Acta Scientiarum
http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v43i1.58474

Antimicrobial activity of the edible mushroom *Pleurotus eryngii* (DC.) Quél grown in liquid medium

Cleudiane Pereira de Andrade[1*], Aldiane Passos de Oliveira[1], Victor Matheus Cabral Vieira[2], Bruna Ketley Paes Frazão[1], Raiana Silveira Gurgel[1,3], Rafael Lopes e Oliveira[2,3] and Larissa de Souza Kirsch[1,2]

[1]Escola Superior de Ciências da Saúde, Universidade do Estado do Amazonas, Av. Carvalho Leal, 1777, 69065-001, Manaus, Amazonas, Brazil. [2]Escola Normal Superior, Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil. [3]Escola Superior de Tecnologia, Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil. *Author for correspondence. E-mail: cleudiane.andrade@hotmail.com

ABSTRACT. Edible mushrooms have a number of medicinal properties and this study aimed to investigate the antimicrobial activity of *Pleurotus eryngii* DPUA1816 in metabolic broths after being grown in submerged cultivation. Mycelial fragments of pure *P. eryngii* culture was inoculated in sweet potato culture medium and incubated at 150 rpm for 15 days at 25°C. *Pleurotus eryngii* was also cultivated for 18 days under the same conditions, the mycelial biomass was separated by filtration for quantification. The supernatant was used in the diffusion test in agar and performed against *Escherichia coli* CCCD-E005, *Staphylococcus aureus* CCCD-S009, *Pseudomonas aeruginosa* CCCD-P004, *Candida albicans* CCCD-CC001, *Candida parapsilosis* CCCD-CC004 and *Candida tropicalis* CCCD-CC002. The samples showed no inhibitory activity against bacteria, however they showed some activity against *C. albicans* (12.17 mm), *C. parapsilosis* (27.67 mm) and *C. tropicalis* (13.67 mm). After being cultivated for 18 days, *P. eryngii* was able to inhibit all yeasts after 12 days of culture, with an inhibition halo of 29.33 mm at 16 days against *C. parapsilosis*. This study demonstrates the antifungal potential filtered liquids from *P. eryngii* cultivated in purple-skinned sweet potato culture medium, which suggests the possibility of the use of this species by the pharmaceutical industry as a natural source of biological action.

Keywords: edible mushrooms; filtered liquids; pathogens; antifungal potential.

Received on April 1, 2021.
Accepted on April 12, 2021.

Introduction

Edible mushrooms are recognized as functional foods due to their remarkable nutritional characteristics (Cohen et al., 2014). These organisms can have valuable resources for maintaining good health, and have numerous scientifically proven properties, including antiviral, immunomodulatory, hyperlipidemic, antioxidant (Brugnari et al., 2016; Acharya, Khatua, & Ray, 2017; Dulay et al., 2017; Finimundy et al., 2018), anti-inflammatory, antitumor (Cao, Liu, Hou, & Li, 2015), hyperglycemic, antithrombotic and antimicrobial properties (Schillaci, Arizza, Gargano, & Venturella, 2013; Owaid, Al-Saeedi, & Al-Assaffii, 2015; 2017; Castillo, Pereira, Alves, & Teixeira, 2017; Das, Saha, Joshi, & Das, 2017; Suresh, Ambika, Noorjahan, & Kalaiselvam, 2017). Such biological properties are conferred to macromolecules and molecules of lower molecular weight, such as polysaccharides, proteins, peptides and cerebrosides, isoflavones, triacylglycerols, steroids, amines, sesquiterpenes, among others (Fu, Liu, & Zhang, 2016).

Among the metabolites produced by these macrofungi, some of these which are derived from their secondary metabolism have antimicrobial activity, and are classified as terpenoids, polyacetylenes and phenolics (Shen, Shao, Chen, & Zhou, 2017). Recent research has indicated that several strains of the genus *Pleurotus* possess antimicrobial properties, such as *Pleurotus japonicus,* which inhibits the pathogen *Bacillus subtilis* via the antibiotic 6-deoxyilludin M (Hara, Yoshida, Morimoto, & Nakano, 1987).

