Revista Información Científica

ISSN 1028-9933
Universidad de Ciencias Médicas de Guantánamo

January 2024 Volume 103 e4364
DOI: https://doi.org/10.5281/zenodo.10456696

ORIGINAL ARTICLE

Cytotoxicity assay of Pleurotusostreatus extracts in different cell lines for immunonutritional applications

Ensayo de citotoxicidad de extractos de *Pleurotusostreatus*en diferentes líneas celulares para aplicacionesinmunonutricionales

Ensaio de citotoxicidade de extratos de *Pleurotusostreatus* em diferentes linhagens celulares para aplicações imunonutricionais

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Received: 11-09-2023 **Accepted:** 20-12-2023 **Published:** 31-01-2024

ABSTRACT

Introduction: bio derivatives proposed as candidates for food ingredients usually require certain evaluations for immunonutritional applications. Edible-medicinal mushrooms are a source of compounds with these potentials. Among them, *Pleurotusostreatus* mushrooms contain bioactive metabolites, with important uses in the food industry and in the therapeutic practice of the medical-pharmaceutical industry. In vitro cytotoxicity assays are valuable methods to evaluate products of natural origin, such as fungal extracts. **Objective:** to evaluate the cytotoxicity of two extracts obtained from the *Pleurotusostreatus* mushroom in different cell lines. **Method:** water-soluble extracts were obtained from the mycelium and fruiting bodies of *Pleurotusostreatus* in laboratories of the Center for Industrial Biotechnology Studies of the Oriente University. The cytotoxicity of the bioproducts was evaluated by the resazurin dye reduction assay on three cell lines at the Laboratory of Microbiology, Parasitology and Hygiene (LMPH) of the University of Antwerp, Belgium. Non-adherent THP-1 cells (human leukemia premonocytes), Caco-2 adherent cells (human colon adenocarcinoma epithelium) and RAW 264.7 adherent cells (murine macrophages) were used. **Results:** *Pleurotusostreatus* extracts were not cytotoxic for any of the human or murine cell lines studied, since they did not cause damage to the viability of the epithelial cells of the gastrointestinal system, or to the immune system cells used. **Conclusions:** this result demonstrates that both fungal bio-derivatives can be safely applied in immunonutritional studies.

Keywords: pleurotusostreatus; aqueous extracts; cytotoxicity; cell lines; immunonutrition

ISSN 1028-9933
Universidad de Ciencias Médicas de Guantánamo

RESUMEN

Introducción: los bioderivados propuestos como candidatos a ingredientes alimentarios suelen requerir ciertas evaluaciones para las aplicaciones inmunonutricionales Los hongos comestibles-medicinales son un surtidor de compuestos con estas potencialidades. Entre ellos, las setas *Pleurotusostreatus*contienen metabolitos bioactivos, con importantes usos en la industria alimenticia y en la práctica terapéutica de la industria médico-farmacéutica. Los ensayos de citotoxicidad *in vitro* constituyen métodos valiosos para evaluarproductos de origen natural, como los extractos fúngicos. **Objetivo:** evaluar la citotoxicidad de dos extractos obtenidos de la seta *Pleurotusostreatus*en diferentes líneas celulares. **Método:** se obtuvieron extractos hidrosolubles a partir del micelio y de los cuerpos fructíferos de *Pleurotusostreatus* en laboratorios del Centro de Estudios de Biotecnología Industrial de la Universidad de Oriente. Se evaluó la citotoxicidad de los bioproductos por el ensayo de reducción del colorante resazurina sobre tres líneas celulares en el Laboratorio de Microbiología, Parasitología e Higiene (LMPH) de la Universidad de Amberes, Bélgica. Se utilizaron células no adherentes THP-1 (pre-monocitos de leucemia humana), células adherentes Caco-2 (epitelio de adenocarcinoma de colon humano) y células adherentes RAW 264.7 (macrófagos murinos). **Resultados:** los extractosde *Pleurotusostreatus* no resultaron citotóxicos para ninguna de las líneas celulares estudiadas humanas o murina, ya que no ocasionaron daños sobre la viabilidad de las célulasepiteliales del sistema gastrointestinal,nisobrelas células del sistema inmune empleadas. **Conclusiones**: este resultado demuestra que ambos bioderivados fúngicos pueden ser aplicados con seguridad en estudios inmunonutricionales.

