Natural Resources for Human Health

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Original Research

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Received 17 April 2024
Revised 06 May 2024
Accepted 21 May 2024
Available online 07 June 2024

Edited by Kannan RR Rengasamy

KEYWORDS:
Antimicrobial activity
Antimutagenic
DPPH radicals
Nutritional characterization
Secondary metabolites

Natr Resour Human Health 2024; 4 (3): 257-268
https://doi.org/10.53365/nrfhh/189170
eISSN: 2583-1194

Oudemansiella cubensis an Edible Mushroom from the Neotropics with Biological and Nutritional Benefits

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ABSTRACT: In this work, we evaluated the antioxidant, toxicological, mutagenic, antigenotoxic and nutritional properties of *Oudemansiella cubensis*, which is a mushroom found in neotropical regions worldwide and native to Paraguay. Nutritional content analyses revealed that *O. cubensis* is a rich source of protein, dietary fiber, and fats. Antimicrobial analyses showed antimicrobial activity against *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Additionally, the species resulted to be nontoxic for human consumption with an LD_{50} value of 37.1 $mg.mL^{-1}$. The ethanolic extract of *O. cubensis* showcased an important antimutagenic activity at a concentration of 20 $mg.mL^{-1}$, which promotes the prevention of genotoxic damage. Regarding its chemical profile, Gas Chromatography – Mass Spectrometry confirmed the presence of compounds such as l-(+)-ascorbic acid 2,6-dihexadecanoate, octacosanol and cyclo(l-leucyl-l-prolyl), which stood out for antioxidant and antimicrobial properties. This study provided further evidence that *Oudemansiella cubensis* is a valuable species because of its potential for biotechnological applications.

1. INTRODUCTION

Mushrooms have been recognized as valuable sources of food and medicine throughout history. However, only recently, they have garnered significant attention due to their abundant biologically active substances and nutritional properties. Currently they are evaluated for their nutritional value and pharmacological properties (Wasser, 2010).

Oudemansiella Speg. (Physalacriaceae, Agaricomycetes, Basidiomycota) has been circumscribed to species with dry to generally viscid pileus, that usually include scattered floccules, white to off-white subdistant lamellae with a central stipe, a lack of veil remnants, and a rudimentary or fugacious veil (Alberti et al., 2021). The genus is widely distributed throughout tropical and temperate regions, and its basidiomata grow on rotting wood Petersen et al. (2010). The number of species within *Oudemansiella* have been reported to range from 25 (Dulay, 2023) to 33 (indexfungorum.org). Seven species have been documented for South America, with *Oudemansiella canarii* (Jungh.) Höhn. and *Oudemansiella cubensis* (Berk. and M.A. Curtis) R.H. Petersen as the most conspicuous and common species in the neotropics (Albertí et al., 2020; Veloso et al., 2021).

Higher Basidiomycete mushrooms contain bioactive compounds in fruiting bodies and mycelia. Several studies already showcased that many of these mushrooms have medicinal properties and activities such as antimicrobial, antiviral, antioxidant, anticancer, immune system enhancer, radical scavenger, anti-parasitic and anti-inflammatory (Wasser, 2017; Zeb & Lee, 2021). However, only a handful of species have been subjected to bioprospecting endeavours aimed at characterizing their chemical and biological profiles or facilitating domestication for intensive cultivation. Notably, *Oudemansiella* species produce oudemansins and strobilurin, which are compounds recognized for their antifungal activity and that thwart competition from other fungi (Anke, 1997;

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Niego et al., 2021). Additional notable metabolites include oudenone, utilized to treat hypertension (Hamao et al., 1974; Tsantrizos & Zhou, 1995), mucidin, an antifungal antibiotic (Nerud et al., 1982; Šubík et al., 1974), bioactive molecules such as lectin (Liu et al., 2013; Matsumoto et al., 2001), and polysaccharides (Wang et al., 2018). In terms of their potential use in biomedical applications, *Oudemansiella* species exhibit potent antimicrobial activity against pathogenic yeasts such as *Candida albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *Cladosporium sphaerospermum* (Rosa et al., 2003). Moreover, several studies have reported moderate anticancer and antioxidant properties (Acharya et al., 2019; Rosa et al., 2003; Veloso et al., 2021).

There is a growing interest about the nutritional and pharmacological properties of wild mushrooms for functional food and alternative drug development worldwide (Dulay, 2023). Some *Oudemansiella* species collected from the wild are known to be edible and a valuable part of regional diets (Magingo et al., 2004). For example, nutritional characterizations have shown that *O. canarii* and *O. cubensis* are excellent sources of nutrients, rich in carbohydrates, proteins, fiber, vitamins, and minerals (Alberti et al., 2021; Dulay, 2023). However, there is scarce information available on the nutritional values of other similar species. Furthermore, there are limited reports regarding safe consumption and their effects on human health.

Anticipating the growing importance of the genus *Oudemansiella* in biotechnological applications, the objective of this work was to evaluate the biological, chemical, and nutritional properties of the neotropical species *Oudemansiella cubensis*. In particular, we focused on evaluating its antioxidant, toxicological, mutagenic, genotoxic, and antigenotoxic properties for its potential role in natural therapeutic or pharmaceutical uses.

2. MATERIALS AND METHODS

2.1. Collection, cultivation, and sample preparation

The wild sample (Figure 1 a) was collected in the Central Department, San Lorenzo city growing on decaying wood and an herbarium reference specimen is kept at FACEN Herbarium, Laboratorio de Recursos Vegetales, Área Micología, Universidad Nacional de Asuncion N°3775 (MC 124). Subsequently, the strain was isolated from the wild fruiting body and codified as FC23 (Figure 1 c). Mycelia were produced from the strain FC23 following Campi, Mancuello, Ferreira, Maubet, et al. (2023) (Figure 1 d). Additionally, this strain was later used to cultivate fruiting bodies using a sawdust and corn cob substrate per Veloso et al. (2021) (Figure 1 b).

