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Research Papers

# *IN VITRO* EVALUATION OF THE CYTOTOXICITY, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF THE SPENT *Pleurotus* MUSHROOM SUBSTRATE
# EVALUACIÓN *IN VITRO* DE LA CITOTOXICIDAD, ACTIVIDAD ANTIOXIDANTE Y ANTIMICROBIANA DEL SUSTRATO AGOTADO DEL HONGO *Pleurotus*

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## ABSTRACT
The biological activities of the spent substrate from *Pleurotus* mushroom cultivated on coffee pulp have been little explored. This research evaluated the *in vitro* cytotoxicity, and the antioxidant/antimicrobial effects, of an aqueous extract (RTA) obtained from the spent substrate of *P. ostreatus* grown on coffee pulp in Cuba. Cytotoxicity was studied in the J774 cell line; total phenols/flavonoids, scavenging of DPPH radical and reducing power were evaluated for the antioxidant activity; and the antimicrobial assays by the disk diffusion method. No changes were found on cell viability. Total phenols and flavonoids were of 79,5 mg GAE/100 g and 48,6 mg QE/100 g, respectively, while the sequestration of DPPH radical was of 18,94 % and the reducing power of 0,11 absorbance units. RTA showed antimicrobial activity against *Bacillus subtilis* and *Bacillus cereus*. The results demonstrate the potentialities of *Pleurotus* spent substrate for applications as antioxidant/antimicrobial additive in animal food and health products.
**Keywords**: animal health; *Pleurotus ostreatus*; spent substrate; antioxidant activity; antimicrobial activity.

## RESUMEN
La actividad biológica del sustrato remanente del hongo *Pleurotus* cultivado sobre pulpa de café ha sido poco explorada. Esta investigación evaluó la citotoxicidad *in vitro* y los efectos antioxidantes/antimicrobianos de un extracto acuoso (RTA) obtenido del sustrato agotado de *P. ostreatus* cultivado sobre pulpa de café en Cuba. La citotoxicidad se estudió en la línea celular J774; para la actividad antioxidante se determinó el contenido de fenoles/flavonoides totales, el secuestro del radical DPPH y el poder reductor; y la antimicrobiana se realizó por el método de difusión en disco. No se observaron cambios en la viabilidad celular. Los fenoles y flavonoides totales fueron de 79,5 mg GAE/100 g y 48,6 mg QE/100 g, respectivamente; mientras que la captación del radical DPPH fue de 18,94 %, y el poder reductor de 0,11 unidades de absorbancia. El extracto RTA mostró actividad antimicrobiana contra *Bacillus subtilis* y *Bacillus cereus*. Los resultados demuestran las potencialidades del sustrato remanente de *Pleurotus* para aplicaciones como aditivo antioxidante/antimicrobiano en alimentos y productos para la salud animal.
**Palabras clave**: salud animal; *Pleurotus ostreatus*; sustrato agotado; actividad antioxidante; antimicrobiana.

## INTRODUCTION

At present there is an upsurge of interest in the search for compounds of natural origin, useful as medicines and foods. In this scenario, edible and medicinal mushrooms (EM) highlight as a promising source of biologically active substances for applications in the medical, nutra/pharmaceutical and food industry [1], as natural antioxidants and antimicrobials.

Edible-medicinal mushrooms (EM) are currently cultivated in more than 100 countries, raising a growth rate of 6-7 % per year.[2] The global market for mushrooms is growing due to its nutritional enrichment, potential usage as a bioremediation, enzyme production, and functional food development. Globally, mushroom farming is booming because of their rich nutritional status.[3] The projected global market for mushroom would be 24,05 million tons by the year 2028.[4]

*Pleurotus* spp. are among the most produced; they can be cultivated on different agro-industrial residues.[5] *Pleurotus* genus is ranked second with respect to mushroom farming after *Agaricus* genus due to its adaptability. Different studies have shown their capacity to lower the ecological issues because of their low-cost production.[6]

*Pleurotus* mushrooms are characterized by their medical, biotechnological, and nutritional attributes.[7,8] Numerous studies have reported the many relevant features of *Pleurotus* spp., which confirm their being an attractive low-cost industrial tool that resolves the pressure of ecological issues.[9,10]