Another species, *P. eryngii,* has attracted the attention of several researchers since it presents a basidiome with desirable organoleptic characteristics that are superior in relation to other mushrooms of the same genus (Iqbal et al., 2018; Ma et al., 2018). These have been reported to include medicinal properties, such as antitumoral, antioxidant, antimicrobial, hypoglycemic, immunomodulatory, antihemolytic properties, among others (Mariga et al., 2014; Xu et al., 2016; Zhang et al., 2016; Kim et al., 2017; Madhanraj et al., 2019). Such attributes may come from the basidiomes themselves or from the liquid filtrates from the cultivation of

this organism (Owaid et al., 2015). Fu et al. (2016) and Zhang et al. (2019) present a brief review of the research in the literature and reaffirm that this mushroom species is interesting for the development of therapeutic remedies, due to the bioactive compounds which are presented. In this context, our study contributes to the continuity of studies regarding this mushroom species, given the continued interest in the search for natural antimicrobial compounds as an alternative to existing antimicrobial agents. Thus, this research evaluated the antimicrobial activity of *P. eryngii* from its filtrates obtained after submerged cultivation.

Material and methods

Microorganism

The *P. eryngii* DPUA 1816 culture used in the assay was provided by the DPUA Culture Collection at the *Universidade Federal do Amazonas* and reactivated in potato dextrose agar (PDA) culture medium with yeast extract (YE) 0.5% (p v^{-1}). The cultures were maintained in the dark at 25°C, for 10 days (Kirsch, Pinto, Porto, Porto, & Teixeira, 2011).

Submerged cultivation

The culture was carried out in a culture medium based on an infusion of 200 g L^{-1} of purple-skinned sweet potato (PSSP), with different concentrations of glucose, in the presence and absence of yeast extract – YE (2 g L^{-1}). The medium containing only sweet potato infusion was used as a control. From the pure cultures, three mycelium fragments (Ø = 1cm) were removed, added to the culture medium and incubated at 150 rpm, for 15 days, at 25°C. At the end of cultivation, samples of the fermented broth were separated from the mycelial biomass by vacuum filtration and placed under refrigeration at 4°C (Rufino et al., 2011).

A second cultivation was carried out to evaluate the growth of macrofungi during 18 days, in medium 4 (M4), under the same conditions as the previous cultivation. Samples, in triplicate, were randomly taken every 2 days, for evaluation of antimicrobial activity. Fungal growth was measured from dehydrated biomass at 60°C until constant weight (Kirsch, Macedo, & Teixeira, 2016).

Preparation of test microorganisms

The antimicrobial activity was evaluated against test microorganisms of the Amazon bacteria collection (CBAM) of the Leonidas and Maria Deane Institute (Fiocruz Amazonas): *Escherichia coli* CBAM 0007, *Staphylococcus aureus* CBAM 0026 and the Cefar diagnostic culture collection (CCCD): *Escherichia coli* CCCD-E005, *Staphylococcus aureus* CCCD-S009, *Pseudomonas aeruginosa* CCCD-P004, *Candida albicans* CCCD-CC001, *Candida parapsilosis* CCCD-CC004 and *Candida tropicalis* CCCD-CC002. The reactivation of the bacteria was performed in Müeller-Hinton agar medium (MH), incubated at 37ºC for 24 hours and the yeast in Sabouraud agar medium (SDA), incubated for 48 hours at 25ºC (Clinical and Laboratory Standards Institute [CLSI], 2009).

Antimicrobial activity

The determination of antimicrobial activity was made using the method of diffusion in agar (CLSI, 2009) with cups cut out of the agar (cup-plates) - according to the recommendation of the Clinical and Laboratory Standards Institute (CLSI, 2009). Aliquots of 100 µL of the microbial suspensions, prepared in sterile, distilled water and standardized using the McFarland scale (1.0), were sown uniformly in the culture mediums using swabs. Then, 100 µL of the samples obtained from the submerged cultivation were added. As a control, itraconazole (30 mg mL^{-1}) and tetracycline hydrochloride (0.05 mg mL^{-1}) were used as positive controls for yeast and bacteria, respectively.

Statistical analyses

The results were evaluated using analysis of variance (ANOVA) to determine significant differences (Tukey test $p < 0.05$), using the Minitab software, version 17 (Minitab Statistical Software, 2014).