Palabras clave: pleurotusostreatus; extractos acuosos; citotoxicidad; líneas celulares; inmunonutrición

RESUMO

Introdução:bioderivados propostos como candidatos a ingredientes alimentícios geralmente requerem determinadas avaliações para aplicações imunonutricionais. *Pleurotusostreatus* contêm metabólitos bioativos, com importantes utilizações na indústria alimentícia e na prática terapêutica da indústria médico-farmacêutica. Ensaios de citotoxicidade in vitro são métodos valiosos para avaliar produtos de origem natural, como extratos de fungos. **Objetivo:** avaliar a citotoxicidade de dois extratos obtidos do cogumelo *Pleurotusostreatus* em diferentes linhagens celulares. **Método:** extratos hidrossolúveis foram obtidos do micélio e dos corpos frutíferos de *Pleurotusostreatus* nos laboratórios do Centro de Estudos de Biotecnologia Industrial da Universidade de Oriente. A citotoxicidade dos bioprodutos foi avaliada pelo ensaio de redução do corante resazurina em três linhagens celulares no Laboratório de Microbiologia, Parasitologia e Higiene (LMPH) da Universidade de Antuérpia, Bélgica. Foram utilizadas células THP-1 não aderentes (pré-monócitos de leucemia humana), células aderentes Caco-2 (epitélio de adenocarcinoma do cólon humano) e células aderentes RAW 264.7 (macrófagos murinos). **Resultados:** os extratos de *Pleurotusostreatus* não foram citotóxicos para nenhuma das linhagens celulares humanas ou murinas estudadas, pois não causaram danos à viabilidade das células epiteliais do sistema gastrointestinal, nem às células do sistema imunológico utilizadas. **Conclusões:** este resultado demonstra que ambos os bioderivadosfúngicos podem ser aplicados com segurança em estudos imunonutricionais.

Palavras-chave: pleurotusostreatus; extratos aquosos; citotoxicidade; linhas de celular; imunonutriçã

How to cite this article:
Lebeque Pérez Y, Llauradó Maury G, Serrat Díaz MJ, Cos P, Barreto Argilagos GA. **Cytotoxicity assay of Pleurotusostreatusextracts in different cell lines for immunonutritional applications**. RevInfCient [Internet]. 2024 [cited Access date]; 103:e4364. Available in: http://www.revinfcientifica.sld.cu/index.php/ric/article/view/4364

INTRODUCTION

Mushrooms constitute sources of compounds with immunopharmacological properties, which have been of much interest for traditional medicine of different civilizations since ancient times.[1] Edible-medicinal mushrooms possess a diverse and robust variety of bioactive molecules that exert more than 200 different medicinal functions.[2] In recent decades, medicinal mushrooms have been used in the development of important health supplements. Consortia of bioactive compounds and their extracts have been reported to be specific for this purpose.[3]

There is a progressive interest in the investigation of immunomodulatory compounds, from natural sources, that lack adverse consequences and have utility in immunotherapeutic practice. From a clinical point of view, new procedures for the development of immunotherapeutics obtained from mushrooms could constitute answers to various immunotherapy demands. Food biotechnology based on the utilization of edible mushrooms is a very promising alternative for obtaining bio-derivatives with immunomodulatory and antitumor effects.[1]

Pleurotus is among the most studied genera as a source of bioactive molecules with the ability to modulate the immune response. However, only 3% of the research on natural bioproducts reaching the preclinical and/or clinical phases is oriented to work with edible mushrooms. A less studied area is the use of Pleurotus in the stimulation of the immune system in relation to nutritional treatments, which is the fundamental purpose of "immunonutrition". This is evidence of the importance of "mycotherapy" as a novel and promising area.[1]

Health is strongly related to a proper balance of immune functions that can be directly mediated by diets, termed "immune modulation by food". These studies have gained utility due to the use of cell lines. Dietary compounds are often integrated into different matrices. After oral consumption, a food matrix passes through the gastrointestinal tract where its bioactivities may have been modified. Therefore, a simulation of gastric digestion in vitro is necessary to study the stimulation of immune cells in vivo and ex vivo (Chanput W. In: Dissertation for the degree of PhD. Wageningen University, Wageningen, NL, 2012).