The circular economy's concept is not only to correctly choose the raw materials that will be the basis of the activity, but also to define correctly all the actions involved during each production phase, in order to apply again the wastes generated. Using this concept in mushrooms production, the main environmental concern would be the spent mushroom substrate (SMS), the principal waste generated in the cultivation.[11]

SMS is a complex mixture of lignocellulose by-products and a rich source of organic matter and mycelium.[9] Studies have shown that 1 kg of fresh mushroom causes 5-6 kg of SMS.[11] The residuals of SMS consist of mycelial material, low commercial-quality mushrooms or primordia, and depleted substrate. The biochemical composition of SMS consists of lignocellulose and its derived enzymes, various organic compounds, such as carbohydrates, fats, and proteins, and inorganic compounds, such as ammonium nitrates. SMS mostly comprises wheat, sawdust, rice straw, and corncobs, which are highly lignocellulosic, and nutrients such as nitrogen (N), phosphorus (P), and potassium (K) (NPK).[12] The composition of the spent mushroom substrate varies depending on produced mushroom species and also the materials used in compost preparation. [13] This material still has the following benefits: as fuel, compost, organic amendment, animal feed, and mulch material, among others.[14] While research has been conducted on these utilization methods, there are still relatively few large-scale industrial applications.

The bulk of mushrooms products are obtained from fruiting bodies and mycelia, and in a lesser extent from filtrates of submerged fermentation.[15] However, SMS-derived products and their biological activities, including those from *Pleurotus* genus, have been little explored, particularly, applications as antioxidant/antimicrobial additives in the fields of animal food and health products. Thus, upcycling SMS via innovative and practical technologies represents a promising approach to transforming organic waste into economic value.

Mushroom science in Cuba allows the valorization of agricultural by-products to address objectives of sustainability and biotechnological development. Much research work done in Cuban eastern region has been performed in the *Pleurotus* genus. This region produces about 80 % of the high-quality coffee in the island, and as a result large amounts of organic by-products, like coffee pulp are generated, which can be used as substrates for *Pleurotus* cultivation.[16]

Given the challenges encountered within the mushroom industry, the objective of this study was to explore the viability of repurposing residues from *Pleurotus* cultivation, specifically SMS, as potential sources of nutraceutical or pharmaceutical natural compounds. The study involved the potential cytotoxicity, antioxidant and antimicrobial activities of a bio-based SMS product (RTA aqueous extract) obtained from the spent *Pleurotus ostreatus* mushroom substrate, resulting of the solid-state fermentation with coffee pulp as substrate.

## MATERIALS AND METHODS

The research was carried out at the Mushroom Research and Production Unit of the Center for Studies on Industrial Biotechnology (CEBI), Universidad de Oriente; the Biochemistry Laboratory of the Center for Toxicology and Biomedicine (TOXIMED), Medical University of Santiago de Cuba; and in the Laboratory of Microbiology,

Parasitology and Hygiene, University of Antwerp, Belgium.

**Obtaining of the SMS-derived bioproduct (RTA extract)**

*Pleurotus ostreatus* CCEBI 3024 strain, deposited at the CEBI's Microbial Culture Collection and the spent substrate resulting from mushroom cultivation was used. The strain was maintained on slants with solid medium of potato dextrose agar (PDA) incubated at 5 °C.

The cultivation process of *Pleurotus ostreatus* took place during 45 days, with three harvests of the fruiting bodies. Then, the spent mushroom substrate was collected, sun dried and finely milled through a 0,5 mm mesh.[17]

For the extraction, 100 g of the previously dried SMS were added to 200 mL of distilled water (ratio 1:2), continuously stirring, at room temperature in a laboratory shaker (Mizard 2001) at 120 rpm for 3 h. The suspension was filtered and then centrifuged at 3000 rpm for 10 min (Kubota 2420, Japan). The resulting bioproduct (RTA) was preserved at 4 °C until use. The determination of the dried matter in RTA was carried out according to the method described by the Association of Official Analytical Chemists.[18]

**Cytotoxicity assay of RTA**

The J774 macrophage cell line from the American Type Culture Collection (ATCC, Manassas, VA) was used, maintained in the Laboratory of Microbiology, Parasitology and Hygiene (LMPH, University of Antwerp, Belgium). Cells were cultured in transparent, smooth-bottom 96-well plates in DMEM: Dulbecco's Modified Eagle's medium (Gibco® Life Technologies, Gent Belgium), supplemented with 10 % fetal bovine serum at 37 °C with 5 % $CO_2$. The medium was replaced three times a week until total confluency of the cells was reached.

