (Murrill) Ryvarden, and *P. badius* (Cooke) G. Cunn. from Sonora, Mexico, showed high values of total phenolics, flavonoids, and antioxidant capacity [8]. Also, the methanolic extract of *P. gilvus* (Schwein.) Pat., *P. rimosus* (Berk.) Pilát, and *P. badius* showed total phenolic contents of 44.7–49.31 mg of gallic acid equivalents (GAE)/g, and total flavonoid contents of 26.48–30.58 mg of quercetin equivalents (QE)/g; these values were positively correlated with their antioxidant activity [9]. Meanwhile, the methanolic extract of *P. gilvus*, rich in phenolic compounds, showed antioxidant properties and exhibited antibacterial properties against Gram-positive (*Staphylococcus aureus* and *Streptococcus mutans*) and Gram-negative bacteria (*Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*) [10]. Also, the extracts of *P. linteus* obtained by sequentially organic solvent extraction (petroleum ether, chloroform, and methanol) presented antibacterial activity. The methanol extracts showed the best antibacterial activity against *Xanthomonas campestris*, *Pseudomonas syringae*, *Agrobacterium tumefaciens*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *and Streptomyces pneumoneae*, followed by chloroform, petroleum ether. The phytochemical characterization of methanolic extracts revealed the presence of alkaloids, tannins, flavonoids, steroids, glycosides, and phenolic compounds [11]. However, the molecular identification of the active compounds and mechanisms of action still needs to be addressed.

Despite the recognized antioxidant and antibacterial activity of *Phellinus* spp. extracts, their antiviral properties have been scarcely investigated. Viral diseases are a significant problem impacting the world, and the production of broad-spectrum antiviral substances is a challenge in the pharmaceutical industry since they must offer protection against emerging and re-emerging diseases [12]. Therefore, the search for novel and efficient antiviral compounds is continuously needed. In this context, phenolic compounds are recognized as antiviral compounds, and their mode of action includes multiple targets in the infectious cycle, such as affecting the viral attachment to the host cell, their entry, replication, and assembly [13]. For example, delphinidin (an anthocyanidin) impaired the attachment and entry of the hepatitis C virus into the host cell surface [14]. Meanwhile, epigallocatechin gallate (commonly found in the green tea plant) showed a broad-spectrum activity against enveloped and non-enveloped viruses; its activity was attributed to the interactions with the virion surface proteins [15]. Also, dicaffeoylquinic acid can bind the HIV-1 integrase and inhibit its activity [16]. Nevertheless, when phenolic compounds receive attention as antiviral agents, most of the studied molecules come from plant tissues, leaving aside the potential of fungi as a source of antiviral compounds [17]. Therefore, the present study aimed to characterize the phenolic composition of *P. badius*, *P. grenadensis*, and *P. fastuosus* extracts and their relation with the antiviral and antibacterial activity against the bacteriophages MS2 (ssRNA), Φ-×174 (ssDNA), *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica* serovar Choleraesuis, and *Escherichia coli* O157: H7.

Materials and methods

Phellinus badius, *P. grenadensis*, and *P. fastuosus* collection and identification

The *Phellinus* species were collected in the Sierra de Álamos-Río Cuchujaqui Biosphere Reserve, Sonora, Mexico (27°03′18.3″N, 109°05′29.3″W), and transported to the CIAD laboratories in Hermosillo. The mushrooms were dried in an oven at 60 °C for 3 days, and the taxonomic identification (Table 1) was carried out in the Laboratory of Fungal Biotechnology of CIAD according to Gilbertson and Ryvarden [18], Larsen and Cobb-Poulle [19], and the information supplied by the Species Index Fungorum database [20].

Extraction of phenolic compounds

Phenolic compounds were extracted from the *Phellinus* spp. with methanol, as previously described by Vazquez-Armenta et al. [21]. The fruiting bodies of *Phellinus* were mechanically ground, and 10 g were placed in 100 mL of methanol: water (7:3) solution; subsequently, the ground mushrooms were macerated for 10 days at 25 °C in darkness. The extracts were then filtered (Whatman No. 1, Springfield Mill, Maidstone Kent, UK), and methanol was removed in a rotatory evaporator (63 rpm and 45 °C) at reduced pressure. Then the residues were hydrolyzed with 10 mL of NaOH (4 M) for 4 h, and the pH was adjusted to 2 with HCl (4 M)

Table 1 Morphologic characteristics used for the identification of *Phellinus badius*, *P. grenadensis*, and *P. fastuosus* (Basidiomycota: Hymenochaetaceae)

	Phellinus badius	*Phellinus grenadensis*	*Phellinus fastuosus*
Hymenial setae	Lacking	Lacking	Lacking
Basidiospore	Subglobose-globose	Oval-ellipsoid	Ellipsoid-subglobose
Basidiocarp	Pileate	Pileate	Pileate
Spore color in KOH	Yellow–brown	Dark brown	Brown
Spore size (μm)	5×4	3×6	5×6
Pores per mm	3	6	8
Shape	Ungulate	Ungulate	Flat-ungulate

and lyophilized for further analysis. The yield of extraction was expressed as a percent.

Total phenolic and flavonoid content

The total phenolic content was measured with the colorimetric method described by Çayan et al. [22]. In a microplate well, 75 μL of Folin–Ciocalteu reagent (1:10) and 60 μL of 7.5% Na_2CO_3 were added to 15 μL of each mushroom extract. The microplate was incubated at 25 °C in darkness for 30 min, and the optical density (OD) was measured at 765 nm with a microplate reader FLUOstar Omega (BMG Labtech, Chicago, IL, USA). Gallic acid was used as standard, and results were expressed in mg of GAE per gram of dry extract. All of the samples were determined by triplicate.

The flavonoid content was determined based on the method described by Zhishen et al. [23], with some modifications. Each mushroom extract (100 μL) was mixed with 430 μL of mixture A (1.8 mL of 5% $NaNO_2$ with 24 mL of distillate water) and incubated for 5 min. Then, 30 μL of 10% anhydrous $AlCl_3$ was added and incubated for 1 min. Later, 440 μL of the mixture B (12 mL of NaOH 1 M with 14.4 mL of distillate water) was added, and 150 μL of this reaction was taken and read at 496 nm with a microplate reader FLUOstar Omega (BMG Labtech, Chicago, IL, USA). The samples were analyzed by triplicate, and results were expressed in mg QE/g.