Wild and cultivated fruiting body samples and the mycelium samples were lyophilized (AE-LGJ-12 Freezer Dryer). Fungal material was macerated for two hours with a ultrasonic bath (Digital ultrasonic cleaner PS-50A), then filtered with Whatman filter paper, and the extracts were prepared according to Campi, Mancuello, Maubet, et al. (2023) while modifying the solvents as ethanol (96%) and ethyl acetate. The extracts were separated from the extraction solvents at 50°C under reduced pressure (RC Ingennery-RE 200A), and any residual water was removed by lyophilization. The resulting samples are later referred to as the ethanolic extract and ethyl acetate extracts, respectively. Genotoxicity and antigenotoxicity studies were performed with ethanolic extracts; antioxidant, total phenolic compounds, antioxidant activity, gas Chromatography-Mass Spectrometry (GC-MS) chemical profile were performed with ethyl acetate extract and antimicrobial activity assay was performed with both extracts.

2.2. Total phenolic compounds

Total phenolic compounds (TPC) were determined by UV-VIS spectrophotometry (760 nm) using Folin-Ciocalteu reagent and gallic acid per Campi, Mancuello, Ferreira, Maubet, et al. (2023). The TPC concentration was quantified as $mg.g^{-1}$ gallic acid equivalents (GAE).

2.3. Antioxidant activity and concentration by the radical scavenging activity assay.

Antioxidant concentrations and activity were determined per Campi et al. (2021) using UV-VIS spectrophotometry (517 nm) and an ascorbic acid standard. The concentration was calculated as:

$$A = (\lambda_{DPPH} - \lambda_{Sol}/\lambda_{DPPH}) \times 100$$

where λ_{DPPH} and λ_{Sol} are the absorbance measurements of DPPH solution and DPPH with extract, respectively.

2.4. Gas Chromatography-Mass Spectrometry (GC-MS) chemical profile

Samples for GC-MS were prepared by dissolving wild, cultivated, and mycelium extracts in acetone at an approximate concentration of 1 $mg.mL^{-1}$. The GC-MS analysis was performed using the Shimadzu GC2010 Plus Gas Chromatograph equipped with a QP2010SE Electron Impact Mass Detector and Supelco SLB-5ms capillary column (I.D. 30 m x 0.25 mm, 0.25 μm). High purity helium (5.0 grade) was the carrier gas operating at a flow rate of 0.87 mL/min and an injection volume of 1.0 μL. The machine settings were set to a 270°C injector temperature, an injection mode of "splitless", a ratio of 10:1, and a 250°C ion-source temperature. The oven temperature was set to a 2-min, 60°C isothermal with an increase of 6°C/min to 280°C and ending with a 20-min isothermal. The mass detector was programmed in full-scan mode 55-550 m/z at 70 eV. A triplicate analysis was performed. Compounds were identified from NIST database library of the GC-MS instrument. All compounds identified with homology above 90% were considered positive and reported in the results.

2.5. Antimicrobial activity assay

Antimicrobial activity was determined by Broth Micro-Dilution Assay following Campi, Mancuello, Ferreira, Ferreira, et al. (2023) and using certificate bacterial and fungal strains *Escherichia coli* WDCM 00012, *Salmonella enterica* WDCM 00031, *Pseudomonas aeruginosa* WDCM 00026, *Klebsiella*

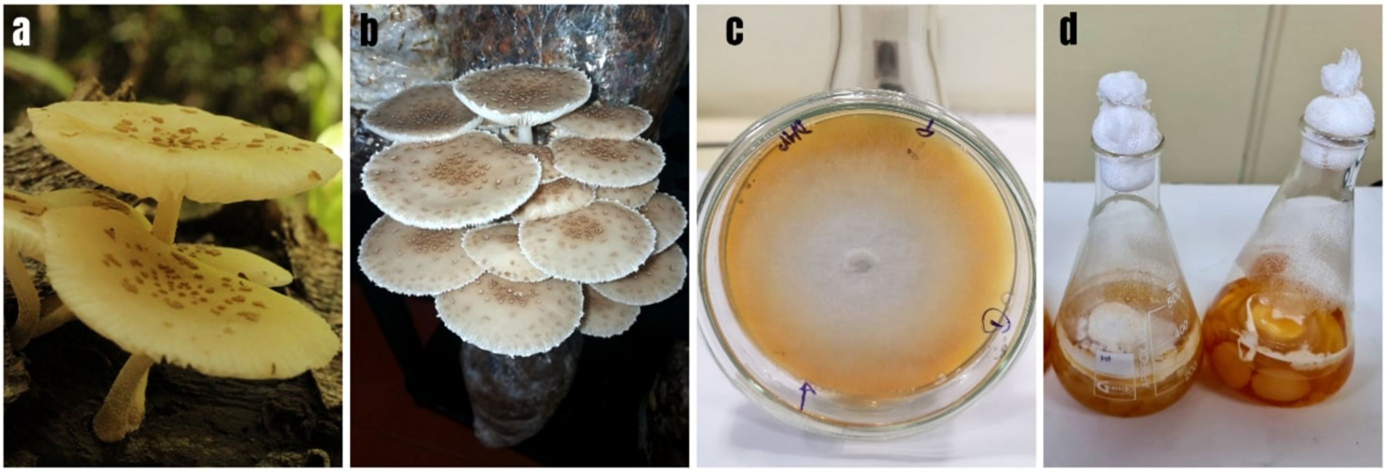

Figure 1. a.Wild fruiting body of *Oudemansiella cubensis* b. Cultivated fruiting body on sawdust/corn substrate c. Iso latedstrain FC23 d. Mycelium in liquid medium

pneumoniae ATCCBAA 1705, *Enterococcus faecalis* WDCM 00087, *Staphylococcus aureus* ATCC 6538, *S. epidermidis* WDCM 00036, and *Candida albicans* WDCM 00054. The positive control was Meropenem (50 mg.mL^{-1}) for gram negative, Ciprofloxacin (25 mg.mL^{-1}) for gram positive, and Nistatin (20 mg.mL^{-1}) for yeast.

