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Biotransformation of Rice Husk into Phenolic Extracts by Combined Solid Fermentation and Enzymatic Treatment †

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Abstract: Biotechnology is essential for developing profitable and productive techniques to obtain metabolites. Two technologies can be used: solid or liquid fermentation and enzymatic treatments. In this context, the objective of this work was to evaluate the use of rice husk, a lignocellulosic material, to obtain bioactive compounds by lignin oxidative transformation and demethoxylation, respectively, through enzymatic treatments of *P. chrysosporium* and *G. trabeum*. In the first step, solid fermentation was used to obtain the enzyme Lig. Peroxidase and methoxyl hydrolase were quantified as 80 UE and 50 UE, respectively. This enzyme concentrate was lyophilized and used to prepare an enzymatic consortium (240 UE LigP and 150 UE metH) applied in the second phase of enzymatic treatment. The overall process involved 20 days in the solid fermentation step and 2 h for the enzymatic treatment. The obtained products were characterized by having veratryl alcohol and veratryl aldehyde at contents of 70.4 ± 0.1 and 23.3 ± 0.3 mg/g, respectively. Moreover, the analyzed products did not show cytotoxicity but revealed antioxidant and bacteriostatic activities. No anti-inflammatory activity was detected. In the context of circular economy, the obtained results pointed out the use of combined solid fermentation and enzymatic treatment as a viable strategy to valorize rice husk. The applications of these bioactive compounds presenting bactericidal and bacteriostatic activity and not showing toxicity are very common in medicine, agriculture, and environmental health, among others, and can be incorporated both in free systems and immobilized in spheres, capsules or biopolymer films, which is an important input for obtaining functionalized materials that are in high demand today.

Keywords: solid fermentation; enzymatic treatment; bioactive phenolic compounds

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1. Introduction

Fungi represent a good substrate transformation tool, such as rice husk residues, which use cellulose, polyphenols, and hemicelluloses as nutrients for their metabolic growth and development. The substrates used are transformed to obtain compounds with properties due to the biological actions of some of their metabolites. Antioxidant, hypocholesterolemic, hypoglycemic, antibacterial, immunomodulatory, anticancer, and regulatory effects of the cardiovascular and antiviral system have been described [1], the

last three being the most relevant. These properties have been attributed to polysaccharides and triterpenoidal compounds, secondary metabolites of some fungi. Many of these compounds are currently being used to produce commercial medicines [2]. However, preliminary research on basidiomycetes, specifically on Shiitake, has shown that these compounds' proportions vary with the fungus's stage and the medium in which it is grown. These skills are presented for fungi such as *Lentinula edodes* (Shitake), *Pleurotus*, *Trametes*, and *Phanaerochaetes*.

It is known that both mycelium and depleted media, as well as different extracts exhibit antibacterial properties, with different effectiveness against Gram-positive compared to Gram-negative bacteria, as is the case for activity against pathogens such as *Bacillus megaterium*, *Streptococcus pyogenes*, and *Staphylococcus aureus*).

Not only intrametabolites present these actions since an exopolymer, a glycoprotein, isolated from Shiitake, when administered in a dose of 200 mg/kg body weight, reduces the plasma level of total cholesterol by 25.1%, while the triglyceride level drops by 44.5%.

Among the compounds that can be obtained are β-glucans, which are non-cellulosic polysaccharides consisting of glucose units linked by glycosidic bonds and with β-1-3 or β-1-6 branches. They are isolated mainly from the fungal cell wall but can also be excreted into the environment. They have immunostimulatory and anticancer activities, and anti-infective, hypocholesterolemic, hypoglycemic, anti-inflammatory, and analgesic properties.

Polyketides are a diverse family of natural products with various pharmacological activities and properties, including antibiotic, antifungal, cytostatic, hypocholesterolemic, antiparasitic, and animal growth and insecticide promotion. Within polyketides statins, polyketides are not aromatic, which was shown to inhibit cholesterol synthesis since they are inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoA), the first enzyme involved in cholesterol biosynthesis.