Phenolic compound identification

The ultra-performance liquid chromatography (UPLC) analysis was carried out by using an ACQUITY Ultra Performance LCTM system (Waters) linked simultaneously to a PDA 2996 photodiode array detector (Waters) as previously reported by Fratianni et al. [24]. This analysis was performed at 30 °C by using a reversed-phase column (BEH C18, 1.7-μm, 2.1×l00-mm; Waters). The used mobile phase consisted of solvent A (7.5 mM acetic acid) and solvent B (acetonitrile) at a flow rate of 250 μL/min. Gradient elution was used starting at 50/0 solvent B for 0.8 min, 5–20% solvent B for 5.2 min, isocratic 20% solvent B for 0.5 min, 20–30% solvent B for 1 min, isocratic 30% solvent B for 0.2 min, 30–50% solvent B for 2.3 min, 50–100% solvent B for 1 min, isocratic 100% solvent B for 1 min, and finally 100–5% solvent B for 0.5 min. At the end of this sequence, the column was equilibrated under the initial conditions for 2.5 min. The applied pressure during the chromatographic run ranged from 6,000 to 8,000 psi. The effluent carrying the phenolic compounds was introduced to a liquid chromatography detector (scanning range, 210–400 nm; resolution, 1.2 nm). Each extract was injected (10 μL) by duplicate, and phenolic compounds were identified and quantified with standard substances and calibration curves. The ultraviolet-detection wavelength was set at 280 nm. The Empower software (Waters) was used to control the instruments and data acquisition and processing.

Antibacterial activity

The antibacterial activity of each *Phellinus* extract was evaluated against *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* subsp. *enterica* serovar Choleraesuis (ATCC 14,028), and *Escherichia coli* O157: H7 (ATCC 43,890). The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of each extract against each bacterium. Stock solutions of each *Phellinus* extract were prepared in trypticase soy broth (TSB), and several concentrations were prepared (0–35 mg/mL). The treatment consisted of mixing 5 μL of bacterial inoculum (1×10^8 CFU/mL) with 295 μL of each extract dilution in sterile 96-well microplates (Costar 96). The inoculum of each bacterium was prepared from overnight cultures; the microplate included a positive (it contained the inocula but not the extract) and negative controls (they contained the extracts but not the inoculum), and then they were incubated at 37 °C for 24 h. The extracts' MIC values were considered the lowest concentrations needed to inhibit the growth of the tested bacteria and confirmed by counting the viable bacteria on agar plates [25]. Also, the growth curves of the tested bacteria (10^8 CFU/mL) were recorded in the presence of the fungi extracts by applying the same experimental conditions. The microplates were incubated at 37 °C for 16 h with intermittent shaking, and the OD readings at 600 nm were recorded every 30 min. The

experiment was replicated three times, and the experimental growth data were fitted to the Baranyi function [26] with a complementary tool for Microsoft Excel (D-model, J. Baranyi, Institute of Food Research, Norwich, UK) and used to calculate the adaptation or lag time (λ; h), maximum growth rate (μ_{max}; OD/h), and maximum growth Ymax (Y_{max}; OD) for each growth curve.

Antiviral activity against MS2 and Φ-X174

The antiviral activity was evaluated by exposing the bacteriophages MS2 (ATCC 15,597-B1) and Φ-×174 (ATCC 13,706-B1) to the presence of the *Phellinus* extracts. The viral dispersions (2 logs of plaque-forming units, PFU/mL) were exposed to 0.9 mg/mL of each extract, mixed and agitated for 10 min; a control mixture of the untreated virus was included. Subsequently, the viral infection was quantified using the double agar layer method. The treated and control virus were added to 10^5 CFU/mL of their respective bacterial host [*Escherichia coli* strain C-3000 (ATCC 15,597) for MS2 and *Escherichia coli* strain C (ATCC 13,706) for Φ-×174]. First, the mixtures were added to a top layer of melted agar (3% tryptic soy broth, 0.5% NaCl, 0.6% agar) and poured onto a bottom layer of solid agar (3% tryptic soy broth, 0.5% NaCl, 1.2% agar). The plates were solidified and incubated 24 h at 37 °C. The PFU in treatments and control were counted, and the percentage of activity was determined by subtracting the titer values of the treated samples from the untreated control [27]. The experiment was replicated three times.

Molecular docking between viral proteins and the identified phenolic compounds

The molecular docking analysis was simulated between each identified phenolic compound of *Phellinus* extracts with the M protein of the MS2 bacteriophage (PDB: 5TC1; chain M) and the G protein of Φ-X174 (PDB: 1CD3; chain G) searching for physicochemical interactions. The M and G proteins are considered responsible for the initial viral attachment to the F-pili and lipopolysaccharides of the host cells, respectively [28, 29]. This analysis was done with the AutoDock Vina application in the UCSF Chimera version 1.13 software (Resource for Biocomputing, Visualization, and Informatics, University of California, San Francisco, USA). The affinity energies (kcal/mol) were selected as those with the lowest root-mean-square deviation (RMSD, Å) between the selected proteins and each of the identified phenolic compounds. Ten binding modes with a 3 level of exhaustiveness search and a 3 kcal/mol level of maximum energy difference were set as basic parameters during the analysis.

Statistical analysis

A completely randomized design was applied to estimate significant differences among *Phellinus* species extracts. A one-way analysis of variance (ANOVA) was used to evaluate the effect of the fungal species on the responses being assessed. Furthermore, the means comparisons (extracts and controls) were made by the Tukey–Kramer test. The Pearson correlation analysis was used to evaluate the relationships between the content of total phenols and total flavonoids vs. the antibacterial and antiviral activity capacity, respectively. The analyses were performed in the statistical package NCSS version 2007 (NCSS, LLC, Utah, USA).

Results and discussion

Identification and quantification of phenolic compounds

The weight yield of the extracts showed values around 18% w/w (Table 2), and they were similar among them ($p > 0.05$); but the *P. badius* had the highest content of total phenolic compounds and flavonoids with values of 209.76 mg GAE/g and 27.61 mg QE/g, respectively. In contrast, the total phenolic and flavonoid contents were not different ($p > 0.05$) between *P. grenadensis* (111.4 mg GAE/g and 13.12 mg QE/g) and *P. fastuosus* (96.96 mg GAE/g and 10.4 mg QE/g). For *P. badius*, the main phenolic compounds were gallic acid (134.15 µg/g), chlorogenic acid (264.15 µg/g), caffeic acid (67.9 µg/g), epicatechin (71.05 µg/g), and ferulic acid (82.45 µg /g) (Fig. 1). The extract of *P. grenadensis* showed five phenolic compounds: gallic acid (28.4 µg/g), catechin (26.95 µg/g), 1,3-dicaffeoylquinic acid (14.3 µg/g), rutin (587.5 µg/g), and ferulic acid (464.75 µg/g). Meanwhile, the extract of *P. fastuosus* showed gallic acid (47.95 µg/g), chlorogenic acid (193.70 µg/g), catechin (26.15 µg/g), epicatechin (116.6 µg/g), 1,3-dicaffeoylquinic acid (27.13 µg/g), *p*-coumaric acid (4.45 µg/g), and ferulic acid (75.85 µg/g). *p*-Coumaric acid was found only in *P. fastuosus*, caffeic acid only in *P. badius*, and rutin only in *P. grenadensis*; the latter was the most abundant phenolic molecule identified by UPLC.