2.6. Nutritional composition

The proximal values of the dry matter content was determined from cultivated fruiting body samples. Crude fat content, moisture, ash, protein, and dietary fiber were determined according to guidelines from the Association of Official Analytical Chemists (AOAC, 2000). Crude fat content was determined using hexane for solvent extraction. The crude protein content was calculated using a 4.38 conversion factor per Chang and Miles (2004). The Anthrone method was applied to determine total carbohydrate content with modifications per Campi, Mancuello, Ferreira, Maubet, et al. (2023), which uses glucose anhydrous as the standard. The energetic value was calculated following Mercosur Technical Regulations (Mercosur/Gmc/Res, 2003).

2.7. Toxicity testing using median lethal dose (LD50)

Five concentrations (10, 40, 70, 90, and 120 mg.mL^{-1}) of the ethanolic wild fruiting body extract were evaluated for toxicity testing. Solutions of 5 mL for each concentration were prepared and utilized to rehydrate 1.5 g of dried sterile mashed potatoes. These samples were administered to 100 third instar *Drosophila melanogaster* Canton S[+] larvae for 72 hours. The median lethal dose (LD50) was estimated with Probit analysis based on mortality percentage and the concentration curve transformed to a logarithmic scale (Finney, 1952).

2.8. Genotoxicity and antigenotoxicity study determined by the Somatic Mutation and Recombination Test (SMART)

Trans-heterozygous (mwh+/+flr^3) *Drosophilla melanogaster* larvae were produced by crossing 400 virgin females of the flare mutation (flr^3 /In(3LR) TM3, ripp sep I(3)89Aa bx34e and BdS) with 200 males of the multiple wing hairs (mwh/mwh) mutation (30). Ethanolic extract samples of wild fruiting body at concentrations of 30, 20, 10 mg.mL^{-1} were evaluated for genotoxicity by treating 100 third instar trans-heterozygous larvae (mwh+/+flr^3) for 72 hours until eclosion, using distilled water as control and 2.61 mg.mL^{-1} cyclophosphamide as mutagen (Graf et al., 1984).

Antigenotoxicity was assessed for the highest concentration of extract with the lowest amount of mutations obtained in a previous genotoxicity evaluation (20 mg.mL^{-}1). Four different treatments were employed: (i) simple treatment, which involved an oral exposure of third instar trans-heterozygous larvae to extract (20 mg.mL^{-1}), (ii) combined treatment, where third instar trans-heterozygous larvae were orally exposed to extract (20 mg.mL^{-1}) and cyclophosphamide (2.61 mg.mL^{-1}) simultaneously for 72 hours, (iii) chronic post-treatment with extract, where second instar trans-heterozygous larvae were orally administered cyclophosphamide (2.61 mg.mL^{-1}) for one hour followed by extract (20 mg.mL^{-1}) for 72 hours, and (iv) chronic pre-treatment with the ethanolic extract, which consisted of orally exposing first instar trans-heterozygous larvae to extract (20 mg.mL^{-1}) for 72 hours followed by a 48 hours treatment with cyclophosphamide (2.61 mg.mL^{-1}) (Graf et al., 1984) (Figure 2).

Once the treatments were complete, we observed the wings of treated adults with a microscope and identified the number (simple single spot, large single spot, twin spots) and type (mwh or flr3) of mutant cells. The percent inhibition of mutagen activity was calculated using the following equation:

% I = ((TM mutagen – (TM extract + TM mutagen)) / (TM mutagen)) x 100 where TM = total number of mutations.

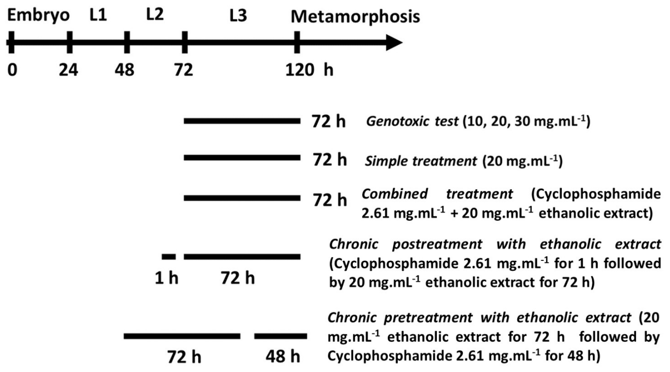

Figure 2. Summary of genotoxic and antigenotoxic treatments applied to *Drosophila melanogaster* in this study.

2.9. Statistical analyses

Analysis of Variance (ANOVA) with a 95% confidence interval and Tukey's test were used to compare the variability and statistical significance between the different fractions of the phenolic and antioxidant compounds, antioxidant and antimicrobial activities analyses. The results were expressed as the mean of triplicate measurements ± Standard Deviation (SD). These analyses were performed using the statistical program Past version 4.03b (Hammer et al., 2001).

The statistical analysis for the genotoxicity and antigenotoxicity tests were performed based on the frequencies of spots found per wing were compared with control according to the statistical table proposed by Frei and Würgler (1988), which corresponds to a conditional binomial statistical model (Kastenbaum-Bowman test) with significance levels α=β=0.05 (Kastenbaum & Bowman, 1970).