On the other hand, cell culture has a relevant role in the research of new treatments for a large number of diseases.[4] The Caco-2 cell line is derived from a human rectal color adenocarcinoma and is characterized by being epithelial type cells. It is frequently used in studies on the absorption of compounds since it exhibits particularities of mature intestinal cells (Romero and Zabaleta. Work for the degree of Pharmaceutical Chemist University of Cartagena, Cartagena de Indias, 2020).

Monocytic THP-1 corresponds to a cancer cell line, whose cells can differentiate into macrophages by exposing them to reagents such as phorbol-12 myristate-13-acetate (PMA), which has been used to study monocytes and macrophages in different inflammatory processes (Gonzaga JF, Master's Thesis Center for Research and Advanced Studies of the National Polytechnic Institute Irapuato, Mexico, 2016).

For their part, RAW 264.7cells belong to a macrophage cell line derived from monocytes of mice with leukemia. The use of this murine macrophage line is also a cellular model of inflammation, commonly used (Arranz EM. TesisdoctoralUniversidad Autónoma de Madrid, 2013).

To assess cytotoxicity, various cell staining techniques are generally used to indirectly estimate the number of live cells following treatment. A correct assay to assess cell viability and cytotoxicity must be simple, rapid, efficient, inexpensive, sensitive, reproducible, safe and effective for the viable cell population, and show no interference with the compounds being evaluated.[5]

The resazurin method is a basic method to assess cell proliferation after treatment and allows complementing the standards used for the evaluation of the cytotoxic effect with the subsequent determination of cell proliferation.[5]

The aim of the present work was to evaluate, through the resazurin dye reduction assay, the cytotoxic activity on different cell lines of aqueous extracts of Pleurotusostreatus to ensure its application in future immunonutritional studies.

METHOD

Sample preparation

The aqueous extracts were previously obtained in the edible mushroom plant and culture laboratory of the Center for Industrial Biotechnology Studies of the OrienteUniversity. From the wet biomass of the mycelium and fruiting bodies of Pleurotusostreatus (P. ostreatus), decoction (90-100 °C) and freeze-dried.(6) The freeze-dried samples were weighed on a balance (METTLER TOLEDO, New ClassicMS, Spain) and resuspended (100 mg/mL) in ultrapure water. Subsequently, biopreparations were obtained in the corresponding culture media at serial concentrations (512, 256, 128, 64, 32 and 16 µg/mL) and stored until use.

Cell cultures

Cell cultures and assays to evaluate the cytotoxicity of the bioproducts were performed at the Laboratory of Microbiology, Parasitology and Hygiene (LMPH) of the Faculty of Pharmaceutical, Biomedical and Veterinary Sciences of the Antwerp University, Belgium.

The fungal extracts were evaluated in three different cell lines: THP-1 non-adherent cells (human leukemia pre-monocytes), Caco-2 adherent cells (human colon adenocarcinoma epithelium) and RAW 264.7 adherent cells (murine macrophages). The lines were acquired from the American Type Tissue Culture Collection (ATCC) (Manassas, VA, USA). Cells were seeded in laminar flow cabinet (LAF® clan, VFRS 1806, The Netherlands), cultured in media according to their specifications and incubated (BINDER, Germany) at 37 °C with humidified atmosphere (5% CO2).

THP-1 cells in RPMI 1640 (1X) medium (Gibco®, USA), supplemented with 10% fetal bovine serum (iFBS) (Gibco®, USA), L-glutamine (2 mM), hygromycin (75 mg/mL) and Penistrep (1%) (Sigma-Aldrich, USA). The Caco-2 cell line was cultured in DMEM(1X) (Gibco®, USA), supplemented with 10 % iFBS (Gibco®, USA), 2 % L-glutamine, D-glucose (4.5 g/L) and Penistrep (1 %) (Sigma-Aldrich, USA). RAW 264.7 macrophages were maintained at 37 °C, 5 % CO2 atmosphere in DMEM medium with FBS (Gibco®, USA)(10 %), L-glutamine (2 %) and 4.5 g/L D-glucose.[7]