Wood-degrading fungi such as *Phellinus* produce lignin-degrading enzymes, for example, lignin peroxidases, manganese-dependent peroxidases, and laccases [30]. Then, the degraded lignin could be used to synthesize phenolic compounds accumulated in the fungal fruiting body. However, enzymatic expression and activity are influenced by several biotic and abiotic factors [30], and it has been demonstrated that the ligninolytic activity of *Phellinus* is also affected by the species [31]. Therefore, the differences in the phenolic composition observed among the studied specimens could

Table 2 Identified phenolic compounds, total phenolic, flavonoids, and weight yield of *P. badius*, *P. grenadensis*, and *P. fastuosus* extracts

Compound	Retention time (mins)	Concentration (µg/g)		
		P. badius	*P. grenadensis*	*P. fastuosus*
Gallic acid	1.04	134.15	28.4	47.95
Chlorogenic acid	3.14	264.15	ND	193.70
Catechin	3.43	ND	26.95	26.15
Caffeic acid	3.81	67.9	ND	ND
Epicatechin	4.03	71.05	ND	116.6
1,3-Dicaffeoylquinic acid	4.54	ND	14.3	27.3
p-Coumaric acid	5.14	ND	ND	4.45
Rutin	5.28	ND	587.5	ND
Ferulic acid	5.47	82.45	464.75	75.85
Extract weight yield (%)		18.4[a]*	17.5[a]	18[a]
Total phenols (mg GAE/g)		209.76[a]	111.4[b]	96.96[b]
Total flavonoids (mg QE/g)		27.61[a]	13.12[b]	10.4[b]

*Different letters in the same row indicate significant differences among extracts ($p < 0.05$)

be caused by the differential expression of enzymes in charge of degrading lignin or phenolic compounds' biosynthesis.

Previous studies highlighted the genus *Phellinus* as a source of phenolic compounds. The production of flavonoids by *Phellinus* sp. was increased with naphthaleneacetic acid and coumarin at 0.03 and 0.02 g/L, respectively [32]. Similarly, Wang et al. [33] improved the production of flavonoids in *P. igniarius* by adding wheat bran, corn silk, yeast extract, KH_2PO_4, and $MgSO_4$. Besides, Ayala-Zavala et al. [9] showed that methanolic extracts from *P. gilvus*, *P. rimosus*, and *P. badius* showed 49.31, 46.51, and 44.7 mg GAE/g, respectively, and total flavonoid contents of 30.58, 28, and 26.48 mg QE/g, respectively. The values of total phenolic contents found in the present study were 2 to fourfold higher than those reported in the studies mentioned above, while the total flavonoid contents were similar. These differences could be attributed to the extraction method because, in the present work, a methanolic maceration for 10 days plus alkaline and acid hydrolysis were employed, which could explain the higher amount of extracted phenolic compounds.

On the other hand, the methanolic extracts of *P. merrillii* showed a total phenolic content of 913.91 mg GAE/g and total flavonoids of 563.83 mg QE/g [34], but this extract was obtained with the same technique used in our work. Therefore, the observed differences could be explained by biotic and abiotic factors surrounding each species [35, 36]. In this way, the results reported here help broaden the knowledge about the phenolic content of *Phellinus* and extraction methods.

Although *Phellinus* species are recognized as a source of phenolic compounds, little is known about their specific composition. Sułkowska-Ziaja et al. [37] identified four phenolic acids (3,4-dihydroxy-phenylacetic, gallic, protocatechuic, and syringic acids) in the methanolic extracts of *P. igniarius*, *P. pini*, *P. pomaceus*, and *P. robustus*, varying their concentration among the species, with protocatechuic acid as the common compound. Similarly, Heleno et al. [38] reported gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, and 3, 4, or 5-*O*-caffeoylquinic acid in *Phellinus linteus*. Gallic and chlorogenic acids and rutin were also identified in *Ganoderma lucidum* extracts but not in *Trametes hirsuta*, which showed catechol and cinnamic acid [39]. This family of compounds is widely recognized for its hepatoprotective, antioxidant, and antiviral activities [40]. To the extent of our literature review, this is the first report of 1,3-dicaffeoylquinic acid in mushrooms, even when it is commonly found in coffee beans, cherries, apples, kiwifruit, and apricots [41]. Therefore, *P. fastuosus* and *P. grenadensis* can be considered sources of this hydroxycinnamic acid derivative.

Antibacterial activity

The antibacterial activities of the extracts are shown in Table 3. *P. badius* showed the lowest MIC (13 mg/mL) against the growth of *E. coli* O157: H7, followed by *P. grenadensis* (20 mg/mL) and *P. fastuosus* extracts (27 mg/mL). The same pattern was observed against *L. monocytogenes*, but this bacterium was more sensitive to the presence of the extracts than *E. coli* O157: H7 (MIC = 10–25 mg/mL). Meanwhile, *S. enterica* ser. Choleraesuis was more susceptible to *P. fastuosus* extract than *P. badius* (MIC = 20 mg/mL) and *P. grenadensis* (MIC = 30 mg/mL). Nevertheless, no inhibitory activity was observed of all the extracts against *S. aureus* at the tested concentrations (0–35 mg/mL).

Pearson's correlations showed a positive relationship with the phenolic content and the antibacterial capacity (Table 3). The total phenol content showed *r* values of 0.88 with the dose needed to inhibit the growth of *E. coli* O157: H7 ($p = 0.0013$), with *L. monocytogenes* of 0.8050