3. RESULTS AND DISCUSSION

3.1. Phenolic compounds, antioxidant concentration, and antioxidant activity

The difference of phenolic compound concentrations of *O. cubensis* FC23 showed Stadistical significance when comparing the ethyl acetate extract from different sources (wild and cultivated fruiting body and mycelia). The cultivated fruiting body extract (36.47 ± 0.51 mg.g^{-1} GAE) had the highest concentration, followed by the mycelium extract (19.41 ± 0mg.g^{-1} GAE) and wild fruiting body extract (10.98 ± 0.32mg.g^{-1} GAE) (Table 1). The phenolic compound value for wild fruiting bodies found in our work are consistent to those found in literature for *O. cubensis* from ethanolic extracts (9.98 mg.g^{-1}GAE) (Veloso et al., 2021). However, the cultivated fruiting bodies was three times higher than the previously obtained value of 10.38 mg.g^{-1}GAE (Veloso et al., 2021). Additionally, comparing the methanolic extracts for the mycelia and cultivated fruiting bodies, our values were lower than the 2.38 and 3.88 mg g^{-1} GAE values reported in literature (Pérez-Chávez et al., 2017).

Previous studies have shown that other species related to *O. cubensis*, such as *O. canarii* and *O. platensis*, resulted in approximate values in the range of 5 mg.g^{-1}GAEfor methanolic extract of wild fruiting bodies (Acharya et al., 2019; Pérez-Chávez et al., 2017), which is twice as low as those obtained for *O. cubensis* in this work. For the mycelia extract, other work has reported phenolic compound values of 12.86 mg.g^{-1} GAE for *O. canarii* aqueous extracts (Contato et al., 2020), which is also lower than observed in this work.

The mean antioxidant compound results ranged between 7.85 to 18.30 mg.g^{-1} AAE with mean antioxidant activity values ranging from 3.22% to 9.14% (Table 1). The cultivated fruiting body extracts had the highest values, again followed by mycelia and wild fruiting body extracts, respectively, which was consistent with the trend exhibited by phenolic compound values.

Other studies have reported antioxidant compound values of 15.4 mg.g^{-1} AAE for the ethanolic extracts of wild fruiting bodies of *O. cubensis*, which is almost twice as much as our results; however, the antioxidant activity (3%) was similar (Veloso et al., 2021). For cultivated fruiting bodies, our antioxidant concentration results were similar to those previously reported for the antioxidants compounds (16 mg.g^{-1} AAE) but for antioxidant activity, our results were over three times higher than the value (3%) reported in literature (Veloso et al., 2021). These results suggest that the antioxidant activity could be correlated to phenolic compounds concentration.

For related species, such as *Oudemansiella platyphyla* and *Xerula radicata*, studies have documented antioxidant activity values of 17% and 5%, respectively (Macáková, 2011). Compared to prior literature, our results suggest that wild and cultivated fruiting bodies and mycelium of *Oudemansiella cubensis* have slightly higher antioxidant capacity and phenolic content compared to other *Oudemansiella* species. Nevertheless, the differences in bioactive compound concentrations could be influenced by several factors, such as the extraction procedure, the choice of solvents, the substrate for growing mushrooms, and the liquid culture of the mycelium (Campi, Mancuello, Ferreira, Maubet, et al., 2023).

3.2. GC-MS chemical profile

A summary of GC-MS results is shown in Table 2, which includes a list of the identified compounds with their name, molecular weight, retention time, and abundance. GC-MS chromatograms are shown in the Supplementary Materials (Figure S1, S2 and S3 inAppendix A). Various compounds were identified in the ethyl acetate fraction of all wild, cultivated, and mycelia samples. Ethyl acetate fractions presented the best biological activity. Our analysis revealed the presence of fatty acids (saturated, monounsaturated, and polyunsaturated), fatty acid esters, sterols, waxes, phenolic compounds, and vitamins.

For the wild fruiting body, 14 compounds were identified (Table 2). One notable compound with biological activity is l-(+)-ascorbic acid 2,6-dihexadecanoate, which is a fat-

Table 1
Phenolic compounds, antioxidant concentration, and antioxidant activity values from extracts of *Oudemansiella cubensis* FC23.

Ethyl acetate extract source	Phenolic compounds (mg.g^{-1} GAE)	Antioxidants compounds (mg.g^{-1} AAE)	Antioxidant activity (%)
Wild fruiting bodies	10.98 ± 0.32^{a}	7.85 ± 0.32^{a}	3.22 ± 0.19^{a}
Cultivated fruiting bodies	36.47 ± 0.51^{b}	18.30 ± 0.48^{b}	9.14 ± 0.29^{b}
Mycelia	19.41 ± 0.00^{c}	12.17 ± 0.73^{c}	5.78 ± 0.54^{c}

GAE: Gallic Acid Equivalents. AAE: Ascorbic Acid Equivalent. Different letter superscripts indicate statistical significance ($p < 0.05$).

Table 2
Identified compounds in the wild and cultivated fruiting body and mycelium from the ethyl acetate fraction of *O. cubensis* FC23.