Assay to evaluate cytotoxicity

The cytotoxic effect was evaluated by the resazurin dye reduction assay on cell viability. Resazurin sodium salt (Sigma-Aldrich, USA) was used as a standard control. Tamoxifen was included as a reference control drug for cytotoxicity and a plate reader (TECAN GENios, Switzerland) was used for readout.[7]

The grown adherent cells (Caco-2) were removed with 0.05 % Trypsin-EDTA and centrifuged at 130 g for 10 min 4 °C in centrifuge (Allegra®, X-15R, USA). For the assay, 200 µL of the cell inoculum (5 x 105 cells/well) were seeded in sterile 96-well microplates and incubated in an incubator (BINDER, Germany) for 24 h at 37 °C and 5 % CO2.

Non-adherent cells (THP-1) were centrifuged at 1 800 rpm/10 min and 4 °C. They were added (5x105 cells/well) with 200 µL of fresh medium for incubation.

10 µL of tamoxifen was added to the positive control wells. The negative control cells were incubated with only 200 µL of the corresponding medium. To the remaining wells the extracts were added at their different concentrations and the plates were incubated for 24-48 h at 37 °C in 5% CO2.After the incubation time, 50 µL of resazurin (2.2 µg/mL) was added to each well and fluorescence was measured after 4 h at 37 °C (excitation λ 550 nm, emission λ 590 nm).

Two independent experiments were performed and samples were assayed in triplicate in each experiment.

Data processing

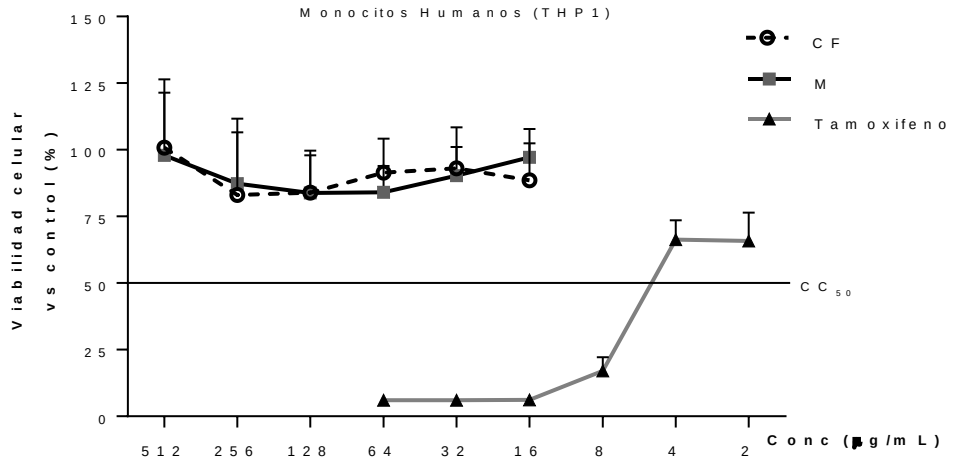
Results were expressed as the arithmetic mean ± standard deviation; from statistical forward analysis performed with Graph PadPrism 7 software (Windows, V.7.04, 2017).The results correspond to the VLIR-UOP-3 Project Natural Products and Pharmaceutical Services, to improve the patient quality of life in Eastern Cuban Hospital's.

RESULTS

The extracts tested in this study constituted material of suitable quality for evaluation.