The cell confluence was checked, with prior subculture under an inverted microscope. Because of cell adhesion, the surface of the culture flask was scraped with a cell scraper (Techno Plastic Products, Trasadingen, Switzer Land) and resuspended in fresh Dulbecco Modified Eagle's Medium (DMEM) using transparent, smooth-bottomed 96-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany). A sample of 10 µL of cells with medium was taken and counted in the 10-grid Kova® counting chamber (Hycor Biomedical Inc., Kassel-Lonfelden, Germany). A cell suspension at a concentration of 5 x $10^5$ cells $mL^{-1}$ was used, adding 100 µL to each well. The cells were incubated in the 96-well plates for 24 h at 37 °C in 5 % $CO_2$. Then, the medium was removed by inverting the plate, and 100 µL of fresh DMEM medium was added. RTA was dispensed in a concentration range of 0,5-128 µg $mL^{-1}$ in triplicate. Two controls were used: a positive control containing cells plus medium and a negative control, only with culture medium, to which 200 µL of DMEM was added. Tamoxifen (Sigma-Aldrich, Bornem, Belgium) was used as a reference substance of cytotoxicity in a range of 0,5-64 µg $mL^{-1}$. The plates were incubated again for 24 h at 37 °C in 5 % $CO_2$ atmosphere.

The resazurin assay was used to evaluate the cytotoxic effect of RTA. For that, 40 µL of resazurin (2,2 µg $mL^{-1}$) (Sigma-Aldrich, St. Louis, MO, USA) was added to each well, and then incubated for 4 h at 37 °C in 5 % $CO_2$ atmosphere. The fluorescence (550 nm excitation and 590 nm emission) was measured in a microplate reader (GeNios, Tecan, Mechelen, Belgium), using the Van X Fluor kopie program, version V4.5. The $IC_{50}$ and $IC_{90}$, which represents the concentration of a compound necessary to inhibit 50 % and 90 % of cell growth were estimated.

**Antioxidant activity of RTA**

**Quantification of total phenols**

The determination of the total phenol content was carried out according to Slinkard and Singleton[19] using the 50 % Folin-Ciocalteu reagent (MERK Millipore, Darmstadt, Germany). The absorbance at 765 nm was recorded in a spectrophotometer (T60 UV Visible, PG Instruments, UK) and compared with a gallic acid calibration curve (Sigma) (y= 0,0152x; $R^2$= 0,9789). The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of the bioproduct (dry basis).

**Quantification of total flavonoids**

The flavonoid content was estimated by the method described by Zhishen.[20] A sample of 250 µL of RTA was mixed with 1,25 mL of distilled water and 75 µL of a 5 % $NaNO_2$ solution (Riedel-de Haën, Berlin). After five minutes, 150 µL of a 10 % aqueous $AlCl_3$ solution (Reachim Ltd., Moscow, Russia) was added to the mixture. Once six minutes had elapsed, 500 µL of 1 mol/L NaOH (Riedel-de Haën) and 275 µL of distilled water were added. The solution was mixed well and the absorbance read at 510 nm in a spectrophotometer (T60 UV-Visible, PG Instruments, UK). Quercetin (Sigma-Aldrich) was used for the calibration curve (y= 0,0149 x; $R^2$= 0,9911).

Results were reported as mg quercetin equivalents (QE) per 100 g of RTA (dry basis).

**Scavenging of the radical 2,2-diphenyl1-picrylhydrazyl (DPPH•)**

The capacity of RTA to capture free radicals was measured with the DPPH• method.[21] The radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (MERCK Millipore, Darmstadt, Germany) was used at a concentration of 1 mM. An amount of 1,5 mL of the DPPH methanolic solution was added to 0,25 mL of RTA at different concentrations (0,06-1 mg/mL). It was vortexed (Heidolph REAX 2000) and kept in the dark for 20 min. Subsequently, the absorbances were read in a spectrophotometer (T60 UV-Visible, PG Instruments, UK) at 517 nm. Ascorbic acid was used as the reference antioxidant substance. The scavenging capacity of the radical was determined as the inhibition percentage using the equation 1:

$$\text{IP DPPH \%} = \frac{\text{AP} - \text{AM}}{\text{AP}} X\ 100 \qquad (1)$$

where

IP DPPH: inhibition percentage (scavenging capacity of DPPH radical).