Name (molecular weight)	Fraction	Retention time (min)	Abundance (%)
Succinic anhydride (100)	Wild	10.164	2.13
3-hydroxy-4,4-dimethyloxolan-2-one (130)	Mycelia	10.762	1.27
4-Tetradecene (196)	Wild	23.356	2.42
1-Pentadecene (210)	Wild	23.496	19.65
7-Octadecene (252)	Wild	27.120	2.05
1-Nonadecene (266)	Wild	27.234	12.79
Isopropyl Myristate (270)	Wild	27.748	1.01
Pentadecanoic acid, ethyl ester (270)	Mycelia	28.863	1.41
Hexadecanoic acid, methyl ester (270)	Wild	29.496	3.29
	Mycelia	29.409	2.58
	Cultivated	28.987	11.36
cyclo(l-leucyl-l-prolyl) (210)	Mycelia	29.954	1.35
	Cultivated	29.494	26.22
l-(+)-Ascorbic acid 2,6-dihexadecanoate (652)	Wild	30.235	1.72
	Cultivated	29.735	11.32
n-Hexadecanoic acid (256)	Mycelia	30.289	14.01
Hexadecanoic acid, ethyl ester (284)	Mycelia	30.538	8.75
7,10-Octadecadienoic acid, methyl ester (294)	Cultivated	31.669	20.28
9,12-Octadecadienoic acid, methyl ester (294)	Mycelia	32.102	1.59
n-Nonadecanol-1(284)	Wild	32.105	2.45
Methyl 16-methyl-heptadecanoate (298)	Cultivated	32.150	9.43
11-Octadecenoic acid, methyl ester (296)	Mycelia	32.201	1.30
9-Octadecenoic acid-, methyl ester (296)	Wild	32.294	1.67
Octadecanoic acid, methyl ester (298)	Wild	32.682	1.31
	Mycelia	32.592	1.06
9,12-Octadecadienoic acid (280)	Wild	32.913	1.32
	Mycelia	32.962	26.1
9,12-Octadecadienoic acid, ethyl ester (308)	Wild	33.205	1.32
Oleic Acid (282)	Mycelia	33.305	1.32
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)- (244)	Cultivated	36.267	7.06
Hexanedioic acid, bis(2-ethylhexyl) ester (370)	Mycelia	36.369	1.71
1-Octacosanol (410)	Wild	36.506	2.85
Docosanoic acid, ethyl ester (368)	Mycelia	41.462	1.30
Squalene (410)	Mycelia	41.720	1.34

soluble vitamin C. The analogues of ascorbic acid formed by esterification and transesterification with fatty acids enhance the antioxidant, anti-inflammatory and antitumor properties of ascorbic acid (Begum et al., 2017; Mohamed et al., 2010). Another identified compound worth mentioning is octacosanol, a common natural aliphatic alcohol (Taylor et al., 2003) that exhibits various biological effects, including anti-fatigue, anti-hypoxia, antioxidant, anti-inflammatory, antitumor, and antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Sengupta et al., 2018; Y. Zhou et al., 2022).

In the cultivated fruiting body, the GC-MS assay identified 6 compounds (Table 2). One of these compounds is pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), also known as cyclo(l-leucyl-l-prolyl), which is noteworthy for its biological properties. This compound was isolated from *Fusarium* sp. and showed inhibitory activity against gram-negative and gram-positive bacteria (Putra & Karim, 2020). The antioxidant capacity of cyclo(l-leucyl-l-prolyl) isolated from mushroom *Sarcodon aspratus* (Berk.) S. Ito has also been reported (Kim et al., 2005). Another compound identified is 7,10-octadecadienoic acid methyl ester, which has been evaluated for cumulative dose-dependent relaxation responses to precontracted penile corpus smooth muscle (PCCSM). This compound was isolated from *Calvatia nipponica* Kawam., which is known as a natural aphrodisiac (Lee et al., 2020). Additionally, L-(+)-ascorbic acid 2,6-dihexadecanoate was also reported in this fraction.

Finally, 14 compounds were identified in the mycelia extract (Table 2). The most abundant compounds identified were fatty acids and their derivatives, some of these compounds have been reported with antimicrobial activities in nature (Zheng et al., 2005). Linoleic acid (9,12-Octadecadienoic acid, palmitic acid (n-hexadecanoic acid), palmitic acid ethyl ester (hexadecanoic acid, ethyl ester) and ethyl tridecanoate were identified. The linoleic acid (9,12-Octadecadienoic acid), also known as omega-6, has been reported to inhibit some gram-positive bacteria (Dilika et al., 2000).

3.3. Antimicrobial activity

Studies have previously shown that *Oudemansiella* species exhibit antimicrobial activity (Anke et al., 1983; Kuhnt et al., 1990; Rosa et al., 2005). Our results were consistent with results from literature and showed that the ethyl acetate extract generally resulted in better inhibitory activity than the ethanolic extract for the tested microorganisms in Figure 3. This could be due to two possible factors. First, the ability of ethanol to extract polar and non-polar compounds resulting in a complex mixture of metabolites that can exert an antagonistic effect on each other and thus suppress efficient antimicrobial activity (Schmid et al., 1999). Secondly, there is a higher concentration of bioactive metabolites in medium polarity solvents, such as ethyl acetate. Regarding the nature of the wild, cultivated, and mycelia extracts, we have not found a correlation between extract source and antimicrobial capacity. We will further discuss the extracts that showed inhibitory activity (above 50%).