In addition to the constitutive compounds present in their growth, fungi have the property of transforming the polyphenols present in the substrate where they grow (lignocellulosic substrates), obtaining functional phenolic units from that catabolism.

These structures derived from complex polyphenols are a source of few natural phenolic aromatic structures. Polyphenols produced by fungi can be depolymerized and oxidized by acquiring new functional groups, such as aldehydes and acids, with various uses. Today, some fungi have very specific activities, such as hydrolyzing the methoxyl of thephenolic ring in position 3 and position 5, which allows for the ability to obtain a greater quantity of building block-type products to re-engineer products. Within the activities of these phenols, the germicidal activity of some fungi and bacteria can allow for the development of products such as polyurethanes with germicidal activity and phenolic extracts of fungicidal activity.

In the present work, phenolic extracts from rice husk production as a renewable substrate were investigated using a combined system of solid fermentation and enzymatic treatment that allows functional compounds (FC) to be obtained in a standardized way, with the aim of a future scale-up.

2. Materials and Methods

2.1. Rice husk Samples

The rice (*Oryza sativa*, Olimar and Gurí) husk was provided by the Uruguayan rice company COOPAR S.A., Lascano, Rocha Departament 15, 27300.

2.2. Strains Obtainment

Phanerochaete chrysosporium and *Gloeophyllum trabeum* strains were obtained from the TNA Laboratory in Biochemistry and Biotechnology of CIN, Faculty of Sciences of the University of the Republic, Uruguay. They were kept in potato dextrose agar (PDA) medium and incubated at 22 ± 2 °C for 5–8 days. For liquid fermentation, GPEL broth (glucose (20 g/L), extract of yeast (2.5 g/L), peptone (2.5 g/L)) adjusted to a pH of 4.5 with 0.1 N HCL and 0.1 N NaOH was used.

2.3. Strain Obtainment

2.3.1. Inoculation and Fermentation

For the pre-inoculum preparation, 200 mL of GPEL broth was poured into 500 mL flasks and then inoculated with 4 mm paper discs obtained from PDA boxes previously invaded with the prepared mycelium. The incubation occurred under orbital stirring (100 rpm) at 28 °C for ten days. Some pellets were taken and weighed from pure cultures that were not contaminated.

The preparation of the semisolid fermentation (FSS) was carried out using fermentation trays containing 5 kg of pasteurized rice husk with a moisture content of 50%, to which 0.5 L of fresh pre-inoculum broth was added, using the prepared pellets with fungal growth. The incubation conditions were set at a temperature between 20 and 30 °C. The fermentation time frame was 20 days.

Samples (10 g) were taken every three days and leached with sterile water. Three replications were made for each sample. The samples were filtered under vacuum to separate the spent medium from the mycelium, which was subsequently weighed and frozen at −18 °C for subsequent lyophilization. Samples were stored and refrigerated in sterile bags until further chemical analysis.

2.3.2. Production of Enzymes

One hundred and fifty milliliters of medium was prepared with 10 g/L of glucose, 0.2 g/L of yeast extract, 0.5 g/L of ammonium tartrate, and 1 mL of Kirk's salts, at pH 5. The medium was sterilized for 15 min at 121 °C and inoculated with 5 mL of growth medium with each fungus. Each culture was maintained at 37 °C for 6 days, and the supernatant was analyzed for enzyme activity.

2.3.3. Analysis of Enzymatic Activity

In the culture supernatant, enzymatic activity assays were performed. The samples were analyzed in triplicate. A Shimadzu spectrophotometer (model UV-1800) was used to determine the following: (a) Lig. Peroxidase: This activity was determined by the Tien and Kirk Method. [3]. A 0.01 M of veratryl alcohol was used as a substrate in sodium tartrate buffer (0.1 M, pH 3.0). The reaction was initiated by adding 4 mM H_2O_2 and monitored by measuring the increase in absorbance at 310 nm that corresponds to veratraldehyde; (b) Methoxyl Hydrolase: The measurement method was applied on a methoxylated model compound. The reaction was followed by spectrophotometric measurements and the determination of the change in the spectrum. Two milliliters of 3,4,5-trimethoxybenzaldehyde 0.1 M, pH 4, was taken and incubated with 0.1 mL of the growth fungi supernatant at 30 °C. The decrease in the substrate was measured by UV spectrophotometry.