AP: absorbance of blank.

AM: absorbance of the sample.

**Estimation of the reducing power**

The technique was performed according to Oyazu[22] To 2,5 mL of the different concentrations of RTA (0,06-1 mg/mL), 2,5 mL of potassium ferricyanide (Reachim) (1 %) was added. The mixture was incubated for 20 min at 50 °C in an incubator (Selecta model, Cham, Switzerland) and once cold, 2,5 mL of trichloroacetic acid (10 %) was added. Then, samples were centrifuged at 2 000 rpm for ten minutes (Kubota 2420, Japan). Subsequently, 5 mL of the supernatant was taken, to which 5 mL of distilled water and 1 mL of ferric chloride (Sigma Aldrich) (1 g/L) were added. Then, the absorbance at 700 nm was measured in a spectrophotometer (T60 UV-Visible, PG Instruments, UK). Ascorbic acid was used as the reference antioxidant substance for the reducing power test.

**Antimicrobial evaluation of RTA**

The method recommended by the Subcommittee on Susceptibility Testing of the NCCLS (National Committee for Clinical Laboratory Standards) was used, based on the procedure originally described by Kirby-Bauer.[23] The following strains belonging to the culture collection of the Center for Studies on Industrial Biotechnology (CEBI) were used. *Staphylococcus aureus* CCEBI 1070, *Pseudomonas aeruginosa* CCEBI 1071, *Escherichia coli* CCEBI 1081, *Bacillus subtilis* CCEBI 1032, and *Bacillus cereus* CCEBI 1033. Streptomycin antimicrobial discs (10 µg/ml) from the "Carlos J. Finlay" Biological Products Company (Havana, Cuba) were used as the reference substance.

In general, 0,1 mL of the suspensions of tested microorganisms were inoculated at the approximate concentration of 1-2 x $10^8$ colony-forming units (CFU)/mL on Mueller-Hinton Agar plates (BIOCEN, Havana, Cuba). The discs (streptomycin or RTA at 10 mg $mL^{-1}$) were placed on the surface of the plates inoculated with the microorganisms, and incubated at 35 ºC for 16-24 h. After that, the diameters (mm) of the inhibition zone were measured. The samples with inhibition zones greater than 6 mm in diameter were considered active.[24] Percentage of inhibition was calculated using the following equation (2):

$$\text{Inhibition (\%)} = \frac{\text{Zone of inhibition for extract}}{\text{Zone of inhibition for standard}} \times 100 \qquad (2)$$

**Statistical analysis**

Samples were evaluated in triplicate in independent experiments. Results were expressed as the arithmetic mean ± standard deviation. The non-parametric Mann-Whitney U test STATGRAPHICS Centurion XV program (Statgraphics Technologies, Inc, USA) was performed to compare antioxidant activity displayed by RTA and ascorbic acid, as well as, the inhibition zones and the percentage of inhibition produced by RTA and streptomycin reference antibiotic in the antimicrobial experiments. A level of $p < 0,05$ was considered as significant.

## RESULTS AND DISCUSSION

Considerable efforts have been dedicated to formulating strategies for the efficient valorization of SMS. One of the most relevant uses lies with enzymes and other bioactive compounds extraction from SMS and its use as animal feed supplements. Also, it is important to understand the potential of these by-products and to develop technologies and/or methodologies to exploit them as products offering additional nutritional or pharmaceutical advantages is equally significant.[25]

It follows the need to develop a more extensive knowledge on the safety and toxicological profile of SMS-derived products, to establish specific qualitative analytical standards. Cytotoxicity in normal cell lines is an important criterion when evaluating the selectivity of the biological activity of a given product. In this study, RTA in the concentration range of 0,5-128 µg $mL^{-1}$ did not affect the cell viability of the J774 murine macrophage cell line, with estimated values of $IC_{50}$ and $IC_{90}$ higher than 128 µg $mL^{-1}$ (Table 1). On the contrary, the cytotoxic standard drug tamoxifen elicited an $IC_{50}$ of 16 µg $mL^{-1}$. In a previous work, values of cell viability higher than 95 % were reported in macrophages RAW 264.7 treated with HW-ES (*Pleurotus* mycelial extract obtained at high temperatures) and CW-P (*Pleurotus* fruiting bodies extract obtained at low temperatures).[26] Since research has tended to focus on the dietary value of species of the genus *Pleurotus*, there is relatively little information pertaining to the anticancer effects of SMS; therefore, future research should address this gap.