Results and discussion

The results indicated that the metabolite broths of *P. eryngii* in all tested mediums were unable to inhibit the growth of the bacterias *E. coli* (CBAM 0007 and CCCD-E005), *S. aureus* (CBAM 0026 and CCCD-S009) and

P. aeruginosa CCCD-P004, as can be seen in Table 1. This result is similar to the study of Yilmaz, Yildiz, Tabbouche, Kiliç, & Can, (2016), who tested *P. ostreatus* against the bacteria *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853. Similarly, Costa, Silva, Araújo, and Carvalho (2018) did not observe the inhibition halo (IH) of organic extracts (hexane, dichloromethane, ethyl acetate and methanol) from *P. ostreatus* against *E. coli*, *P. aeruginosa* and *S. aureus*, although they did achieve IH in tests with β-glucan, isolated from this species.

Table 1. Antimicrobial activity of metabolites from submerged cultivation of *Pleurotus eryngii* in different culture medium.

Medium culture	Bacterias					Fungi		
	Inhibition halo (mm)							
	E. coli (CBAM 0007)	*E. coli* (CCCD-E005)	*S. aureus* (CBAM 0026)	*S. aureus* (CCCD-S009)	*P. aeruginosa* (CCCD-P004)	*C. albicans* (CCCD-CC001)	*C. parapsilosis* (CCCD-CC004)	*C. tropicalis* (CCCD-CC002)
Control	-	-	-	-	-	12.00±0.0^{a}	24.50±0.5^{a,b}	11.50±0.5^{b,c}
M1	-	-	-	-	-	-	27.66±1.9^{a}	13.50±0.9^{a}
M2	-	-	-	-	-	11.00±1.3^{a}	25.17±1.9^{a,b}	-
M3	-	-	-	-	-	11.50±0.5^{a}	23.00±0.5^{b}	11.83±0.3^{b,c}
M4	-	-	-	-	-	13.50±1.3^{a}	26.33±1.3^{a,b}	12.50±0.5^{a,b}
M5	-	-	-	-	-	10.33±0.3^{a}	25.83±0.8^{a,b}	11.00±0.0^{c,d}
M6	-	-	-	-	-	11.00±0.0^{a}	24.33±1.9^{a,b}	9.83±0.3^{d}
M7	-	-	-	-	-	12.50±0.5^{a}	5.50±1.5^{a,b}	11.83±0.3^{b,c}
M8	-	-	-	-	-	12.17±0.3^{a}	25.00±0.0^{a,b}	11.33±0.3^{b,c}

Key: Control (200 g L^{-1} of purple-skinned sweet potato); M1, M2, M3 or M4 (200 g L^{-1} of purple-skinned sweet potato PSSP, 10, 20, 30 and 40 g L^{-1} of glucose and 2 g L^{-1} of YE); M5, M6, M7 and M8 (200 g L^{-1} of purple-skinned sweet potato , 10, 20, 30 or 40 g L^{-1} of glucose and 0 g L^{-1} of YE). (-) no inhibition halo; (±) standard deviation. In each column, averages that share the same letter do not differ from each other by the Tukey test ($p < 0.05$).

Although the bacteria tested were resistant in this study, the inhibition of *P. aeruginosa* using biomass extracted from *P. eryngii*, *P. sajor-caju*, *P citrinopileatus*, *P. ostreatus* and *P. florida* in hot water has been mentioned in the literature, and presented an IH which was equivalent to 8.8, 9.0, 8.1, 9.4 and 9.7 mm, respectively (Özdal, Gülmez, Gür-Özdal, & Algur, 2019). Similarly, Fasoranti, Ogidi, and Oyetayo (2018) verified the sensitivity of *P. aeruginosa* (IH= 14 and 15 mm), *S. aureus* (IH= 13.7 and 15.7 mm) *and E. coli* (IH= 15.7 and 14 mm), in relation to the mushrooms *P. pulmonarius* and *P. ostreatus*. Sathyan, Majeed, Majitha, and Rajeswary (2017) evaluated the extracts Dimethyl Ether, chloroform and methanol from *P. ostreatus*, *P. eryngii* and *P. djamor*, respectively, and found that the first extract had an IH of 12.20 mm against *E. coli*, chloroform had an of 17.5 mm and methanol an HI of 12.2 mm against *P. aeruginosa*. Despite this, *E. coli* was in fact resistant to *P. eryngii* and *P. djamor* in our study.

However, antifungal assays revealed more promising data (Table 1), since, with the exception of the sample M1 against *C. albicans* CCCD-CC001 and the sample M2 against *C. tropicalis* CCCD-CC002, the others were able to form inhibition halos against all the evaluated yeasts. Of these, we can highlight: *C. albicans* CCCD-CC001 from samples M4, M7 and M8, without statistical difference between them, with an IH of 13.5, 12.5 and 12.17 mm respectively; *C. tropicalis* CCCD-CC002, in samples M1, M4 and M7, presented an IH greater than 11 mm; and finally, samples M1 (27.67 mm), M4 (26.33 mm) and M5 (25.83 mm) against *C. parapsilosis* CCCD-CC004, the latter being the most sensitive yeast in the samples tested.