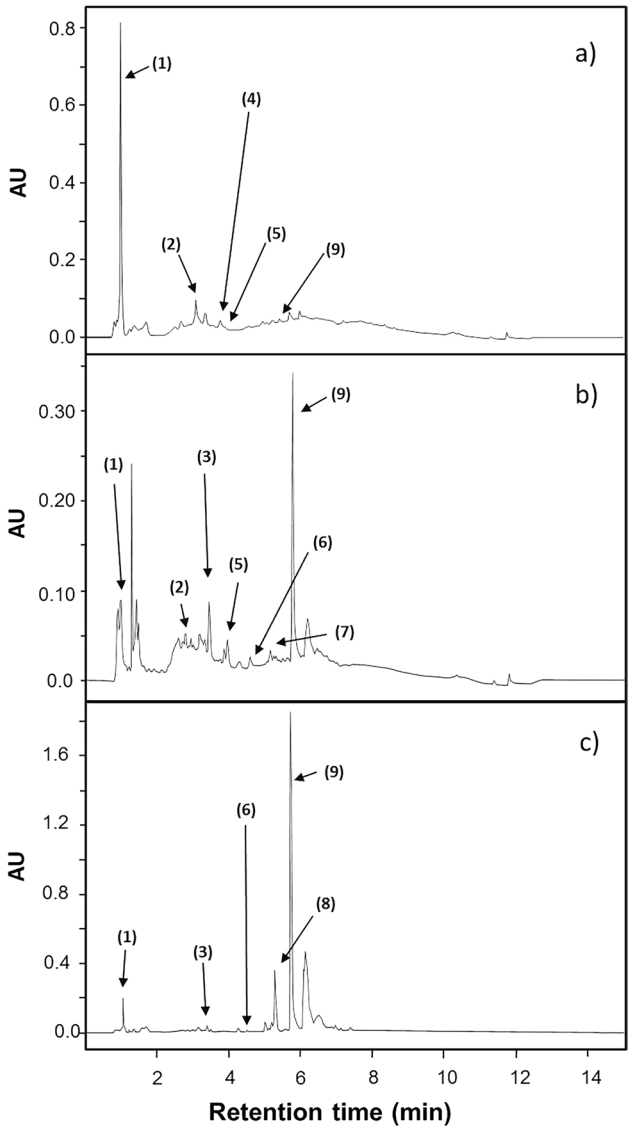

Fig. 1 UPLC-DAD chromatograms of **a** *P. badius*, **b** *P. grenadensis*, and **c** *P. fastuosus* extract showing the identified phenolic compounds: (1) gallic acid, (2) chlorogenic acid, (3) catechin, (4) caffeic acid, (5) epicatechin, (6) 1,3-dicaffeoylquinic acid, (7) *p*-coumaric acid, (8) rutin, and (9) ferulic acid

($p = 0.0088$), and with *S.* Choleraesuis of 0.0552 ($p = 0.880$). Meanwhile, the flavonoid content showed *r* values of 0.904 with the doses needed to inhibit the growth of *E. coli* O157: H7 ($p = 0.0008$), *L. monocytogenes* of 0.8260 ($p = 0.0061$), and *S.* Choleraesuis of 0.0291 ($p = 0.9410$). The high correlation coefficient with the MICs against *E. coli* O157: H7 and *L. monocytogenes* with a low probability ($p < 0.05$) indicates the significant relationship between the phenolic compounds and the antibacterial capacity.

The growth kinetics evidenced how the studied *Phellinus* extracts affected bacterial growth (Table 4). *P. badius* extended the lag phase of *E. coli* O157: H7 about 5.5 times compared to non-treated bacteria, while for *S.* Choleraesuis and *L. monocytogenes*, the extension was 1.8 and 2.3 times, respectively. *P. fastuosus* caused the lag phase extension by 3.7, 3.1, and 2.0 times for *E. coli* O157: H7, *L. monocytogenes*, and *S.* Choleraesuis, respectively, compared with untreated bacteria. Similarly, *E. coli* O157: H7 was more susceptible to *P. grenadensis* extending the lag phase 3.3 times, followed by *S.* Choleraesuis (2.1 times) and *L. monocytogenes* (2.0 times). The extracts also affected the maximum growth rates (μmax) of the tested bacteria. For example, *E. coli* O157: H7 showed a reduction of 62% on this parameter in the presence of *P. badius* extract, compared with the control, followed by *P. grenadensis* (57%) and *P. fastuosus* (52%). For *S.* Choleraesuis, μ_{max} values were more drastically affected by the extracts, with a 99% reduction caused by *P. badius*, 90% by *P. grenadensis*, and 80% by *P. fastuosus.* Finally, μ_{max} of *L. monocytogenes* was reduced 84 and 81% by *P. grenadensis* and *P. fastuosus* extracts, but to a lesser extent by *P. badius* (43%). The lag phase extension and the μ_{max} reduction caused by the *Phellinus* extracts affected the maximum bacterial growth (y_{max}). Considering that the lag phase (λ) is the time taken by bacteria in adapting to a new environment, therefore, the extracts affected the ability of bacteria to adapt to the growing media; usually, this occurs by altering the membrane functionality and nutrient absorption. The influence of the treatments on the growth rate indicated that the extracts affected the treated bacteria's reproductive capacity, possibly by inhibiting the activity and production of enzymes necessary for cellular reproduction.

The antibacterial activity of *Phellinus* extracts can be attributed to the presence of phenolic compounds. It has been reported that chlorogenic acid found in the extracts of *P. badius* and *P. fastuosus* showed bacteriostatic and bactericidal effects against *E. coli* O157: H7. This effect was dose-dependent and associated with exposure time [42]. Vazquez-Armenta et al. [21] reported that *L. monocytogenes* was more susceptible to ferulic, caffeic, and gallic acids (MIC = 3–4 mg/mL) than to catechin and rutin (MIC > 7 mg/mL). Meanwhile, gallic and ferulic acids, found in all *Phellinus* extracts, inhibited the bacterial growth of *P. aeruginosa*, *S. aureus*, and *L. monocytogenes* by damaging their cytoplasmic membranes [43]. A similar effect was observed in the cytoplasmic membrane of uropathogenic *E. coli* treated with catechin, protocatechuic, and vanillic acids [44]. Catechin was more effective than protocatechuic and vanillic acids at disturbing membrane permeability. These results were confirmed using a phospholipids membrane model monolayer, where catechin also showed the most fluidizing effect on the monolayers, followed by protocatechuic and vanillic acids [44].

The disruption of the cytoplasmic membrane could lead to the internalization of phenolic compounds to

Table 3 Minimum inhibitory concentrations (MIC) of *P. badius*, *P. grenadensis*, and *P. fastuosus* extracts against pathogenic bacteria, and Pearson correlations of the antibacterial activity with the total phenol and flavonoid contents

Bacteria	MIC (mg/mL)		
	P. badius	*P. grenadensis*	*P. fastuosus*
E. coli O157: H7	13	20	27
S. Choleraesuis	20	30	15
L. monocytogenes	10	15	25
S. aureus	> 35	> 35	> 35
Correlated responses		Pearson coefficient (*r*)	*p*-value
Polyphenols *Vs*	*E. coli* O157: H7	0.888	0.001
	S. Choleraesuis	0.055	0.888
	L. monocytogenes	0.805	0.009
Flavonoids *Vs*	*E. coli* O157: H7	0.904	0.001
	S. Choleraesuis	0.029	0.941
	L. monocytogenes	0.826	0.006

Table 4 Effect of extracts of *P. badius*, *P. grenadensis*, and *P. fastuosus* on the growth parameters of *E. coli* O157: H7, *S.* Choleraesuis, and *L. monocytogenes*