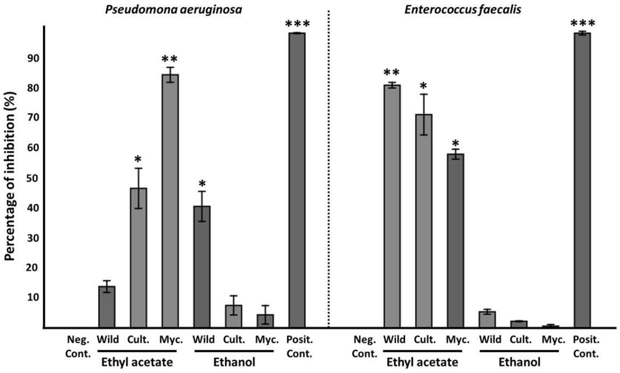

Figure 3. Comparison of antimicrobial activity of ethanol and ethyl acetate extract in wild, cultivated and mycelia samples of *Oudemansiella cubensis* with notable inhibition of microorganisms. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to negative control

The results from microdilution assay are shown in Table 3. *Oudemansiella cubensis* exhibited inhibitory activity against *Pseudomona aeruginosa* 84% (mycelia) and *Enterococcus faecalis* 71% (cultivated fruiting body) and 58% (mycelia). However, in studies for similar species, *Oudemansiella canarii* did not show activity against the same microorganisms for the mycelia ethyl acetate extract (Rosa et al., 2003). Additionally, low antimicrobial activity for ethanolic extracts of the fruiting body of *O. cubensis* against *Staphylococcus aureus* was found in previous studies (Costa et al., 2021), while our results showed no activity. Previous work also reported inhibition against four *Candida* species, *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis* (Rosa et al., 2003), while our study resulted in low activity against *C. albicans*.

Our results report the presence of compounds with known antimicrobial properties such as l-(+)-ascorbic acid 2,6-dihexadecanoate, cyclo(l-leucyl-l-prolyl), octocosanol and linolenic acid which could explain the inhibitory activity observed in the ethyl acetate fractions of the wild and cultivated fruiting bodies and mycelium. These results support that *Oudemansiella* is a genus with important antimicrobial properties. Literature additionally mentions isolated molecules of the *Oudemansiella* genus with antimicrobial activity such as Oudemansin A and B, Dihydroxerulin, Xerulinic acid and Strobilurin C (Anke et al., 1983; Kuhnt et al., 1990; Rosa et al., 2005).

3.4. Nutritional composition

The approximate nutritional composition of *O. cubensis* is summarized in Table 4 and was consistent with existing literature of *Oudemansiella* despite the different substrate used for cultivation. Our analyses revealed that *O. cubensis* is high in protein (25%) and high in dietary fiber (24%). The fat content for *Oudemansiella* species is reported to be higher (7-10%) compared to other commercial edible mushrooms (1.7-3.0%) such as *Agaricus bisporus*, *Pleurotus ostreatus*, and *Agrocybe cylindracea* (Alberti et al., 2021). The resulting fat content in this

Table 3
Percentage of inhibition of ethanolic and ethyl acetate extracts from different samples of *Oudemansiella cubensis*.

Microorganism	Percent inhibition (%)						
	Ethanolic extracts			**Ethyl acetate extracts**			**Positive Control**
	Wild fruiting bodies	**Cultivated fruiting bodies**	**Mycelia**	**Wild fruiting bodies**	**Cultivated fruiting bodies**	**Mycelia**	
Gram-Negative Bacteria							
Escherichia coli WDCM 00012	-	-	-	-	-	-	98.26 ± 0.12
Salmonella enterica WDCM 00031	2.03 ± 0.62	-	-	16.44 ± 1.90	9.35 ± 0.07	-	98.65 ± 0.90
Pseudomonas aeruginosa WDCM 00026	40.50 ± 5.01	9.12 ± 2.00	6.11 ± 0.25	13.73 ± 1.93	46.49 ± 6.69	84.37 ± 2.49	98.19 ± 0.06
Klebsiella pneumoniae ATCC BAA-1705	1.16 ± 0.42	-	1.41 ± 0.30	-	8.72 ± 1.37	-	98.68 ± 0.26
Gram-Positive Bacteria							
Enterococcus faecalis WDCM 00087	10.59 ± 3.15	-	-	49.7 ± 9.11	71.09 ± 6.79	57.89 ± 1.68	98.25 ± 0.63
Staphylococcus epidermidis WDCM 00036	23.26 ± 0.00	12.76 ± 2.51	-	44.47±0.37	18.05 ± 1.43	30.01 ± 4.89	99.13 ± 0.18
Staphylococcus aureus ATCC 6538	-	-	-	8.52±2.36	-	-	98.54 ± 1.43
Yeast							
Candida albicans WDCM 00054	4.88 ± 0.13	2.18 ± 0.14	0.85 ± 0.30	8.30±0.74	5.75 ± 0.99	18.61 ± 2.08	61.70 ± 2.51

(-) Without inhibition.

Table 4
Bibliographic comparison of compositions on a dry basis (% dry weight) and energy (kcal/100g) of dried cultivated fruiting bodies of edible *Oudemansiella* species from literature and this study.

Species	*O. cubensis* Paraguay	*O. cubensis* Argentina	*O. canarii* Argentina	*O. canarii* China	*O. submucida* Thailand
	(Current)	Alberti et al. (2021)	Alberti et al. (2021)	Xu et al. (2016)^A	S. Zhou et al. (2015)
Substrate of cultivation	Sawdust, corncob	Wheat straw	Wheat straw	Cotton seed hull, sawdust, corncob wheat bran and lime	No data
Moisture	92.49 ± 0.51	–	93.1 ± 0.0	-	3.91 ± 0.1
Ash	8.6 ± 0.11	10.65 ± 0.07	12.5 ± 0.28	7-8	11.48 ± 0.46
Crude protein	25.52 ± 0.50	11.50 ± 0.30	13.22 ± 0.63	16-18	14.70 ± 0.17
Fat	13.19 ± 0.74	10.45 ± 0.03	7.30 ± 0.51	1-3	7.10 ± 0.08
Dietary fiber	38.04 ± 0	32.35 ± 0.45	31.76 ± 0.06	33-35	-
Crude fiber	–	–	–	–	3.59 ± 0.57
Carbohydrates	12.3 ± 1 Anthrone method	23.72 ± 0.55 Difference method	23.44 ± 0.88 Difference method	30-33 Difference method	27.41 ± 0.44% Difference method
Energy kcal/100g	270	235^B	212^B	193-239^B	232^B

[A] Range obtained from four different substrates.
[B] Calculated from data of the references mentioned.