These data are superior to those of Nwachukwu and Uzoeto (2010), who tested ethanolic extracts from *P. squarrosolus* against *C. albicans* and obtained a maximum IH of 7.10 mm. Similarly, in the study by Akyuz, Onganer, Erecevit, and Kirbag (2010), extracts of *P. eryngii* var. *eryngii*, *P. eryngii* var. *ferulae*, *P. ostreatus* and *P. sajor-caju* were evaluated and obtained IH results of 7.5 and 8.5 mm against *C. albicans* for only the first two fungi mentioned. Fasoranti et al. (2018) were not successful with organic extracts of *P. pulmonarius* and *P. ostreatus* against the yeasts *C. albicans* and *C. parapsilosis*, since both presented resistance to the tested species. This research is similar to that of Popa, Voaides, Cornea, and Zagrean (2016), who also indicated resistance of *C. albicans* ATCC 10321 and *C. parapsilosis* CBS 604 to *P. eryngii*.

After observing that the metabolic broths of *P. eryngii,* obtained from the culture medium M4, produced larger inhibition halos against two of the three tested yeasts, despite there being no statistical difference, this medium was selected for mushroom cultivation during 18 days. However, even after a longer cultivation period, none of the samples inhibited the growth of bacteria (Table 2).

Table 2. Antimicrobial activity of metabolites derived from the cultivation of *Pleurotus eryngii* in M4 medium during a cultivation period of 18 days.

Culture period (in days)	Bacterias					Fungi		
	Inhibition halo (mm)							
	E. coli CBAM 0007	*E. coli* CCCD-E005	*S. aureus* CBAM 0026	*S. aureus* CCCD-S009	*P. aeruginosa* CCCD-P004	*C. albicans* CCCD-CC001	*C. parapsilosis* CCCD-CC004	*C. tropicalis* CCCD-CC002
2	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
12	-	-	-	-	-	16.33±1.2^{a}	28.00±0.0^{a}	13.67±1.0^{a}
14	-	-	-	-	-	11.83±0.6^{b}	23.00±3.3^{b}	11.67±0.8^{a,b}
16	-	-	-	-	-	9.83±0.3^{c}	29.33±0.6^{a}	10.50±0.5^{a,b}
18	-	-	-	-	-	11.17±0.3^{b}	21.17±1.4^{b}	7.00±6.1^{b}

Key: (-) no inhibition halo presented; (±) standard deviation. In each column, averages that share the same letter do not differ from each other by the Tukey test ($p < 0.05$).

However, there was antifungal activity which appears to be favorable for all samples from the 12th day of culture onwards. This activity presented greater IH against *C. albicans* CCCD-CC001 and *C. tropicalis* CCCD-CC002 in 12 days and against *C. parapsilosis* CCCD-CC004 after 16 days of cultivation (Figure 1).

Comparatively, the IH obtained from the M4 sample during 18 days of cultivation (Table 2) were lower against all yeasts tested than those of 15 days (Table 1). This result may be related to the growth phases of the fungus, since, according to Figure 1, it is possible to associate the growth phases of *P. eryngii* in 18 days of cultivation with the antifungal activity presented. This indicates antifungal potential from the end of the exponential phase and beginning of the stationary phase, and as the fungal growth decreases, the antifungal action also decreases.