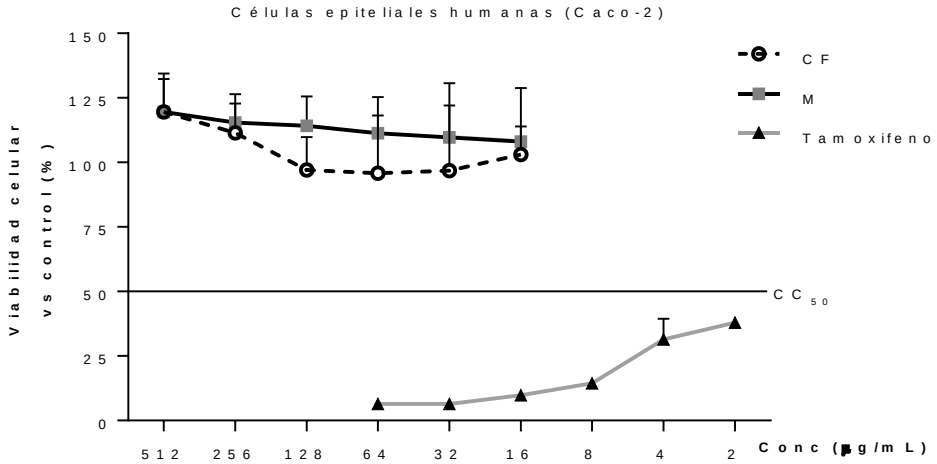
The effect of the fungal extracts was evaluated on the cell viability of three secondary cell lines: human leukocyte line (THP-1), intestinal epithelial line (Caco-2) and murine macrophage line (RAW 264.7) (Figure 1, Figure 2 and Figure 3). The resazurin reduction assay was applied as a test indicative of possible damage at the mitochondrial level and, therefore, in the processes of cellular respiration or metabolic activity.(7)

The dye resazurin (non-fluorescent blue) is reduced to resofurin (highly fluorescent pink) by oxidoreductases present mainly in the mitochondria of living cells. Resofurinase is excreted into the medium and allows the observation of cell proliferation or cytotoxicity of compounds on different cell types, including fungal cells.(5)

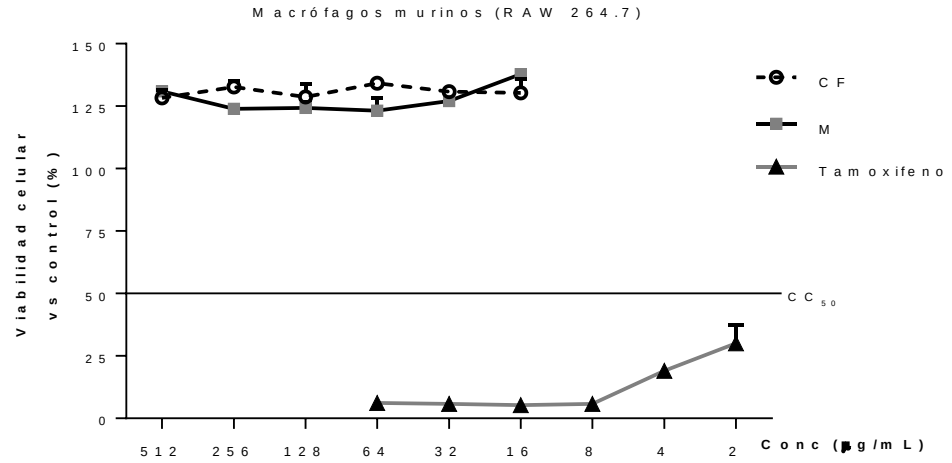


Graph 1 Effect of Pleurotus extracts (Cf and M) on cell viability of human monocytesTHP1. Results expressed as the mean ± SD of 6 replicates (n=6).Cf(fruiting bodies) M (mycelium)Tamoxifen (reference control for cytotoxicity)

ISSN 1028-9933
Universidad de Ciencias Médicas de Guantánamo



Graph2 Effect of P. ostreatus extracts on cell viability of human intestinal adenocarcinoma epithelial cells Caco-2. Results expressed as the mean ± SD of 6 replicates (n=6). Cf (fruiting bodies),M (mycelium),Tamoxifen (reference control for cytotoxicity).



Graph 3 Effect of Pleurotus extracts (Cf and M) on cell viability of immune system cells (murine macrophages RAW 264.7). Results expressed as the mean ± SD of 6 replicates (n=6). Cf (fruiting bodies),M (mycelium),Tamoxifen (reference control for cytotoxicity).

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DISCUSSION

The extracts of P. ostreatus tested are bio-derivatives with the capacity to enhance the immune response.[8] The carbohydrates and other bioactive compounds present in them[8,9,10,11] could be responsible for their potential prebiotic effects and guarantee their possible immunonutritional applications, once the absence of cytotoxicity on selected cells of the gastrointestinal and immune systems has been proved.