2.3.4. Production of Functional Compounds from Lignin

The production of the FC units was carried out in a semi-solid fermentation system for 20 days in a controlled chamber. The rice husk (25 kg) was inoculated with the propagated fungi Phanerochaete chrysosporium and Gloeophyllum trabeum previously tested for enzyme production capacity. The semi-solid fermentation process was performed in an open vessel without stirring. inside a controlled oven (humidity: 60–70%; temperature: 20–30 °C; pH 5–6). The activity of the inoculated fungi was controlled by measuring the enzymatic activity in the material of semi-solid fermentation. For this, 2 g of inoculated rice husk was taken and suspended in 10 mL of water. After 1 h, the enzymatic activity was determined in the liquid: lignin peroxidase at an average of 80 EU/mL and methoxyl hydrolase at an average of 50 UE/mL. Twenty days after enzymatic action, a volume equal to that occupied by the solid material was decanted for 2 h and centrifuged, and the supernatant was analyzed by UV spectrophotometry, which was also used to determine the units of FC (veratryl alcohol and veratryl aldehyde). The samples were analyzed in triplicate. The equipment used was a spectrophotometer of the Shimadzu brand (model

UV-1800). The FC extracts were lyophilized in a Biobase BK-FD10S vacuum freeze dryer and stored at 4 °C.

2.3.5. Enzymatic Process

From the analyzed samples, it was possible to determine the enzymatic activity in the extracts of both Lig peroxidase and methoxyl hydrolase enzymes. The extracts were lyophilized, and an enzymatic consortium was formulated in an aqueous medium with 0.2 M citrate buffer, pH 4.5, containing the following enzymatic constitution: 240 UE/mg Lig peroxidase and 150 UE/mg methoxy hydrolase. This enzymatic solution was the medium in which the residual lignocellulosic of the FSS was added in a proportion of 100 g of the solid substrate (40% moisture) in 200 mL of medium. This procedure was performed in shaker shaking at 30 °C for 2 h. After the oxidative depolymerization and hydrolysis of the methoxy groups by the acting enzymes, the material was filtered, and the supernatant liquid was stored at −18 °C for subsequent characterization analysis.

2.4. Characterization of the Obtained Product

2.4.1. Phenolic Compound Profile

The lyophilized extract was redissolved in ethanol/water (80:20, *v/v*), to determine the phenolic compounds profiles by chromatographic analysis using a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA), following the protocol previously described by Bessada, Barreira, Barros, Ferreira, & Oliveira, (2016) [4]. Detection was carried out using a diode array detector (DAD) using the preferred wavelengths of 280 nm and 370 nm. Data acquisition was carried out using the Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA). The identification was performed with the available standard compounds and by using literature information regarding the fragmentation pattern. Quantification was performed using seven-level calibration curves obtained from commercial standard compounds. The results were expressed in mg per g of extract.

2.4.2. Antioxidant Activity

Two cell-based assays were performed to evaluate the in vitro antioxidant activity of the samples. Trolox (Sigma-Aldrich, St. Louis, MO, USA) was the positive control used in both assays. The thiobarbituric acid reactive substances (TBARS) formation inhibition capacity of the extract was evaluated using porcine brain cell homogenates following the in vitro assay previously described by [5]. The results were expressed as EC50 values (μg/mL), i.e., extract concentration providing 50% of antioxidant activity. The antihemolytic activity of the extracts was evaluated using sheep erythrocytes by OxHLIA assay, as previously described by [6]. The results were given as IC50 values (μg/mL) for the Δt of 60 and 120 min, i.e., extract concentration required to keep 50% of the erythrocyte population intact for 60 and 120 min.