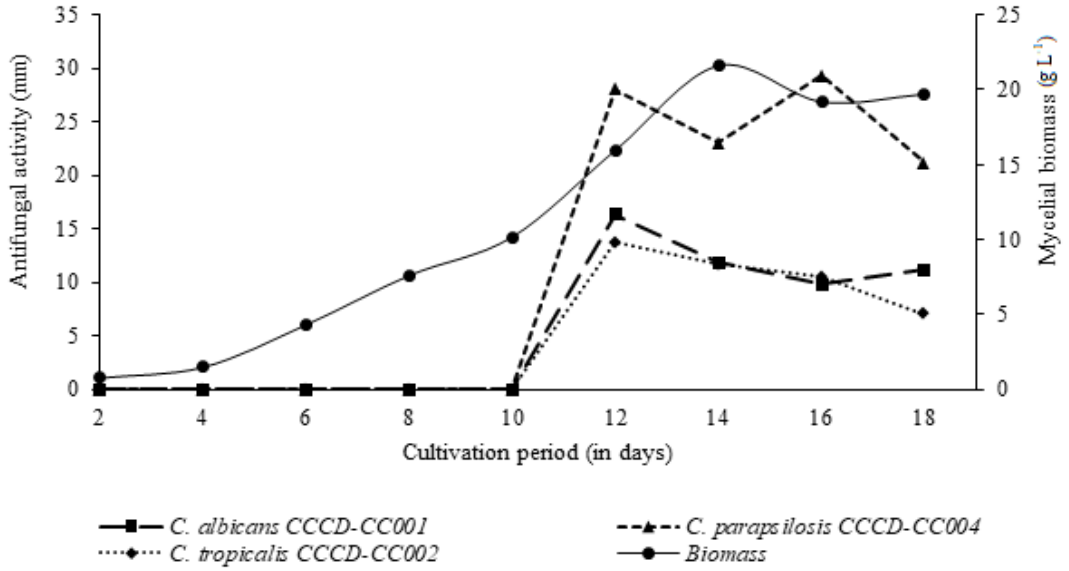

Figure 1. Antifungal activity of metabolites obtained from *Pleurotus eryngii* culture in M4 medium for 18 days

The results of this research are in accordance with those found by Subrata, Gunjan, Prakash, Mandal, and Krishnendu (2012), who verified antifungal activity against *C. albicans* using methanolic extracts of *P. djamor*, although no activity was noted for *S. aureus*, *E. coli* and *P. aeruginosa*. Extracts obtained using methanol, ethanol and water may also be favorable in antimicrobial tests, and may present satisfactory results against *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans* and *C. glabrata* (Kalu & Kenneth, 2017). The use of extracts makes it possible to perform antimicrobial tests, such as minimum inhibitory concentration, in addition to the isolation and purification of substances capable of inhibiting pathogenic microorganisms (Schillaci et al., 2013; Yehia & Al-Sheikh, 2014; Li & Shah, 2014; Finimundy et al., 2018; Musa et al., 2018).

Conclusion

The results of this study demonstrate that the metabolic broths obtained from the submerged cultivation of *P. eryngii* DPUA 1816 did not inhibit the growth of the bacteria tested, however, all samples after the 12th day of cultivation showed promising IH against the yeasts tested in the culture medium M4. These results show that the species studied has evident antifungal properties and, as such, future studies should be carried out in order to evaluate the production, purification and physico-chemical characterization of the bioactive constituent of pharmacological interest.

Acknowledgements

The authors appreciate the support granted by the *Fundação de Amparo a Pesquisas do Estado do Amazonas* (FAPEAM) and the technical support of *Universidade do Estado do Amazonas* (UEA-ESA) and *Laboratório de Química Aplicada a Tecnologia* (UEA-EST-QAT).

References

Acharya, K., Khatua, S., & Ray, S. (2017). Quality assessment and antioxidant study of *Pleurotus djamor* (Rumph. ex Fr.) Boedijn. *Journal of Applied Pharmaceutical Science*, *7*(6), 105-110. DOI: http://dx.doi.org/10.7324/JAPS.2017.70614

Akyuz, M., Onganer, A. N., Erecevit, P., & Kirbag, S. (2010). Antimicrobial activity of some edible mushrooms in the eastern and southeast Anatolia region of Turkey. *Gazi University Journal of Science, 23*(2), 125-130.

Brugnari, T., Kato, C. G., Correa, V. G., Freitas, E. N., Nolli, M. M., & Souza, C. G. M. (2016). Atividade antioxidante do extrato aquoso do cogumelo comestível *Pleurotus ostreatus*. *Revista UNINGÁ Review*, *25*(3), 46-50. Recovered from: http://34.233.57.254/index.php/uningareviews/article/view/1784

Cao, X. L., Liu, J. L., Hou, X., & Li, Q. J. (2015). Antitumor activity of polysaccharide extracted from *Pleurotus ostreatus* mycelia against gastric cancer *in vitro* and *in vivo*. *Molecular Medicine Reports, 12*(2), 2383-2389. DOI: http://dx.doi.org/10.3892/mmr.2015.3648

Castillo, T. A., Pereira, J. R. G., Alves, J. M. A., & Teixeira, M. F. S. (2017). Mycelial growth and antimicrobial activity of species of genus *Lentinus* (Agaricomycetes) from Brazil. *International Journal of Medicinal Mushrooms, 19*(12), 1135-1143. DOI: http://dx.doi.org/10.1615/IntJMedMushrooms.2017024708

Clinical and Laboratory Standards Institute [CLSI]. (2009). Performance standards for antimicrobial disk susceptibility test: Wayne, PA: CLSI.