Bacteria	Treatment	λ (h)	μ_{max} (OD/h)	y_{max} (OD)	R^{2*}
E. coli O157: H7	Control	3.4 ± 0.10^{d}	0.209 ± 0.006^{d}	2.46 ± 0.074^{d}	0.98
	P. badius	18.5 ± 0.55^{a}	0.079 ± 0.002^{a}	0.37 ± 0.014^{a}	0.96
	P. grenadensis	11.2 ± 0.34^{c}	0.100 ± 0.003^{c}	0.59 ± 0.018^{c}	0.81
	P. fastuosus	12.6 ± 0.38^{b}	0.089 ± 0.003^{b}	0.48 ± 0.011^{b}	0.96
S. Choleraesuis	Control	9.8 ± 0.29^{d}	0.383 ± 0.011^{d}	1.29 ± 0.039^{d}	0.99
	P. badius	18.0 ± 0.54^{c}	0.005 ± 0.001^{a}	0.08 ± 0.002^{a}	0.98
	P. grenadensis	21.0 ± 0.63^{a}	0.079 ± 0.002^{c}	0.29 ± 0.009^{c}	0.92
	P. fastuosus	19.8 ± 0.59^{b}	0.037 ± 0.001^{b}	0.16 ± 0.005^{b}	0.99
L. monocytogenes	Control	4.5 ± 0.13^{d}	0.230 ± 0.007^{d}	2.35 ± 0.070^{c}	0.98
	P. badius	10.6 ± 0.32^{b}	0.131 ± 0.004^{c}	0.41 ± 0.012^{a}	0.84
	P. grenadensis	9.2 ± 0.28^{c}	$0.037^{a} \pm 0.001^{a}$	0.40 ± 0.012^{a}	0.85
	P. fastuosus	13.8 ± 0.41^{a}	$0.063^{b} \pm 0.002^{b}$	0.59 ± 0.018^{b}	0.98

Data are the mean of three independent experiments. Different letters in each column indicate differences ($p < 0.05$) among treatments for each bacterium. λ corresponds to the lag phase, μ_{max} is the maximum growth rate, and y_{max} is the maximum growth of bacteria

*R^2 = Coefficient of determination

reach multiple targets. For example, the flavonoid quercetin (aglycone form of rutin) at 0.8 mM increased the permeability of *L. monocytogenes* membrane ~ 80% after 30 min of treatment [25]. Quercetin is an inhibitor of the bacterial DNA gyrase [45] and d-alanine:d-alanine ligase [46] implicated in nucleic acid synthesis and assembly of cell wall precursors. Whereas rutin selectively promoted the cleavage of topoisomerase IV, an essential enzyme related to bacterial survival, this result evidences phenolic compounds' intercellular action [47]. On the other hand, caffeoylquinic acid was also identified in *P. fastuosus* and *P. grenadensis* extracts. However, this compound exhibited weakly or no antimicrobial activity against Gram-positive and Gram-negative bacteria; it also inhibited the efflux pump systems of *S. aureus*, *E. faecalis*, and *B. cereus*, in charge of expelling antimicrobial activity agents, including phenolic compounds [48, 49]. In this way, the presence of 1,3-dicaffeoylquinic acid in *Phellinus* extracts could enhance the other phenolic acids and flavonoids' antimicrobial activity. Consequently, the variety of phenolic compounds in the extracts of *Phellinus* could act via multiple modes of action; nevertheless, more studies must be conducted to get a deeper insight into this idea.

Previous studies explored the antibacterial properties of *Phellinus* species using different extraction techniques. Yoon and Jang [10] obtained a methanol extract from *P. gilvus* with values of total phenolic and flavonoid contents of 31.17 mg GAE/g and 15.29 mg QE/g, respectively; this extract inhibited the growth of *S. aureus*, *Streptococcus mutans*, *Bacillus subtilis*, *E. coli*, and *Pseudomonas aeruginosa*, with MIC values ranging from 50 to 200 mg/mL. Those MIC values are higher than the MIC values obtained in the present work (10–35 mg/mL), and this could be attributed to the lower phenolic and flavonoid

Table 5 Antiviral activity of *P. badius*, *P. grenadensis*, and *P. fastuosus* extracts, and Pearson correlation of the antiviral activity with the total phenol and flavonoid contents

Extract (0.9 mg/mL)	Plaque inhibition (%)		
	MS2		*Φ-X174*
P. badius	41.12 ± 0.67^{a}		37.73 ± 0.07^{a}
P. grenadensis	37.38 ± 0.14^{b}		33.02 ± 0.08^{b}
P. fastuosus	28.97 ± 0.17^{c}		26.41 ± 0.15^{c}
Correlated responses		Pearson coefficient (r)	p-value
Polyphenols *Vs*	MS2	0.837	0.005
	Φ-X174	0.887	0.001
Flavonoids *Vs*	MS2	0.852	0.004
	Φ-X174	0.902	0.001

content of *P. gilvus* compared with *P. badius*, *P. fastuosus*, and *P. grenadensis.*

Kodiyalmath and Krishnappa [11] evaluated the antimicrobial activity of *P. linteus* by using different solvents (petroleum ether, chloroform, and methanol) and tested against *Salmonella typhi*, *S. aureus*, and *E. coli* using the disk diffusion technique. The tested bacteria were more sensitive to methanol > chloroform > petroleum ether extract; a comparison of these results with those of the present study is difficult considering the different antimicrobial tests used, but it can be highlighted that the methanolic and chloroform extracts of *P. linteus* showed phenolic compounds, while the petroleum ether extracts did not show phenolic compounds. Leyva et al. [34] evaluated the antibacterial activity of fractionated (polar and non-polar fractions) methanolic extracts of *P. merrillii*. The non-fractionated methanolic extract showed the highest phenolic content (913.91 mg GAE/g), flavonoids (563.83 mg QE/g), and the highest inhibition of *S. aureus*, *E. coli* O157: H7, *Salmonella* Choleraesuis, and *L. monocytogenes*, followed by the non-polar fraction and the polar fraction. However, this study did not determine the extracts' MICs nor evaluated the changes in bacterial growth parameters as the present work did. It is important to mention that the studies discussed above did not identify the phenolic compounds.

Antiviral activity

Phellinus extracts at 0.9 mg/mL showed antiviral activity against both bacteriophages (Table 5), and *P. badius* showed the highest activity ($p < 0.05$) by inhibiting 41.1 and 37.7% of the plaque formation of MS2 and Φ-X174 compared with their respective controls. Meanwhile, *P. grenadensis* and *P. fastuosus* inhibited 37.38 and 28.97% for MS2, and 33.02 and 26.41% for Φ-X174, respectively. Pearson's coefficient between total phenols and the antiviral activity against MS2 was 0.837 ($p = 0.005$), and against Φ -X174, it was 0.887 ($p = 0.001$). Besides, Pearson's coefficient between total flavonoid contents and the antiviral activity against MS2 was 0.852 ($p = 0.004$), and 0.902 ($p = 0.001$) against Φ -X174. The significant coefficients ($p < 0.05$) indicated that these compounds are potentially responsible for antiviral activity.