Table 5
SMART test data obtained with the ethanolic extract of *O. cubensis* evaluated.

Treatment	Wing number	Small single spots (1-2 cells) m = 2	Large single spots (>2 cells) m = 5	Twin spots m = 5	Total spots m = 2	Percent Inhibition (%)
Distilled water	20	0.00 (00)	0.00 (00)	0.00 (00)	0.00 (00)	-
Cyclophosphamide 2,61 mg.mL^{-1}	20	2.20 (44) +	0.65 (13) +	0.25 (05) +	3.10 (62) +	-
Genotoxicity test						
10 mg.mL^{-1}	20	0.00 (00) I	0.00 (00) i	0.00 (00) i	0.00 (00) i	-
20 mg.mL^{-1}	20	0.00 (00) I	0.00 (00) i	0.00 (00) i	0.00 (00) i	-
30 mg.mL^{-1}	20	0.10 (02) I	0.00 (00) i	0.00 (00) i	0.10 (02) i	-
Antimutagenicity test						
i. Simple treatment	20	0.05 (01) I	0.00 (00) i	0.00 (00) i	0.05 (01) i	-
ii. Combined treatment	20	0.25 (05) +	0.05 (01) i	0.00 (00) i	0.30 (06) +	90.32
iii. Chronic post-treatment of ethanolic extract	20	0.00 (00) i	0.00 (00) i	0.00 (00 i	0.00 (00) i	100
iv. Chronic pre-treatment of ethanolic extract	20	0.15 (03) i	0.10 (02) i	0.00 (00) i	0.25 (05) +	91.93

Diagnosis according to Frei and Würgler (56), the table indicates the following: +, positive; -, negative; i, inconclusive. m, multiplication factor. Significance levels α=β=0.05, even for single flr^3 rare spots.

study is also higher (13%) than those reported for *O. cubensis* from Argentina (10%). A more detailed study of a fatty acid profile would be necessary for a better characterization of the fat content. The protein content was around twice as high compared to *O. cubensis* and *O. canarii* from Argentina (Alberti et al., 2021) and *O. submucida* from Thailand (Table 4). Finally, dietary fiber content found in this work (38%) is similar than those reported in the literature for *Oudemansiella* species (Table 4). Edible mushrooms are considered as a novel source of dietary fiber, in fact, the functional characteristics of mushrooms are mainly due to the presence of dietary fiber, specifically chitin and beta-glucans (Cheung, 2013; Waktola & Temesgen, 2018).

For carbohydrates, our results obtained with the anthrone method were 12% dry weight. Typically, carbohydrates are a major component in mushrooms with total content ranges from 35% to 70% dry weight (Cheung, 2010; Dimopoulou et al., 2022). However, previous studies of *Oudemansiella* species reported generally lower values of 23% to 27% (Alberti et al., 2021; Xu et al., 2016; S. Zhou et al., 2015). Although these results are lower than others, they are incomparable, as those percentages reported in the literature were calculated with the difference method (Alberti et al., 2021; Xu et al., 2016; S. Zhou et al., 2015). This difference in methods could be the cause for inaccurate determinations of carbohydrate composition in mushrooms (Campi, Mancuello, Maubet, et al., 2023). Additionally, ash content resulted in a lower value (8.6%) than other edible *Oudemansiella* species. For example, the ash values of *O. cubensis* obtained are within the values tolerated by some food codes for edible mushrooms (i.e. less than 10% in the Argentine Food Code).

3.5. Median lethal dose (LD50), genotoxicity, and antimutagenicity

The toxicity test based on Probit analysis resulted in an LD50 concentration of 37.1 mg.mL^{-1} ($\chi^2 > 0.05$, R^2=0.79, P=0.049) of *O. cubensis* (Figure 4).

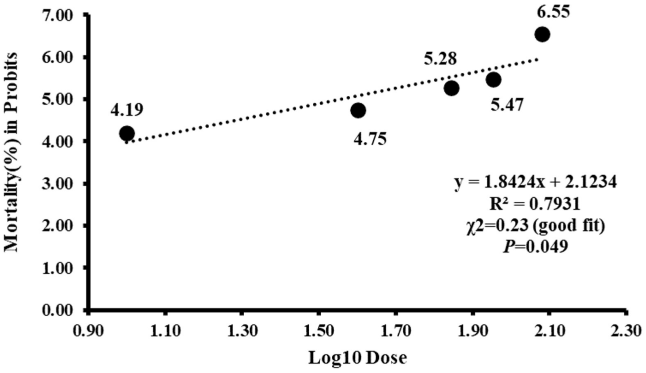

Figure 4. Toxicity tests, plot of log conc. versus probit for 72 h of treatment.

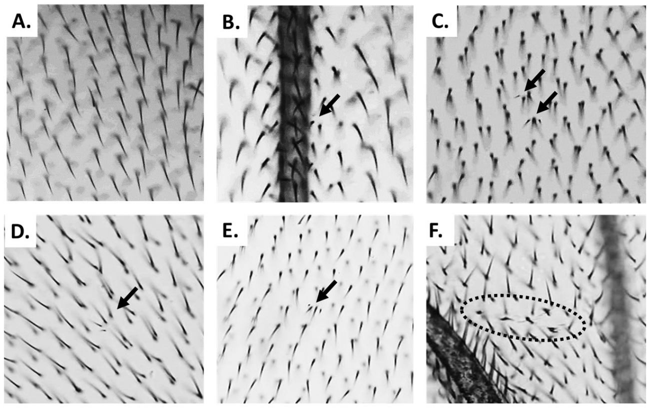

Figure 5. Microscopic view of the following wing morphologies: A. normal cell, B.-F. Mutant cell clones, B.-E. mwh phenotype, and F. flr^3 phenotype

Table 5 summarizes the results obtained from experimental treatments for genotoxic and antigenotoxic studies of *O. cubensis* compared to control groups. The wild fruiting body ethanolic extract showed no genotoxic effect at tested doses of 10 mg.mL^{-1} and 20 mg.mL^{-1}. Two mutant cell clones were found in samples treated with a an extract concentration of 30 mg.mL^{-1} (Figure 5). Statistical analysis showed inconclusive results for the assessment of genotoxicity, so the analysis of antimutagenic activity was carried out with the highest concentration that did not shown mutations (20 mg.mL^{-1}).