Cohen, N., Cohen, J. Asatiani, M. D. Varshney, V. K., Yu, H. T., Yang, Y. C., ... Wasser, S. P. (2014). Chemical composition and nutritional and medicinal value of fruit bodies and submerged cultured mycelia of culinary-medicinal higher Basidiomycetes mushrooms. *International Journal of Medicinal Mushrooms, 16*(3), 273-291. DOI: http://dx.doi.org/10.1615/IntJMedMushr.v16.i3.80

Costa, A. C., Silva, K. M. R., Araújo, E. T. H., & Carvalho, M. L. (2018). Avaliação da atividade antibacteriana do *Pleurotus ostreatus* isolados de *Staphylococcus aureus*. *Pseudomonas aeruginosa* e *Escherichia coli*. *Revista Prevenção de Infecção e Saúde, 4*(1), 6892-6899. DOI: http://dx.doi.org/10.26694/repis.v4i0.6892

Das, A. R., Saha, A. K., Joshi, S. R., & Das, P. (2017). Wild edible macrofungi consumed by ethnic tribes of Tripura in Northeast India with special reference to antibacterial activity of *Pleurotus djamor* (Rumph. Ex Fr.) Boedijn. *International Food Research Journal, 24*(2), 834-838.

Dulay, R. M. R., Miranda, L. A., Malasaga-Sofronio, J. S., Kalaw, S. P., Reyes, R. G., & Hou, C. T. (2017). Antioxidant and antibacterial activities of acetonitrile and hexane extracts of *Lentinus tigrinus* and *Pleurotus djamor*. *Biocatalysis and Agricultural Biotechnology, 9*(1), 141-144. DOI: http://dx.doi.org/10.1016/j.bcab.2016.12.003

Fasoranti, O. F., Ogidi, C. O., & Oyetayo, V. O. (2018). Phytochemical constituents and antimicrobial evaluation of ethanolic extracts from *Pleurotus* spp. cultivated on substrate fortified with selenium. *Microbial Biosystems,3*(2), 29-39.

Finimundy, T. C., Barrosa, L., Calhelha, R. C., Alves, M. J., Prieto, M. A., Abreu, R. M. V., ... Ferreira, I. C. F. R. (2018). Multifunctions of *Pleurotus sajor-caju* (Fr.) singer: a highly nutritious food and a source for bioactive compounds. *Food Chemistry, 245*(1), 150-158. DOI: http://dx.doi.org/10.1016/j.foodchem.2017.10.088

Fu, Z., Liu, Y., & Zhang, Q. (2016). A potent pharmacological mushroom: *Pleurotus eryngii*. *Fungal Genomics and Biology, 6*(1), 1-5. DOI: http://dx.doi.org/10.4172/2165-8056.1000139

Hara, M., Yoshida, M., Morimoto, M., & Nakano, H. (1987). 6-Deoxylludin M. a new antitumor antibiotic: Fermentation. isolation and structural identification. *The Journal of Antibiotics, 40*(11), 1643-1646. DOI: http://dx.doi.org/10.7164/antibiotics.40.1643

Iqbal, W., Asma, M. M., Ayyub, C. M., Khan, N. A., Samin, G., & Khatana, M. (2018). Optimization of king oyster mushroom (*Pleurotus eryngii*) production against cotton waste and fenugreek straw. *Pakistan Journal of Phytopathology, 31*(2), 149-154. DOI: http://dx.doi.org/10.33866/phytopathol.030.02.0435

Kalu, A. U., & Kenneth, O. C. (2017). Antimicrobial activity of *Pleurotus squarrosulus* on clinical pathogenic bacteria and fungi. *Journal of Advances in Microbiology, 4*(3), 1-9. DOI: http://dx.doi.org/10.9734/JAMB/2017/34644