**Table 1-** $IC_{50}$/ $IC_{90}$ values of RTA on J774 murine macrophages and phenol/ flavonoid concentrations in the extract

| Parameters | Values |
|---|---|
| Cytotoxicity, $IC_{50}$ (µg/mL) | >128 |
| Cytotoxicity, $IC_{90}$ (µg/mL) | >128 |
| Total phenols (mg GAE/100 g) | 79,5 ± 1,33 |
| Flavonoids (mg QE/100 g) | 48,6 ± 0,06 |

The cytoprotection exerted by mushroom extracts from *Pleurotus* has been reported previously, and this effect could be related with the antioxidant properties of tested bioproducts with a high concentration of phenols and flavonoids.[27] It is noteworthy that there is limited research available on the antioxidant capacity of various spent mushroom substrates.

A considerable range of total phenols, spanning from 85,54 to 250,92 in terms of mg of gallic acid equivalent, was reported for *Lentinula edodes'* Spent Mushroom Substrate depending on the treatment (short or long extraction -24 h), temperature, use of ultrasound and the type of solvent (distilled water or ethanol 50 %).[25] The highest levels were reached by these authors with ethanol 50 %, 24 h extraction with high temperature (50 °C). The phenol content in RTA (79,5 mg GAE/ 100 g) was lower than reported in the *Lentinula edodes'* SMS in analogue conditions to our study (distilled water, low temperature and short extraction). One factor that could influence the obtained results is the SMS composition,[28] particularly in phenols, but the referred paper did not mention the nature of *Lentinula edodes'* SMS used (FLORESTA.VIVA company, Amarante, Portugal).

The spent substrate is rich in bioactive components, such as polysaccharides, vitamins and some trace elements. In particular, *Pleurotus ostreatus* is considered a primary decomposer, due to its ability to degrade lignin-cellulose-containing substrates and other components. Some researchers report that coffee pulp also contains various phenolic compounds, such as chlorogenic acid, caffeic acid, ferulic acid, coumaric acid, gallic acid.[29]

Species of the genus *Pleurotus* released several enzymes, such as laccases, peroxidases, manganese peroxidases, peptidases, glycosyl hydrolases, esterases, and lipases. It was shown that during the cultivation of *Pleurotus ostreatus* in coffee pulp, the concentration of phenols in this residue decreased, probably associated with the production of the laccase enzyme, which could be the responsible for pulp transformation together with other enzymes.[30]

Not much research can be found on the flavonoid content of different spent mushroom substrates. In the present study, flavonoid content in RTA agreed with values informed for *Lentinula edodes'* SMS between 25,80 and 90,93, in terms of mg of catechin equivalents.[25] Other studies were found to assess the flavonoid content, but expressing it in different units (mg of catechin equivalent per gram -CAT/g). Since the units are not the same, these values cannot be directly compared with the ones obtained in this study. For example, flavonoid content of 3,58 and 5,46 mg CAT/100 g, was reported previously in primordia and fruiting bodies, respectively, of *Pleurotus ostreatus* by our group.[31] Other aspect to take into consideration for comparing phenol/flavonoid content with other studies is the lack of information on the extract yield, in our case 1 % (on dried basis).