The total number of mutant cell clones in wings of *Drosophila melanogaster* flies was variable depending on the given treatment. For the (i) simple treatment, we identified only one mutant cell clone, which agrees with the other characterizations performed in this study. In the (ii) combined treatment, six mutant cell clones were registered with an inhibition of 90.32% of the mutant agent activity. The (iii) chronic post-treatment with ethanolic extract showed no mutant cell clone and 100% of mutagenic activity inhibition. Finally, the (iv) chronic pre-treatment with ethanolic extract revealed five mutant cell clones in the wings of treated flies and showed 91.93% of the mutagenic activity inhibition. These results suggest that ethanolic extract of *O. cubensis* at the evaluated concentrations (10, 20, and 30 mg.mL^{-1}) has no genotoxicity effect. Additionally, this study reveals an important antimutagenic activity of the ethanolic extract of *O. cubensis* at the concentration of 20 mg.mL^{-1} promoting the prevention of the genotoxic damage caused by the mutagenic agent before, during and after mutations were acquired or fixed (Gaivão et al., 2021; Graf et al., 1998, 1984). This damage prevention activity might be related to the presence of antioxidant compounds since there is an argument for a correlation between antioxidant and antigenotoxic activity (De Flora, 1998; Kelloff et al., 1994; Malik et al., 2022).

4. CONCLUSIONS

The chemical profile of *O. cubensis* obtained by GC-MS showcased the presence of compounds such as l-(+)-ascorbic acid 2,6-dihexadecanoate and cyclo(l-leucyl-l-prolyl). Compounds analogous of ascorbic acid contain antioxidant and antitumor properties, which may be attributed to the results of this study. The presence of cyclo(l-leucyl-l-prolyl) also may contribute to the antioxidant properties, as described by other studies (Begum et al., 2017; Kim et al., 2005; Mohamed et al., 2010).

Several studies argue that antioxidant activities are required for natural compounds to demonstrate antigenotoxic/antimutagenic activities, as a mechanism of steps to be performed to obtain chemoprevention by studied molecules against genotoxic damage (De Flora, 1998; Kelloff et al., 1994; Malik et al., 2022). Antioxidant activities have also been described in other related species such as *O. radicata*, *O. canarii* and *O. melanotricha* (Dulay, 2023; Pauli, 2010; Wang et al., 2018). This study, despite to be a first approach, has shown that *O. cubensis* also has antioxidant properties. Deep further studies are needed to, understand and characterize the antioxidant potential and antimutagenic mechanisms.

Oudemansiella is a genus of economic importance and it is already widely utilized as a source of food, cultivated, and marketed in several countries around the world. *Oudemansiella cubensis* is classified as edible, and our nutritional composition analysis revealed that is rich in protein (25%) and dietary fiber content (24%) while being low in fat (13%). Ethyl acetate and ethanolic extracts of *O. cubensis* were found to exhibit moderate antioxidant activity and antimicrobial activity against *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Additionally, bioactive compounds such as cyclo(l-leucyl-l-prolyl) and l-(+)-ascorbic acid 2,6-dihexadecanoate were identified in the extracts of wild and cultivated fruiting bodies and the mycelium. Finally, *O. cubensis* also showed important antimutagenic activity in its ethanolic extract.

CONFLICTS OF INTEREST

The authors declare there are no competing interests to declare.

ACKNOWLEDGMENTS

The authors thank FungiParaguay and Fundación Fungicosmos for their support. They also acknowledge the assistance of FACEN-UNA, which supported facilities used in this project. Two authors are members of Red Iberoamericana de Investigadores en Plantas Medicinales: Aromáticas y Condimenticias (REDIIMAC) and Red Iberoamericana de Investigadores en Micología (RIIMICO). We thank Francisco Ferreira, Alfredo Acosta, Braulio Vantrate, Alexander Ramos, and Dario Benítez for technical support. Finally, the authors would like to kindly thank Sarah Torhan for her revision and comments that helped improve the writing of this paper in English.

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A. APPENDIX: SUPPLEMENTARY INFORMATION

Supplementary data to this article can be found online at https://doi.org/10.53365/nrfhh/189170.

FUNDING

This work was financed by the project CONACYT (Consejo Nacional de Ciencias y Tecnología) PINV01-230 "Nuevos recursos endógenos del Paraguay: Bioprospección de compuestos antimicrobianos en hongos lignícolas nativos del neotrópico" and the project PINV01-218 "Caracterización del potencial nutricional y perfil químico de los hongos nativos silvestres, comestibles y medicinales del Paraguay y desarrollo de productos derivados para la industria alimenticia"

AUTHOR CONTRIBUTIONS

CM, MC, EC, GR - Research concept and design, CM, MC, AT, LM, EG - Collection and/or assembly of data, CM, MC, BV, LM, EG - Data analysis and interpretation, MC, YM, EC - Writing the article, MC, YM, EC, BV, GR - Critical revision of the article, CM, MC, YM, EC, BV, GR, LM - Final approval of the article.

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