Different cell lines have been commonly used as models in coculture assays and monoculture systems designed to explore toxicological effects[12] and as a strategy to evaluate microorganism-host interactions.[13] In our research, one of the first studies to be performed is to rule out a possible cytotoxic effect of fungal bio-derivatives on cells involved in the study of prebiotic candidates, capable of stimulating local immunity in the intestine (e.g. phagocytosis) or interfering with pathogen adhesion mechanisms and favoring the adhesion of beneficial bacteria to the intestinal epithelium.

The monocytic THP-1 cell line is the most frequently used cell line for investigations of human macrophage function. The inability to expand macrophage populations ex vivo and their limited lifespan in culture have made THP-1 cells the in vitro model to overcome the drawbacks of using primary macrophages and has been the most widely used cell line to determine the response of human macrophages to proinflammatory stimuli.[14]

Caco-2 and HT-29 cell cultures have been widely used as models for the study of specific functions in intestinal epithelial cells. These cell lines allow the phenotype of absorptive and mucosecretory cells to be reproduced.[15]

On the other hand, the resazurin dye reduction method proved to be suitable and reproducible for the evaluation of the cytotoxicity of fungal bio-derivatives. It was possible to verify the metabolic activity expressed in the viability levels of the cells treated with the experimental substances (extracts), in comparison with the negative effects observed on the cell viability of the compound (tamoxifen), used as a reference control.

According to Escobar, et al.[5] the resazurin dye is not very toxic to cells and allows the continuity of studies on the same cells, which saves time and money, especially in primary cultures where cells are very scarce and precious. In addition, the method is sensitive and highly reproducible.

The results of this study suggest that none of the extracts evaluated caused damage to the metabolic processes of the cells, since they did not cause a decrease in cell viability. On the contrary, a slight tendency towards concentration-dependent stimulation of cell proliferation was observed in human monocyte (THP-1) and epithelial cell (Caco-2) lines. Meanwhile, in murine macrophages (RAW 264.7) a very stable viability trend was observed at the different concentrations applied. This evidences the non-cytotoxic effect of the fungal bio-derivatives on these cell populations.

Resazurin reduction has been described as a variable method depending on the cell line assessed. One study identified how this occurs by assessing CuSO4 at different concentrations in three cell lines (mouse hepatocytes, HepG2, and HeLa).Here the method detected faster cytotoxicity in the former lines; however, in the HeLa cell line, the result was opposite. This may indicate a different sensitivity of this line. For this reason, some authors state the importance of assessing the cell lines individually, since each one has unique metabolic properties that must be characterized to determine different experimental parameters.[5]

Llauradó, et al.[8] evaluated the cytotoxicity of a P. ostreatus extract (HW-Pm) obtained at 95 °C. The effect was determined by the resazurin dye reduction assay, using various concentrations (2, 4, 8, 16, 32, 32, 64, 128, 256, 512 and 1024 µg/mL) of the product. The result showed that HW-Pm was not cytotoxic to RAW 264.7 cells, as stability in cell viability was observed at the different concentrations tested.

In an investigation carried out to evaluate the antimicrobial activity of an aqueous extract of the microalgaePhorphyridiumcruentum, it was also demonstrated that it did not exert cytotoxic activity on human THP-1 monocytes and murine RAW 264.7 macrophages. The resazurin reduction method and the same concentrations (512, 256, 128, 128, 64, 32 and 16 µg/mL) used to evaluate the product of our assay were used by these authors.[16]

In the present study, it can also be observed that the percentages of cell survival obtained were very similar between the cells treated with the different extracts. Meanwhile, in the evaluation of the cytotoxicity of three synthetic compounds on HEp-2, HT-29 and HeLa cells, the results indicated that in none of the cell lines did the compounds cause significant decrease in cell survival with the use of the resazurin method. It was also found that the percentage survival responses obtained were similar when the different compounds were evaluated.[5]

It is important that biopreparations for immunonutritional applications do not show cytotoxicity. This study ensures that Pleurotus extracts can be applied in successive investigations without risk of possible adverse effects. A probable limitation, however, is the use of a single method for the evaluation of cell viability.

CONCLUSIONS

The Pleurotusostreatus extracts evaluated did not affect the viability of human cells (leukocytes and intestinal epithelial cells), as well as murine immune cells (macrophages). These results support their use as possible prebiotic candidates and guarantee their safety for further immunonutritional studies, such as co-culture assays between leukocytes, epithelial cells and beneficial or pathogenic bacteria.