Previous studies evidenced the antiviral properties of phenolic compounds obtained from natural sources; for example, Miguel et al. [50] reported that the infectivity of Qβ bacteriophage, an enteric viral surrogate, was reduced by a honey-based product named água-mel. The higher antiviral activity was observed in the samples that showed the highest polyphenol content. Similarly, Gescher et al. [51] tested procyanidins against the herpes simplex virus and observed a significant effect of the molecular size; the bigger the size, the higher antiviral effect, gallic acid having the lowest antiviral activity

Table 6 Binding free energy and physicochemical characteristics of phenolic compounds found in *P. badius*, *P. grenadensis*, and *P. fastuosus* extracts with the M and G proteins of MS2 and Φ-×174

Compound	LogP	TPSA (Molecular polar surface area)	Hydrogen bonds donors (nOHNH)	Hydrogen bonds acceptors (nON)	Number of rotatable bonds (Nrotb)	Binding Free Energy (Kcal/mol)	
						M	*G*
Rutin	−1.06	269.43	10	16	6	−7.3	−7.9
1,3-Dicaffeoylquinic acid	1.42	211.28	7	12	9	−7.2	−6.4
Chlorogenic acid	−0.45	164.74	6	9	5	−6.3	−6.4
Epicatechin	1.37	110.37	5	6	1	−6.0	−6.1
Catechin	1.37	110.37	5	6	1	−5.9	−6.2
Ferulic acid	1.25	66.76	2	4	3	−5.4	−5.0
Caffeic acid	0.94	77.75	3	4	2	−5.3	−5.1
Gallic acid	0.59	97.98	4	5	1	−5.0	−4.8
p-Coumaric acid	1.43	57.53	2	3	2	−4.8	−4.9

Source: https://pubchem.ncbi.nlm.nih.gov/

Table 7 Correlation between the physicochemical properties of phenolic compounds identified in *Phellinus* spp. extracts, and their respective binding energy

Parameter	Correlation coefficient (*r*)	*p*-value
LogP	0.5496	0.0181
TPSA	−0.9254	0.0000
Hydrogen bonds donors	−0.9315	0.0000
Hydrogen bonds acceptors	−0.9285	0.0000
Rotatable bonds	−0.6823	0.0018

because of the simplicity of its structure. The extracts of the present study were hydrolyzed, and a similar effect cannot be discarded. In the case of mushrooms, the study of antiviral properties has been increased in recent years [1]. It has been reported that extracts from *Fomes fomentarius*, *P. igniarius*, and *P. pini* exerted antiviral activity against enveloped viruses such as Herpes simplex 1(HSV-1) [52]. Also, mycelial extracts of *Pleurotus ostreatus*, *F. fomentarius*, *Auriporia aurea*, and *Trametes versicolor* demonstrated activity against HSV-2 [53]. Among the studied *Phellinus*, Lee et al. [54] reported that *P. igniarius* extracts affected the influenza virus's replication by interfering with the viral attachment to the host cell surface. Nevertheless, the studies of antiviral activity of *Phellinus* against non-enveloped viruses are limited; in this regard, these results showed that *P. badius*, *P. fastuosus*, and *P. grenadensis* were effective against this kind of viruses.

On the other hand, the binding energies of phenolic compounds with the M and G proteins of the MS2 and Φ-×174 bacteriophages are shown in Table 6. Phenolic compounds showed binding energy values ranging from −7.9 to −4.8 kcal/mol. Rutin showed the highest binding affinity with both proteins (−7.9 and −7.3 kcal/mol with the G and M protein, respectively), followed by 1,3-dicaffeoylquinic acid (−7.2 kcal/mol with the M protein and −6.4 kcal/mol with the G protein), and chlorogenic acid (−6.4 kcal/mol with the M protein and −6.3 kcal/mol with the G protein). Meanwhile, *p*-coumaric acid showed the lowest affinity (−4.8 kcal/mol with the M protein), similar to gallic acid with the G protein (−4.8 kcal/mol). As can be seen in Table 7, strong and significant coefficients (*r*) were found among the number of hydrogen bond donors (nOHNH), number of hydrogen bond acceptors (nON), molecular polar surface area (TPSA), number of rotatable bonds (Nrotb), and the binding energies of phenolic compounds with the viral proteins. The number of hydrogen bond donors showed an $r = -0.9315$, explaining the higher affinity of rutin and 1,3-dicaffeoylquinic acid with both proteins; both phenolics and proteins have more –OH groups (hydrogen donors) in their molecules. Therefore, the antiviral properties of the *Phellinus* extracts studied here could be attributed to their phenolic content.

Conclusions

Phellinus extracts showed antibacterial activity against *E. coli* O157: H7, *L. monocytogenes*, and *S.* Choleraesuis; this activity was higher for *P. badius* than *P. grenadensis* and *P. fastuosus*. Besides, *Phellinus* extracts showed antiviral activity against the MS2 bacteriophage and Φ-X174; the extract of *P. badius* had the highest antiviral activity. Moreover, the evaluated bioactivities were well correlated with the phenolic content of *Phellinus* extracts. Finally, the in silico analysis showed that rutin, 1,3-dicaffeoylquinic acid, and chlorogenic acid were the most probable active phenolics in binding the M and G proteins of the MS2 and Φ-×174 bacteriophages. Therefore, this research indicates the potential uses of *P. badius*, *P. grenadensis*, and *P. fastuosus* as a source of antibacterial and antiviral agents.

Acknowledgements The authors thank the Mexican Council for Science and Technology CONACYT (Project number CB-2008-01-103105) for their financial support.

Author contribution FJVA: conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing. JML: investigation, writing—review and editing. VMH: funding acquisition, supervision, writing—review and editing. GAGA: funding acquisition, supervision, writing—review and editing. MRCV: formal analysis, funding acquisition, writing—review and editing. ME: funding acquisition, supervision, writing—review and editing. AG: funding acquisition, investigation, writing—review and editing. FN: formal analysis, funding acquisition, investigation, writing—review and editing. FF: formal analysis, investigation, writing—review and editing. RGH: formal analysis, visualization, writing—review and editing. JFAZ: Conceptualization, funding acquisition, project administration, resources, supervision, validation, visualization, writing—review and editing. All authors discussed and agreed with the submitted version of the manuscript.

Funding Consejo Nacional de Ciencia y Tecnología, CB-2008-01-103105, Jesus Fernando Ayala-Zavala

Data availability Data can be found on the institution repository (https://ciad.repositorioinstitucional.mx/jspui/handle/1006/346) or requested to the corresponding author. The used fungal material can be located at the fungal collection "Dr. Martín Esqueda Valle" at the herbarium of the Sonoran State University.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors consent for publication.

Competing interests The authors declare no competing interests.