The antioxidant activity of RTA was assayed through the scavenging of the DPPH radical and reducing power estimation. The percentages of scavenging DPPH radical by RTA, compared with ascorbic acid as the reference substance is shown in Figure 1. The results showed that in RTA, the uptake percentage was in the range of 11-18 %, lower than ascorbic acid (60-96 %). However, results in that range have been informed in other works evaluating mushrooms products from mycelia or fruiting bodies. In a study, in which 24 *Basidiomycetes* strains were evaluated to determine their free-radical scavenging capacity in

submerged cultivation, the scavenging capacity of the extracts against DPPH• varied from 1 to 85 % depending on the mushroom species, solvent used and concentration. For example, three water extracts from *Pleurotus* species (*P. citrinopileatus*, *P. nebrodensis* and *P. eryngii*) showed scavenging abilities between 11 and 14 % at 0,5 mg $mL^{-1}$.[32]

On the other hand, he representative curves of the reducing power of RTA are shown in Figure 2. It was observed that even at the highest concentration (1 mg $mL^{-1}$), the reducing power of RTA was significantly lower than ascorbic acid. An in-depth analysis of the findings presented in the literature unveils noteworthy disparities among various extraction methods. As mentioned before, these results are dependent on the treatment (short or long extraction -24 h), temperature, use of ultrasound and the type of solvent (distilled water or ethanol 50 %). For antioxidant capacity, the most remarkable outcomes were consistently obtained through the 24 h extraction process at 50 °C employing a 50 % (v/v) ethanol solution (et-LE-HT) in all assessments ($ABTS^{•+}$, DPPH• and FRAP.[25]

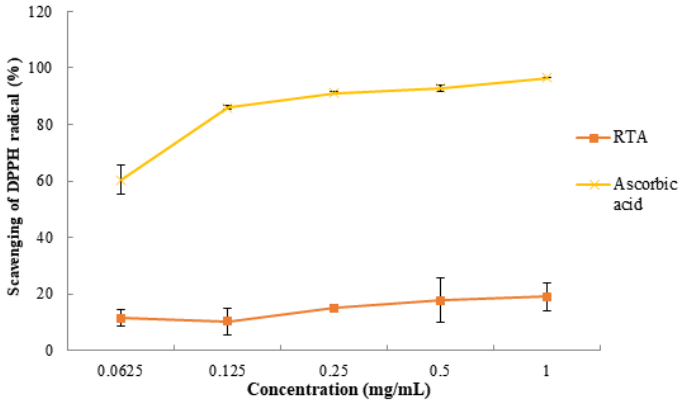


**Fig. 1-** Scavenging of DPPH radical by RTA and ascorbic acid as reference substance
All values were significant higher for ascorbic acid in the Mann-Whitney U test ($p < 0,05$).

These results could be of relevance for novel applications of RTA, considering the growing interest of antioxidants in animal nutrition as feed additives, especially in poultry farming due to their potential as agents with antimicrobial and antioxidant activity, contributing to food safety. In livestock, antioxidants are used to prevent certain diseases and improve animal production efficiency.[33]

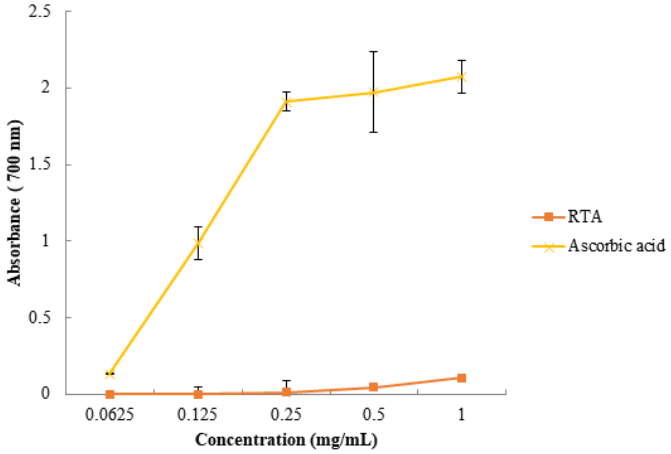


**Fig. 2-** Reducing power of RTA and ascorbic acid as reference substance
All values were significant higher for ascorbic acid in the Mann-Whitney U test ($p < 0,05$).

Concerning the potential role of spent mushroom substrate as a natural antibacterial agent, the present study revealed that RTA exhibited no significant effect on *Staphylococcus aureus* CCEBI 1070, *Pseudomonas aeruginosa* CCEBI 1071, and *Escherichia coli* CCEBI 1081. In contrast, a bacteriostatic activity (moderate antibacterial effect) was found against the strains of *Bacillus subtilis* CCEBI 1032 and *Bacillus cereus* CCEBI 1033 with inhibition zones of 7,21 ± 1,06 and 7,24 ± 0,35, respectively (Table 2 and Figure 3). In addition to their role in the antioxidant activity, the antibacterial properties of SMS could be related to the presence of phenolic compounds.