The cytotoxic activity was evaluated using four human tumor cell lines, namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma). All cell lines were commercially acquired from DSMZ (Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) and routinely maintained with RPMI-1690 medium enriched with 10% fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 μg/mL). The cells were incubated under a humidified atmosphere at 37 °C and 5% CO_2, and were only used when they reached 80 to 90% confluence. A primary culture of non-tumor cells was also tested from a freshly harvested porcine liver (PLP2) and established according to the previously described [7]. The studied extracts were re-dissolved in water (8 mg/mL) and further diluted to obtain the range of concentrations to be tested (0.125–8 mg/mL). An aliquot (10 μL) of the extract's different concentrations was incubated for 48 h with the different tested cell lines (190 μL, 10,000 cells/mL). The cytotoxic potential was analyzed using the sulforhodamine B colorimetric assay and according to the procedure previously

described [8]. Ellipticine was used as a positive control, and the results were expressed as extract concentration that causes 50% inhibition of cell proliferation (GI50 values).

2.4.3. Anti-Inflammatory Activity

The evaluation of the anti-inflammatory activity was performed determining the capacity of the extracts to inhibit the lipopolysaccharide (LPS)-induced nitric oxide (NO) production in a murine macrophage cell line (RAW 264.7). The extracts were re-dissolved and further diluted following the previously described (Section 2.4.2.). The procedure was performed according to what was previously described [9]. Dexamethasone corticosteroid (50 mM) was used as positive control, and cells with and without LPS were considered negative controls. The obtained results were expressed in IC_{50} values (µg/mL), corresponding to the concentration of the extracts responsible for 50% of NO production inhibition.

2.4.4. Antibacterial Activity

The antimicrobial activity was evaluated using six Gram-negative bacteria: Escherichia coli, Escherichia coli ESBL, Klebsiella pneumoniae, Klebsiella pneumoniae ESBL, Morganella morganii, and Pseudomonas aeruginosa; and four Gram-positive bacteria: Enterococcus faecalis, Listeria monocytogenes, MRSA (Methicillin resistant Staphylococcus aureus), and MSSA (methicillin susceptible Staphylococcus aureus). The clinical isolates were obtained from patients hospitalized in various departments at the North-eastern local health unit (Bragança, Portugal) and Hospital Center of Trás-os-Montes and Alto Douro (Vila Real, Portugal). To maintain the exponential growth phase, all microorganisms were incubated at 37 °C in an appropriate fresh medium for 24 h before analysis. The extracts were redissolved in water (100 mg/mL) and further diluted to obtain a range of seven concentrations below the stock solution to determine the antibacterial activity. The minimal inhibitory concentration (MIC) determination was conducted using a colorimetric assay using p-iodonitrotetrazolium chloride according to a previously described procedure [10]. Two negative controls were prepared, one with Mueller-Hinton Broth (MHB). Two positive controls were prepared with MHB; each contained inoculum, but one contained the medium, antibiotic, and bacteria for Gram-negative bacteria. Ampicillin and imipenem were used as positive controls, while ampicillin and vancomycin were used for Gram-positive bacteria.

To determine the MBC (Minimum Bactericidal Concentration), 10 µL of liquid from each well that showed no change in color was plated on a solid medium, Blood agar (7% sheep blood), and incubated at 37 °C for 24 h. The lowest concentration that yielded no growth was the MBC. The MBC is defined as the lowest concentration required to kill bacteria.

2.5. Statistical Analysis

The experiments were carried out in triplicate, and the results were expressed as mean ± standard deviation. SPSS Statistics software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA: IBM Corp.) was used to assess significant differences ($p < 0.05$) among samples by applying a two-tailed paired Student's *t*-test at a 5% significance level.