References

1. Hyde KD, Xu J, Rapior S et al (2019) The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Divers 97:1–136. https://doi.org/10.1007/s13225-019-00430-9
2. Shirouzu T, Matsuoka S, Doi H, Nagata N, Ushio M, Hosaka K (2020) Complementary molecular methods reveal comprehensive phylogenetic diversity integrating inconspicuous lineages of early-diverged wood-decaying mushrooms. Sci Rep 10:e3057. https://doi.org/10.1038/s41598-020-59620-0
3. Kawashte G, Sonawane H, Mittal S, Borde M 2021 Medicinal fungi: a natural source of pharmacologically important metabolites. In Recent Developments in Microbial Technologies. Ed. Springer. p 379 – 394. https://doi.org/10.1007/978-981-15-4439-2_18
4. He P, Zhang Y, Li N (2021) The phytochemistry and pharmacology of medicinal fungi of the genus *Phellinus*: a review. Food Funct 12:1856–1881. https://doi.org/10.1039/D0FO02342F
5. Varghese R, Dalvi YB, Lamrood PY, Shinde BP, Nair CKK. Historical and current perspectives on therapeutic potential of higher basidiomycetes: an overview. 3 Biotech, 9:362. https://doi.org/10.1007/s13205-019-1886-2
6. Sandargo B, Chepkirui C, Cheng T, Chaverra-Muñoz L, Thongbai B, Stadler M, Hüttel S (2019) Biological and chemical diversity go hand in hand: Basidiomycota as source of new pharmaceuticals and agrochemicals. Biotechnol Adv 37:e107344. https://doi.org/10.1016/j.biotechadv.2019.01.011
7. Shi L, Tan Y, Sun Z, Ren A, Zhu J, Zhao M (2019) Exogenous salicylic acid (SA) promotes the accumulation of biomass and flavonoid content in *Phellinus igniarius* (Agaricomycetes). Int J Med Mushrooms 21:955–963. https://doi.org/10.1615/IntJMedMushrooms.2019032557
8. Ayala-Zavala JF, Pérez-Carlón JJ, Esqueda M et al (2012) Polar fractionation affects the antioxidant properties of methanolic extracts from species of genus *Phellinus* Quél. (higher Basidiomycetes). Int J Med Mushrooms 14:563–573. https://doi.org/10.1615/intjmedmushr.v14.i6.40
9. Ayala-Zavala JF, Silva-Espinoza BA, Cruz-Valenzuela MR et al (2012) Antioxidant and antifungal potential of methanol extracts of *Phellinus* spp. from Sonora. Mexico Rev Iberoam Micol 29:132–138. https://doi.org/10.1016/j.riam.2011.09.004
10. Yoon KN, Jang HS (2016) Antioxidant and antimicrobial activities of fruiting bodies of *Phellinus gilvus* collected in Korea. Korean J Clin Lab Sci 48(4):355–364. https://doi.org/10.15324/kjcls.2016.48.4.355
11. Kodiyalmath JK, Krishnappa M (2017) Evaluation of antimicrobial activity of *Phellinus linteus* (Berk. & MA Curtis.) with their wild collections from Western Ghats of India. Tropical Plant Res 4(2):351–7. https://doi.org/10.22271/tpr.2017.v4.i2.046
12. Andersen PI, Ianevski A, Lysvand H et al (2020) Discovery and development of safe-in-man broad-spectrum antiviral agents. Int J Infec Dis 93:268–276. https://doi.org/10.1016/j.ijid.2020.02.018
13. Ben-Shabat S, Yarmolinsky L, Porat D, Dahan A (2020) Antiviral effect of phytochemicals from medicinal plants: applications and drug delivery strategies. Drug Delivery Transl Res 10:354–367. https://doi.org/10.1007/s13346-019-00691-6
14. Calland N, Sahuc ME, Belouzard S et al (2015) Polyphenols inhibit hepatitis C virus entry by a new mechanism of action. J Virol 89:10053–10063. https://doi.org/10.1128/JVI.01473-15
15. Chowdhury P, Sahuc ME, Rouillé Y et al (2018) Theaflavins, polyphenols of black tea, inhibit entry of hepatitis C virus in cell culture. PLoS ONE 13:e0198226. https://doi.org/10.1371/journal.pone.0198226
16. Makola MM, Dubery IA, Koorsen G et al (2016) The effect of geometrical isomerism of 3,5-Dicaffeoylquinic acid on its binding affinity to HIV-integrase enzyme: a molecular docking study. J Evidence-Based Complementary Altern Med 2016:e4138263. https://doi.org/10.1155/2016/4138263
17. Elhusseiny SM, El-Mahdy TS, Awad MF, Elleboudy NS, Farag MMS, Aboshanab KM, Yassien MA (2021) Antiviral, cytotoxic, and antioxidant activities of three edible agaricomycetes mushrooms: *Pleurotus columbinus, Pleurotus sajor-caju*, and *Agaricus bisporus*. J of Fungi 7(8):645. https://doi.org/10.3390/jof7080645
18. Gilbertson RL, Ryvarden L 1987 North American Polypores. Vol. 2. *Megasporoporia – Wrightoporia*. Fungiflora, Oslo.
19. Larsen MJ, Cobb-Poulle LA 1989 *Phellinus* (Hymenochaetaceae). A survey of the world taxa. Fungiflora, Oslo.
20. Index Fungorum 2019 Richmond, Royal Botanic Gardens Kew. http://indexfungorum.org. Accessed 10 November 2019
21. Vazquez-Armenta FJ, Bernal-Mercado AT, Lizardi-Mendoza J et al (2018) Phenolic extracts from grape stems inhibit *Listeria monocytogenes* motility and adhesion to food contact surfaces. J Adhes Sci Technol 32:889–907. https://doi.org/10.1080/01694243.2017.1387093
22. Çayan F, Deveci E, Tel-Çayan G, Duru ME (2020) Identification and quantification of phenolic acid compounds of twenty-six mushrooms by HPLC-DAD. J Food Meas Charact 14:1690–1698. https://doi.org/10.1007/s11694-020-00417-0
23. Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64:555–559. https://doi.org/10.1016/S0308-8146(98)00102-2
24. Fratianni F, Coppola R, Nazzaro F (2011) Phenolic composition and antimicrobial and antiquorum sensing activity of an ethanolic extract of peels from the apple cultivar Annurca. J Med Food 14:957–963. https://doi.org/10.1089/jmf.2010.0170
25. Vazquez-Armenta FJ, Bernal-Mercado AT, Tapia-Rodriguez MR et al (2018) Quercetin reduces adhesion and inhibits biofilm development by *Listeria monocytogenes* by reducing the amount of extracellular proteins. Food Control 90:266–273. https://doi.org/10.1016/j.foodcont.2018.02.041
26. Baranyi J, McClure PJ, Sutherland PJ, Roberts TA (1993) Modeling bacterial growth responses. J Ind Microbiol 12:190–194. https://doi.org/10.1007/BF01584189
27. Bae J, Schwab KJ (2008) Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. Appl Environ Microbiol 74:477–484. https://doi.org/10.1128/AEM.02095-06
28. Hardy ME (2005) Norovirus protein structure and function. FEMS Microbiol Lett 253:1–8. https://doi.org/10.1016/j.femsle.2005.08.031
29. Sun Y, Roznowski AP, Tokuda JM et al (2017) Structural changes of tailless bacteriophage Φx174 during penetration of bacterial cell walls. Proc Natl Acad Sci USA 114:13708–13713. https://doi.org/10.1073/pnas.1716614114
30. Cajthaml T (2015) Biodegradation of endocrine-disrupting compounds by ligninolytic fungi: mechanisms involved in the degradation. Environ Microbiol 17:4822–4834. https://doi.org/10.1111/1462-2920.12460
31. Okino LK, Machado KM, Fabris C, Bononi VL (2000) Ligninolytic activity of tropical rainforest basidiomycetes. World J Microbiol Biotechnol 16:889–893. https://doi.org/10.1023/A:1008983616033
32. Ma XK, Li L, Peterson EC, Ruan T, Duan X (2015) The influence of naphthaleneacetic acid (NAA) and coumarin on flavonoid production by fungus *Phellinus* sp.: modeling of production kinetic profiles. Appl Microbiol Biotechnol 99:9417–9426. https://doi.org/10.1007/s00253-015-6824-6