Kim, Y. H., Jung, E. G., Han, K. I., Patnaik, B. B., Kwon, H. J., Lee, H. S., ... Han, M. D. (2017). Immunomodulatory effects of extracellular beta-glucan isolated from the king oyster mushroom *Pleurotus eryngii* (Agaricomycetes) and its sulfated form on signaling molecules involved in innate immunity. *International Journal of Medicinal Mushrooms, 19*(6), 521-533. DOI: http://dx.doi.org/10.1615/IntJMedMushrooms.v19.i6.40

Kirsch, L. S., Macedo, A. J. P., & Teixeira, M. F. S. (2016). Production of mycelial biomass by the Amazonian edible mushroom *Pleurotus albidus*. *Brazilian Journal of Microbiology (Online), 47*(3), 658-664. DOI: http://dx.doi.org/10.1016/j.bjm.2016.04.007

Kirsch, L. S., Pinto, A. C. S., Porto, T. S., Porto, A. L. F., & Teixeira, M. F. S. (2011). The influence of different submerged cultivation conditions on mycelial biomass and protease production by *Lentinus citrinus* Walleyn et Rammeloo DPUA 1535 (Agaricomycetidae). *International Journal of Medicinal Mushrooms, 13*(2), 185-192. DOI: http://dx.doi.org/10.1615/IntJMedMushr.v13.i2

Li. S., & Shah, N. P. (2014). Antioxidant and antibacterial activities of sulphated polysaccharides from *Pleurotus eryngii* and *Streptococcus thermophilus* ASCC 1271. *Food Chemistry, 165*(1), 262-270. DOI: http://dx.doi.org/10.1016/j.foodchem.2014.05.110

Ma, G., Kimatu, B. M., Zhao, L., Yang, W., Pei, F., & Hu, Q. (2018). Impacts of dietary *Pleurotus eryngii* polysaccharide on nutrient digestion. metabolism. and immune response of the small intestine and colon-an ITRAQ-based proteomic analysis. *Proteomics, 18*(7), e1700443. DOI: http://dx.doi.org/10.1002/pmic.201700443

Madhanraj, R., Ravi, K. K., Maya, M. R., Ramanaiah, I., Venkatakrishna, K., Rameshkumar, K., ... Balaji, P. (2019). Evaluation of anti-microbial and anti-hemolytic activity of edible basidiomycetes mushroom fungi. *Journal of Drug Delivery and Therapeutics*, *9*(1), 132-135. DOI: http://dx.doi.org/10.22270/jddt.v9i1.2277

Mariga, A. M., Pei, F., Yang, W., Zhao, L., Shao, Y., Mugambi, D. K., & Hu, Q-H. (2014). Immunopotentiation of *Pleurotus eryngii* (DC. ex Fr.) Quél. *Journal of Ethnopharmacology, 153*(3), 604-614. DOI: http://dx.doi.org/10.1016/j.jep.2014.03.006

Minitab Statistical Software. (2014). *LEAD technologies. Inc. Version 17.0*. State College, PA: Minitab LLC.

Musa, S. F., Yeat, T. S., Kamal, L. Z. M., Tabana, Y. M., Ahmed, M. A., Ouweini, A. E., ... Sandai, D. (2018). *Pleurotus sajor-caju* can be used to synthesize silver nanoparticles with antifungal activity against *Candida albicans*. *Journal of the Science of Food and Agriculture, 98*(3), 1197-1207. DOI: http://dx.doi.org/10.1002/jsfa.8573

Nwachukwu, E., & Uzoeto, H. O. (2010). Antimicrobial activity of some local mushrooms on pathogenic isolates. *Journal of Medicinal Plants Research, 4*(23), 2460-2465. DOI: http://dx.doi.org/10.5897/JMPR10.154

Owaid, M. N., Al-Saeedi, S. S. S., & Al-Assaffii, I. A. A. (2015). Antimicrobial activity of mycelia of oyster mushroom species (*Pleurotus* spp.) and their liquid filtrates (*in vitro*). *Journal of Medical and Bioengineering, 4*(5), 376-380. DOI: http://dx.doi.org/10.12720/jomb.4.5.376-380

Owaid, M. N., Al-Saeedi, S. S. S., & Al-Assaffii, I. A. A. (2017). Antifungal activity of cultivated oyster mushrooms on various agro-wastes. *Summa Phytopathologica, 43*(1), 9-13. DOI: http://dx.doi.org/10.1590/0100-5405/2069

Özdal, M., Gülmez, Ö., Gür-Özdal, Ö., & Algur, Ö. F. (2019). Antibacterial and antioxidant activity of mycelial extracts of different *Pleurotus* species. *Food and Health, 5*(1), 12-18. DOI: http://dx.doi.org/10.3153/FH19002