3. Results and Discussion

3.1. Protein and Phenolic Compounds Yield

Protein concentrations of 1.2 mg/mL was obtained in the filtrates of enzyme production by mixed cultures of *G. trabeum* and *P. chrysosporium.* The enzymatic activities obtained are shown in Table 1. Enzymatic activity of lignin peroxidase and methoxyl hydrolase was determined, being higher for lignin peroxidase.

Table 1. Enzymatic activity obtained in the liquid system of *Gloeophyllum trabeum* and *Phanerochaete chrysosporium*.

Enzyme	*G. trabeum* (UE/mL)	*P. chrysosporium* (UE/mg)
Lignin peroxidase	80	50
Methoxyl hydrolase	50	31

Solid fermentation (SF) was carried out on 500 g of rice husk, with an enzymatic extract of 80 UE/mL, and 50 UE/mL with a specific activity of 50 y 30 UE/mg of proteins. Particularly, 50 g of rice husk in SSF was lixiviated with 200 mL of water, and 200 mL of aqueous extract equivalent to 6.5 g FC (determined after the lyophilization) was obtained. The performance of the process reveals that from 100 g of lignocellulosic material 56 g of material at the end of the transformation was obtained. The other 44 g were solubilized and depolymerized to obtain the phenols extract, 12% of the rice husk, and 85% of the rice husk lignin content (14%). The products of the rice husk treatment were lyophilized and characterized to identify and quantify the compounds present in the mixture (mg/g extract).

The biotransformation yields have been very interesting from the point of view of the % of the oligomers solubilized in lignin since 45% of oligomers and soluble monomers were obtained at pH 7. This fraction represents the modification obtained in the combined solid fermentation process followed by an enzymatic transformation that allows for shorter treatment times. In solid fermentation processes, only 40% can be obtained in 60 days, while the time could be shortened to 20 days in this new stage procedure.

3.2. Phenolic Compounds Profile

Two organic compounds were found in the studied samples of rice husk (Table 2), which were identified by comparing their retention time and maximum spectra with two available standard compounds, namely veratryl alcohol (peak 1) and veratryl aldehyde (peak 2), that have identified in rice husk samples [11–13].

Table 2. Retention time (Rt), wavelengths of maximum absorption in the visible region (λmax), tentative identification, and quantification (mg/g extract) of the organic compounds present in rice husk (mean ± SD).

Peak	Rt (min)	λmax (nm)	Identification	Quantification (mg/g Extract)
1	11.46	276	Veratryl alcohol	70.4 ± 0.1
2	21.77	278/sh309	Veratryl aldehyde	23.3 ± 0.3
-	-	-	Total	93.7 ± 0.2

Standard calibration curves: veratryl alcohol (y = 51,266x + 414,240, R^2 = 0.999) and veratryl aldehyde (y = 43,916x + 305,634, R^2 = 0.999).

3.3. Bioactive Properties

The antioxidant activity was measured in vitro using two cell-based bioassays, namely TBARS and OxHLIA, which assess the extracts' ability to inhibit the formation of thiobarbituric acid reactive substances (TBARS), such as malondialdehyde (MDA), which results from the degradation of lipid peroxidation products (TBARS assay), and to protect the erythrocyte membranes from oxidative hemolysis initially induced by 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH)-derived free radicals (OxHLIA assay), respectively. The results obtained with these assays are shown in Table 3, where the extract concentration required to provide 50% of antioxidant activity via TBARS formation inhibition or to keep 50% of the erythrocyte population intact for 60 and 120 min is presented. Thus, the lower the extract concentration (EC_{50} or IC_{50}), the greater its antioxidant activity. Furthermore, when measuring the antioxidant activity of natural extracts, some antioxidants may react more quickly and become depleted in the system, while others may offer prolonged antioxidant protection. The OxHLIA bioassay thus allowed for a distinction to be made between short-term (60 min Δt) and long-term (120 min Δt) antioxidant protection.