33. Wang Y, Yuan Z, Tan X, Ran Z, Jin H 2018 Optimization of medium components for the production of flavonoids and soluble protein with *Phellinus igniarius* in liquid culture. In: Liu H, Song C, Ram A (eds) Advances in applied biotechnology. Springer, Singapore, pp 421–431 https://doi.org/10.1007/978-981-10-4801-2_43
34. Leyva JM, Pérez-Carlón JJ, González-Aguilar GA, Esqueda M, Ayala-Zavala JF (2013) Funcionalidad antibacteriana y antioxidante de extractos hidroalcohólicos de *Phellinus merrillii*. Rev Mex Micol 37:11–17. https://doi.org/10.33885/sf.2013.3.1108
35. Li J-M, Liang H-Q, Qiao P, Su K-M, Liu P-G, Guo S-X, Chen J (2019) Chemical composition and antioxidant activity of *Tuber indicum* from different geographical regions of China. Chem Biodivers 16(3):e1800609. https://doi.org/10.1002/cbdv.201800609
36. Fogarasi M, Socaci SA, Dulf FV, Diaconeasa ZM, Fărcaș AC, Tofană M, Semeniuc CS (2018) Bioactive compounds and volatile profiles of five Transylvanian wild edible mushrooms. Molecules 23(12):3272. https://doi.org/10.3390/molecules23123272
37. Sułkowska-Ziaja K, Maślanka A, Szewczyk A, Muszyńska B (2017) Physiologically active compounds in four species of *Phellinus*. Nat Prod Commun 12:363–366. https://doi.org/10.1177/1934578X1701200313
38. Heleno SA, Martins A, Queiroz MJ, Ferreira IC (2015) Bioactivity of phenolic acids: Metabolites versus parent compounds: a review. Food Chem 173:501–513. https://doi.org/10.1016/j.foodchem.2014.10.057
39. Sheikh IA, Vyas D, Ganaie MA, Dehariya K, Singh V (2014) HPLC determination of phenolics and free radical scavenging activity of ethanolic extracts of two polypore mushrooms. Int J Pharm Pharm Sci 6:679–684
40. Deshpande S, Matei MF, Jaiswal R, Bassil BS, Kortz U, Kuhnert N (2016) Synthesis, structure, and tandem mass spectrometric characterization of the diastereomers of quinic acid. J Agric Food Chem 64:7298–7306. https://doi.org/10.1021/acs.jafc.6b02472
41. Nagy Á, Abrankó L (2016) Profiling of hydroxycinnamoylquinic acids in plant extracts using in-source CID fragmentation. J Mass Spectrom 51:1130–1145. https://doi.org/10.1002/jms.3847
42. Kabir F, Katayama S, Tanji N, Nakamura S (2014) Antimicrobial effects of chlorogenic acid and related compounds. J Korean Soc Appl Biol Chem 57:359–365. https://doi.org/10.1007/s13765-014-4056-6
43. Borges A, Ferreira C, Saavedra M, Simões M (2013) Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. Microb Drug Resist 19:256–265. https://doi.org/10.1089/mdr.2012.0244
44. Bernal-Mercado AT, Gutierrez-Pacheco MM, Encinas-Basurto D et al (2020) Synergistic mode of action of catechin, vanillic and protocatechuic acids to inhibit the adhesion of uropathogenic *Escherichia coli* on silicone surfaces. J Appl Microbiol 128:387–400. https://doi.org/10.1111/jam.14472
45. Wu T, Zang X, He M, Pan S, Xu X (2013) Structure–activity relationship of flavonoids on their anti-*Escherichia coli* activity and inhibition of DNA gyrase. J Agric Food Chem 61:8185–8190. https://doi.org/10.1021/jf402222v
46. Wu D, Kong Y, Han C et al (2008) D-Alanine: D-alanine ligase as a new target for the flavonoids quercetin and apigenin. Int J Antimicrob Agents 32:421–426. https://doi.org/10.1016/j.ijantimicag.2008.06.010
47. Bernard FX, Sable S, Cameron B et al (1997) Glycosylated flavones as selective inhibitors of topoisomerase IV. Antimicrob Agents Chemother 41:992–998. https://doi.org/10.1128/AAC.41.5.992
48. Blanco P, Hernando-Amado S, Reales-Calderon JA et al (2016) Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. Microorganisms 4:1–19. https://doi.org/10.3390/microorganisms4010014
49. Fiamegos YC, Kastritis PL, Exarchou V et al (2011) Antimicrobial and efflux pump inhibitory activity of caffeoylquinic acids from *Artemisia absinthium* against Gram-positive pathogenic bacteria. PLoS ONE 6:e18127. https://doi.org/10.1371/journal.pone.0018127
50. Miguel MG, Faleiro L, Antunes MD, Aazza S, Duarte J, Silvério AR (2013) Antimicrobial, antiviral and antioxidant activities of "água-mel" from Portugal. Food Chem Toxicol 56:136–144. https://doi.org/10.1016/j.fct.2013.02.007
51. Gescher K, Hensel A, Hafezi W, Derksen A, Kühn J (2011) Oligomeric proanthocyanidins from *Rumex acetosa* L. inhibit the attachment of herpes simplex virus type-1. Antiviral Res 89:9–18. https://doi.org/10.1016/j.antiviral.2010.10.007
52. Doğan HH, Karagöz S, Duman R (2018) *In vitro* evaluation of the antiviral activity of some mushrooms from Turkey. Int J Med Mushrooms 20:201–212. https://doi.org/10.1615/IntJMedMushrooms.2018025468
53. Krupodorova T, Rybalko S, Barshteyn V (2014) Antiviral activity of *Basidiomycete* mycelia against influenza type A (serotype H1N1) and herpes simplex virus type 2 in cell culture. Virol Sin 29:284–290. https://doi.org/10.1007/s12250-014-3486-y
54. Lee S, Kim JI, Heo J et al (2013) The anti-influenza virus effect of *Phellinus igniarius* extract. J Microbiol 51:676–681. https://doi.org/10.1007/s12275-013-3384-2