Popa, G., Voaides, C., Cornea, P., & Zagrean, V. (2016). Antimicrobial properties of *Pleurotus eryngii* and *Lentinus edodes* hydro-alcoholic extract. *Bulletin UASVM Animal Science and Biotechnologies, 73*(2), 259-260. DOI http://dx.doi.org/10.15835/buasvmcn-asb:12284

Rufino, R. D., Luna, J. M., Sarubbo, L. A., Rodrigues, L. R. M., Teixeira, J. A. C., & Campos-Takaki, G. M. (2011). Antimicrobial and anti-adhesive potential of a biosurfactant Rufisan produced by *Candida lipolytica* UCP 0988. *Colloids and Surfaces B-Biointerfaces, 84*(1), 1-5. DOI: http://dx.doi.org/10.1016/j.colsurfb.2010.10.045

Sathyan, A., Majeed, K. A., Majitha, V. K., & Rajeswary, K. R., (2017). A comparative study of antioxidant and antimicrobial activities of *Pleurotus ostreatus*. *Pleurotus eryngii* and *Pleurotus djamor*. *International Journal of Agriculture Innovations and Research, 5*(6), 907-912.

Schillaci, D., Arizza, V., Gargano, M. L., & Venturella, G. (2013). Antibacterial activity of Mediterranean oyster mushrooms. species of genus *Pleurotus* (higher basidiomycetes). *International Journal of Medicinal Mushrooms, 15*(6), 591-594. DOI: http://dx.doi.org/10.1615/IntJMedMushr.v15.i6.70

Shen, H., Shao, S., Chen, J. C., & Zhou, T. (2017). Antimicrobials from mushrooms for assuring food safety. *Comprehensive Reviews in Food Science and Food Safety, 16*(2), 316- 329. DOI: http://dx.doi.org/10.1111/1541-4337.12255

Subrata, G., Gunjan, B., Prakash, P., Mandal, S. C., & Krishnendu, A. (2012). Antimicrobial activities of basidiocarps of wild edible mushrooms of West Bengal. India. *International Journal of PharmTech, 4*(4), 1554-1560.

Suresh, N., Ambika, J., Noorjahan, A., & Kalaiselvam, M. (2017). Pink oyster mushroom (*Pleurotus djamor*) and its efficacy against human pathogen. *International Journal of Science Inventions Today, 6(*6), 749-757.

Xu, D., Wang, H., Zheng, W., Gao, Y., Wang, M., Zhang, Y., & Gao, Q. (2016). Charaterization and immunomodulatory activities of polysaccharide isolated from *Pleurotus eryngii*. *International Journal of Biological Macromolecules, 92*(1), 30-36. DOI: http://dx.doi.org/10.1016/j.ijbiomac.2016.07.016

Yehia, R. S., & Al-Sheikh, H. (2014). Biosynthesis and characterization of silver nanoparticles produced by *Pleurotus ostreatus* and their anticandidal and anticancer activities. *World Journal of Microbiology and Biotechnology, 30*(11), 2797-2803. DOI: http://dx.doi.org/10.1007/s11274-014-1703-3

Yilmaz, A., Yildiz, S., Tabbouche, S., Kiliç, A. O., & Can, Z. (2016). Total phenolic content. antioxidant and antimicrobial properties of *Pleurotus ostreatus* grown on lime (*Tilia tomentosa*) leaves. *Hacettepe Journal of Biology and Chemistry, 44*(2), 119-124. DOI: http://dx.doi.org/10.15671/HJBC.20184417585

Zhang, B., Li, Y., Zhang, F., Linhardt, R. J., Zeng, G., & Zhang, A. (2019). Extraction, structure and bioactivities of the polysaccharides from *Pleurotus eryngii:* A Review. *International Journal of Biological Macromolecules, 150*, 1342-1347. DOI: http://dx.doi.org/10.1016/j.ijbiomac.2019.10.144

Zhang, C., Li, S., Zhang, J., Hu, C., Che, G., Zhou, M., & Jia, L. (2016). Antioxidant and hepatoprotective activities of intracellular polysaccharide from *Pleurotus eryngii* SI-04. *International Journal of Biological Macromolecules, 91*(1), 568-577. DOI: http://dx.doi.org/10.1016/j.ijbiomac.2016.05.104