The extracts from the spent mushroom extracts of *Lentinula edodes* exhibited antimicrobial activity ranged from 30,4 % (*Vibrio fluvialis* RimA1TCBS) to up to 83 % (*Pseudomonas aeruginosa* C3GSPR1) of the positive control (gentamicin CN10). No inhibitory effect was showed against the majority of Gram-positive bacteria, namely *Staphylococcus aureus* (C511 and C612 strains) and *Enterococcus faecium* (C1 and C14 strains). Nonetheless, some extracts

**Table 2-** Antibacterial assay relative to inhibition zone diameter and percentage of inhibition

| Bacterial strains | Inhibition zones (mm) | | Inhibition percentage (%) | |
|---|---|---|---|---|
| | RTA | Streptomycin | RTA | Streptomycin |
| *Bacillus subtilis* CCEBI 1032 | 7,21 ± 1,06 | 12,02 ± 1,03* | 60,0 | 100 |
| *Bacillus cereus* CCEBI 1033 | 7,24 ± 0,35 | 13,12 ± 0,24* | 55,2 | 100 |

*The values were significant higher for streptomycin in the Mann-Whitney U test ($p < 0,05$)

inhibited the bacterial growth of *S. aureus* (ATCC 23235), achieving a reduction of 38,9 % compared to the antibiotic used (gentamicin). Regarding the studied Gram-negative isolates, the extracts alone showed antibacterial effect in all strains, except in *E. coli* (ATCC 25922).[25]

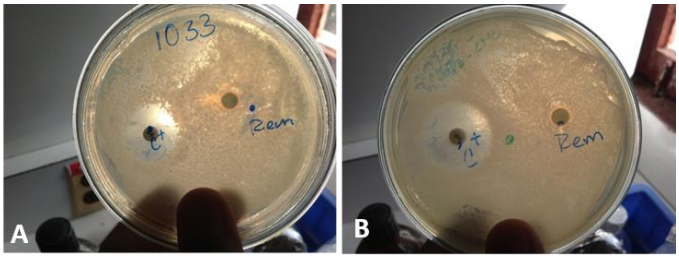


**Fig. 3**- Antimicrobial activity of the RTA against strains A: *Bacillus subtilis* CCCEBI 1032 and B: *Bacillus cereus* CCEBI 1033

The antimicrobial activity of a product depends on several factors such as the type of mushroom species, the concentrations of the extracts and the microorganisms evaluated. Some fungal peptides are capable of stimulating the microbial autolytic system. In this regard, it was informed the antimicrobial activity of an aqueous extract obtained at high temperatures from the mycelium of *Pleurotus* sp. against four bacterial strains: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25953, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus cereus* ATCC 11776.[34]

One of the main limitations of this preliminary study was the low number of bacterial strains tested. There is a need for a more in-depth exploration of the specific bioactive components present in SMS, elucidating their role in conferring antibacterial properties.

The obtained results are particularly important in the case of *Bacillus cereus*, which is often responsible for foodborne diseases and both local and systemic infections in humans and animals. Likewise, this bacterium causes multiple (but uncommon) cases of mastitis in dairy sheep and goats, and recently a *B. cereus* feed-related outbreak that caused the death of 6,234 pigs was reported in Italy.[35] Moreover, our findings hint at the possibility of using SMS as a prebiotic in animal diet; however, more research should be foreseen on this matter, and the repercussions on the gut microbiota, yet unexplored, necessitate a thorough investigation to discern any unforeseen effects.

## CONCLUSIONS

The obtained results suggest that the transformation of the coffee pulp by *Pleurotus ostreatus* into a spent substrate could be taken into consideration for developing bio-based products rich in phenolic compounds and with different biological activities, such as antioxidant and antimicrobial, attractive for potential applications in the food industry and human and animal health in the context of the "One Health" approach.

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## INTEREST CONFLICT

The authors express that there are no conflicts of interest in the submitted manuscript.

## AUTHOR'S CONTRIBUTION

Yaixa Beltrán Delgado: conceptualization, methodology, formal fnalysis, research, writing-draft preparation, writing-review and editing.
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