The rice husk extract yielded an EC_{50} value of 804 μg/mL in the TBARS bioassay, and IC_{50} values of 136 and 341 μg/mL in OxHLIA for Δt of 60 and 120 min, respectively (Table 3). These values are higher than Trolox's, but while this synthetic analog of α-tocopherol is a pure antioxidant, the rice husk extract is a complex mixture of various compounds, including non-active constituents. In general, it appeared that the extract performed better in the OxHLIA bioassay than in the TBARS, as the result was closer to the positive control.

Table 3. Antioxidant, anti-inflammatory, and cytotoxic activities of rice husk extract and positive control (mean ± SD).

		Rice Husk	Positive Control
Antioxidant activity			Trolox
TBARS inhibition (EC_{50}, μg/mL)		804 ± 39	5.8 ± 0.6
OxHLIA (IC_{50}, μg/mL)	60 min Δt	136 ± 5	19 ± 2
	120 min Δt	341 ± 17	41 ± 2
Anti-inflammatory activity			Dexamethasone
NO production inhibition (EC_{50}, μg/mL)		>400	16 ± 1
Cytotoxicity (GI_{50}, μg/mL)			Ellipticine
MCF-7 (breast carcinoma)		310 ± 6	0.91 ± 0.04
NCI-H460 (non-small cell lung carcinoma)		>400	1.03 ± 0.09
HeLa (cervical carcinoma)		>400	1.91 ± 0.06
HepG2 (hepatocellular carcinoma)		239 ± 3	1.1 ± 0.2
PLP2 (non-tumor porcine liver primary cells)		>400	3.2 ± 0.7
Anti-inflammatory activity (IC_{50} values, μg/mL)			
RAW 264.7 (murine macrophage)		>400	16 ± 1

Statistical differences ($p < 0.05$) between extract and positive control were found using a two-tailed paired Student's *t*-test.

The antiproliferative capacity of rice husk extract was tested against several cell lines. The obtained results are presented in Table 3 as the extract concentration required to inhibit the cell proliferation in 50% (GI_{50} values). The selection of the tested tumor cell lines was based on the higher incidence associated with those types of cancer. The studied extract only demonstrates the capacity to inhibit the proliferation of MCF-7 and HepG2 cell lines. The highest susceptibility was observed for the HepG2 cell line, exhibiting the lowest GI_{50} values (GI_{50} = 293 μg/mL). No activity was observed for the remaining tumor cell lines tested (NCI-H460, HeLa) as they showed GI_{50} values higher than the highest concentration tested (GI_{50} > 400 μg/mL). No cytotoxicity was observed regarding the primary culture of non-tumor cells (GI_{50} > 400 μg/mL), highlighting the security associated with the studied agro-waste material.

Similar results were described by Gao and co-workers [11], having demonstrated the antiproliferative potential of rice musk for the HepG2 cell line. The cytotoxic potential of this agro-waste material is scarcely studied. Most of those studies evaluated the cytotoxic properties of peptides obtained from rice husk. The highest cytotoxic power was described for samples with the highest hydrolyzed protein content. The authors considered that the presence of glutamic acid and proline are the main ones responsible for the reported activity [12]. Further studies are needed to understand the compounds responsible for the demonstrated potential and the mechanisms involved.

The anti-inflammatory potential of rice husk extract was assessed through the NO inhibition production by the LPS-stimulated murine macrophage cells (RAW 246.7). The extract concentration with the capacity to inhibit in 50% de NO production (IC_{50} values) is exhibited in Table 3. No activity was observed with the cell-based assay used at the tested concentrations (between 400 and 6.25 μg/mL). The extract does not exhibit a capacity to inhibit the NO production with GI_{50} values >400 μg/mL.

The phenolic compounds identified in rice samples, namely ferulic acid and quercetin, are described as potent anti-inflammatories [13]. Also, studies with rice bran describe their

components as having anti-inflammatory potential [14]. Although the activity has not been demonstrated, its study is very scarce, and it would be interesting to use assays that evaluate other mechanisms involved in the inflammatory process.