Recommendations

To evaluate the activity of these tested extracts on the immunological function of leukocyte cells, as well as the possible inhibitory effect on the adhesion mechanisms of pathogenic and beneficial bacteria.

Acknowledgments

We thank the Cuban Ministry of Science, Technology and Environment (Territorial Project PT241SC003-003 of the Program "Development of Health Products and Services") for its monetary contribution. As well as to the Belgian Cooperation for Development (Project VLIR-UO, Flemish Interuniversity Council-University Cooperation for Development) of the Institutional University Cooperation Program with the OrienteUniversity. This program provided a research stay and guaranteed material resources for the realization of the research.

REFERENCES

1. Morris-Quevedo HJ, Llauradó-Maury G, Bermúdez-Savón RC, Cos P, Lebeque-Pérez Y, Beltrán-Delgado Y, *et al*. Evaluación de la Actividad Inmunomoduladora de bioproductos obtenidos de la seta comestible-medicinal *Pleurotusostreatus*. An AcadCiencCuba[Internet]. 2018 [cited 26 Jun 2023]; 8(1):1-10. Available in: https://revistaccuba.sld.cu/index.php/revacc/article/view/436/428
2. Rathore H, Prasad Sh, Kapri M, Tiwari A, Sharma S. Medicinal importance of mushroommycelium: Mechanismsandapplications. J FunctFoods [Internet]. 2019 [cited 2 Mar 2023]; 56(2019):182-193. DOI: https://doi.org/10.1016/j.jff.2019.03.016
3. Martin KR, Pence JC, Bloomer RJ. Vitamin D –enhancedMushrooms as DietarySupplementsandNutraceuticals: A NutritionallySensible Trade-off for the Consumer. Clin J NutrDiet [Internet]. 2020 [cited 10 Mar 2023]; 3(1):1-7. Available in: https://www.memphis.edu/healthsciences/pdfs/pdfs2020/2020_cjnd_mushrooms.pdf
4. Montoya EL, Juárez E. El Cultivo Celular en la búsqueda de nuevas estrategias de Combate contra el Cáncer. RevMéd U Ver [Internet]. 2022 [cited 10 Mar 2023]; 22(1):39-44. Available in: https://www.medigraphic.com/pdfs/veracruzana/muv-2022/muv221d.pdf
5. Escobar L, Rivera A, Aristizábal F. A. Estudio comparativo de los métodos de resazurina y MTT en estudios de citotoxicidad en líneas celulares tumorales humanas. Vitae [Internet]. 2010 [cited 10 Mar 2023]; 17(1):67-74. Available in: http://www.scielo.org.co/pdf/vitae/v17n1/v17n1a09.pdf
6. Lebeque-Pérez Y, Fong-Lores O, Rodríguez-Leblanch E, Llauradó-Maury G, Serrat-Díaz MJ. Evaluación *in vivo* de la pirogenicidad de bioproductos fúngicos con potencial prebiótico. Rev InfCient [Internet]. 2022 [cited 15 Mar 2023]; 101(3):e3791. Available in: http://www.revinfcientifica.sld.cu/index.php/ric/article/view/3791
7. Llauradó G, Morris HJ, Heykers A, Lanckacker E, Cappoen D, Delputte P, *et*

al.DifferentialInductionPatternTowardsClassicallyActivatedMacrophages in Response to an Immuno modulatory Extract from *Pleurotusostreatus* Mycelium. J Fungi [Internet]. 2021 [cited 15 Mar 2023]; 7:206. DOI: https://doi.org/10.3390/jof7030206