The antioxidant activity of rice husk subjected to solid-state fermentation with different strains of *Pleurotus sapidus* was previously evaluated by Pinela et al. (2020) [15] using the same bioassays. With OxHLIA, the authors reached higher IC_{50} values (179 and 259 μg/mL for 60 min Δt when using monokaryotic and dikaryotic strains, respectively) than in the present work. Curiously, the residual or spent mushroom substrate obtained after the fructification of the dikaryotic strain had a lower IC_{50} value (83 μg/mL). Still, the antihemolytic activity of unfermented rice husk (IC_{50} of 42.8 μg/mL for 60 min Δt) was higher than that of fermented samples. For TBARS, both unfermented and monokaryotic strain-fermented samples presented the same result (~155 μg/mL), which are much higher what was observed in the present work (Table 1) in the spent mushroom substrate (317 μg/mL). In general, the authors concluded that the fermentation process decreased the antioxidant activity, and the content of phenolic compounds (mainly phenolic acids).

Regarding the results obtained for antibacterial activity (Table 4), the extract of rice husk presented the capacity to inhibit the growth of all tested bacteria in a range of 20 to 5 mg/mL. Methicillin-resistant *Staphylococcus aureus* was the microorganisms with better antimicrobial results in a MIC of 5 mg/mL. These results were expected because *Staphylococcus aureus* is a Gram-positive bacteria and was more sensitive than Gram-negative bacteria due to differences in the chemical and physical properties of the cell wall. Moreover, the presence of sugar parts of lignin can interact with the peptidoglycan of the cell membrane of bacteria. The results obtained are in accordance with Tran et al., 2021 [16], who focused their study on the sustainably rice-husk-extracted lignin, nano-lignin (n-Lignin), lignin-capped silver nanoparticles (LCSNs), n-Lignin-capped silver nanoparticles (n-LCSNs), and lignin-capped silica-silver nanoparticles (LCSSNs), using them for antibacterial activities. The results showed that the antimicrobial activity of all compounds was better against Gram-positive bacteria, *S. aureus*, than against Gram-negative bacteria, *E. coli*.

Table 4. Antibacterial activity (MIC and MBC values mg/mL) of rice husk (mean ± SD).

	Rice Husk		**Ampicillin**		**Imipenem**		**Vancomycin**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria								
Escherichia coli	10	>20	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
Klebsiella pneumoniae	10	>20	10	20	<0.0078	<0.0078	n.t.	n.t.
Morganella morganii	10	>20	20	>20	<0.0078	<0.0078	n.t.	n.t.
Proteus mirabilis	20	>20	<015	<0.15	<0.0078	<0.0078	n.t.	n.t.
Pseudomonas aeruginosa	10	>20	>20	>20	0.5	1	n.t.	n.t.
Gram-positive bacteria								
Enterococcus faecalis	10	>20	<0.15	<0.15	n.t.	n.t.	<0.0078	<0.0078
Listeria monocytogenes	10	>20	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
MRSA	5	>20	<0.15	<0.15	n.t.	n.t.	0.25	0.5

MIC and MBC correspond to the minimal sample concentration inhibiting the bacterial growth or killing the original inoculum. n.t.—Not tested.

The tested sample presented a bacteriostatic effect but not bactericidal.

4. Conclusions

From the tests carried out, it can be concluded that using an FSS process for rice residues can be an important management and recovery solution for rice husk that can provide circularity to the processes, obtaining products of great added value for bioremediation. On the other hand, the extraction of the enzymes produced by the microorganisms allows for their use in a second liquid process where compounds such as veratraldehyde and veratrylic alcohol are obtained with bacteriostatic properties with an interesting activ-

ity for Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Morganella morganii*, and Gram-positive bacteria, such as *Enterococcus faecalis* and *Listeria monocytogenes*. The use of veratraldehyde alcohol and veratrylic aldehyde in the obtained extracts marks an interesting application for materials to be functionalized with germicidal properties, such as food containers and clothing and sanitary accessories. In future works, the encapsulation of the extracts for their controlled release and incorporation into different matrices for different applications will be studied.

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