8. Morris HJ, Llauradó G, Beltrán Y, Bermúdez RC, García N, Lebeque Y. Biotecnología de hongos comestibles: fuente de alimentos funcionales/nutracéuticos en sistemas agroalimentarios sostenibles de origen microbiano. Rev Congreso Univ [Internet]. 2020 [cited 15 Mar 2023]; 9(3).
9. Lebeque Y, Morris HJ, Beltrán Y, Llauradó G, Gaime-Perraud I, Meneses M, *et al.*Proximal Composition, NutraceuticalProperties, and AcuteToxicityStudy of Culinary-Medicinal OysterMushroomPowder, *Pleurotusostreatus* (Agaricomycetes). Int J MedMushrooms [Internet]. 2018 [cited 15 Mar 2023]; 20(12):1185-1195. DOI: https://doi.org/10.1615/IntJMedMushrooms.v20.i12.60
10. Beltrán-Delgado Y, Morris-Quevedo HJ, Llauradó-Maury G, Bermúdez-Savón RC, García-Oduardo N. Procedimientos para la producción de setas del género *Pleurotus* con potencial aplicación farmacológica. RevCubanaQuím[Internet]. 2020 [cited 15 Mar 2023]; 32(2):245-261. Available in: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S2224-54212020000200245
11. Beltrán Y, Morris H, Domínguez O, Batista P, Llauradó G. Composición micoquímica y actividad antioxidante de la seta *Pleurotusostreatus* en diferentes estados de crecimiento. ActaBiolColomb [Internet]. 2021 [cited 15 Mar 2023]; 26(1):89-98. DOI: http://dx.doi.org/10.15446/abc.v26n1.84519
12. Arezki Y, Cornacchia J, Rapp M, Lebeau L, Pons F,Ronzani C. A Co-Culture Model of the Human RespiratoryTracttoDiscriminate the ToxicologicalProfile of CationicNanoparticlesAccordingtoTheir Surface ChargeDensity. Toxics [Internet].2021 [cited 4 Jul 2023]; 9(210). DOI: https://doi.org/10.3390/toxics9090210
13. De Rudder Ch, Calatayud M, LebeerS, Van de Wielea T. Dual and Triple EpithelialCocultureModelSystemswithDonor-Derived Microbiota and THP-1 MacrophagesToMimic Host-MicrobeInteractions in the Human SinonasalCavities. mSphere. 2020 [cited 4 Jul 2023]; 5(1):e00916-19. DOI: https://doi.org/10.1128/mSphere.00916-19
14. Lundÿ ME, Joyce A, O'Brien BA, Donnelly S. La elección del protocolo de diferenciación de forbol 12-miristato 13-acetato influye en la respuesta de los macrófagos THP-1 a un estímulo proinflamatorio. RevMétInmunol [Internet]. 2016 [cited 15 Mar 2023]; 430(2016):64-70. DOI: http://dx.doi.org/10.1016/j.jim.2016.01.0120022-1759/
15. Rodríguez P. Estrategias para mitigar la toxicidad asociada a la exposición crónica al mercurio a través de la dieta [Tesis Doctoral]. España: UniversitatPolitècnica de València; 2023. [cited 25 Ago 2023]. Available in: http://hdl.handle.net/10251/192865
16. Ferrer-Salas D, Llauradó-Maury G, Fernández-Duharte G, Lebeque-Pérez Y. Antimicrobialevaluation of the aqueousextract of the biomass of the microalga *Phorphyridiumcruentum*. RevCubanaQuím [Internet]. 2023 [cited 15 Mar 2023]; 35(1):83-99. Available in: https://cubanaquimica.uo.edu.cu/index.php/cq/article/view/5303

Conflicts of interest:
The authors declare that there are no conflicts of interest.

Author contributions:
Yamila Lebeque Pérez: data curation, formal analysis, research, project management, validation, visualization, writing original draft, writing-revising and editing.
Gabriel Llauradó-Maury: conceptualization, formal analysis, supervision, validation, drafting-revision and editing.
Manuel de JesúsS errat-Díaz: research, methodology, writing-revising and editing.
Paul Cos: methodology, project management, resources, writing-revising and editing.
Guillermo Antonio Barreto Argilagos: conceptualization, methodology, project management, writing-revising and editing.

Financing:
Funding was received from the VLIR-UO Project "Natural Products and PharmaceuticalServicestoimprove the patientquality of life in Eastern Cuban Hospital's". Sponsored by the Belgian Development Cooperation through the Laboratory of Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical Biomedical and Veterinary Sciences of the University of Antwerp, Belgium

Complementary file (Open Data):
Base de datos sobre ensayo de citotoxicidad de extractos de *Pleurotusostreatus* en diferentes líneas celulares para aplicaciones inmunonutricionales