applied sciences

MDPI

Article

Global Phenolic Composition and Antioxidant Capacity of Extracts from the Endophytic Fungi *Cophinforma mamane* with Potential Use in Food Systems: The Effects of Time, Temperature, and Solvent on the Extraction Process

Raiana S. Gurgel [1,2], Dorothy I. M. Pereira [2], Bárbara Martins [3], Lucas S. Falcão [2], Caroline D. Lacerda [2], Cláudia M. B. Neves [3], António F. Pinto [3], António M. Jordão [3,*] and Patrícia M. Albuquerque [1,2,*]

[1] Graduate Program in Biodiversity and Biotechnology of the Bionorte Network, School of Health Sciences, Amazonas State University, Manaus 69050-010, Brazil; raianagurgel@hotmail.com
[2] Research Group on Chemistry Applied to Technology, School of Technology, Amazonas State University, Manaus 69050-020, Brazil; dorothyivila@gmail.com (D.I.M.P.); lucas.sfalcao@hotmail.com (L.S.F.); cdutralacerda@gmail.com (C.D.L.)
[3] CERNAS Research Center, Polytechnic University of Viseu, 3500-606 Viseu, Portugal; barbaradanielasantos@gmail.com (B.M.); cmneves@esav.ipv.pt (C.M.B.N.); apinto@esav.ipv.pt (A.F.P.)
* Correspondence: antoniojordao@esav.ipv.pt (A.M.J.); palbuquerque@uea.edu.br (P.M.A.)

Abstract: Endophytic fungi are promising producers of phenolic compounds; however, the process of extracting these molecules from the culture medium is a crucial step for obtaining these metabolites. In this context, the best conditions for extracting phenolic compounds produced by *Cophinforma mamane* CF2-13, isolated from an Amazonian plant, were evaluated. The fungus was cultivated in a liquid medium to obtain its metabolites. The effects of the solvent, time, and temperature were investigated, as well as the use of ultrasound. The total phenolics and the antioxidant activity were assessed to define the best extraction conditions. The optimized extract was chemically characterized and used to analyze its potential in protecting olive oil from lipid oxidation. The best results were found using ethyl acetate at 20 °C for 2 h. These conditions led to 61.61 mg GAEq/g of extract of total phenolics, and an antioxidant capacity of 0.47, 0.45, and 0.29 mmol TEq/g of extract using the DPPH, ABTS, and FRAP methods, respectively. In addition, the optimized extract presented caffeic acid and was able to delay the lipid oxidation of olive oil. The potential of endophytic fungi to produce phenolic compounds is promising for the food industry since natural bioactive compounds have many benefits for human health.

Keywords: antioxidant activity; endophytes; fungal metabolites; phenolic compounds; lipid oxidation; caffeic acid

Citation: Gurgel, R.S.; Pereira, D.I.M.; Martins, B.; Falcão, L.S.; Lacerda, C.D.; Neves, C.M.B.; Pinto, A.F.; Jordão, A.M.; Albuquerque, P.M. Global Phenolic Composition and Antioxidant Capacity of Extracts from the Endophytic Fungi *Cophinforma mamane* with Potential Use in Food Systems: The Effects of Time, Temperature, and Solvent on the Extraction Process. *Appl. Sci.* **2024**, *14*, 8784. https://doi.org/10.3390/app14198784

Academic Editors: Barbara Borczak and Joanna Kapusta-Duch

Received: 31 August 2024
Revised: 19 September 2024
Accepted: 26 September 2024
Published: 29 September 2024

1. Introduction

Phenolic compounds comprise a group of substances known in the literature for their various benefits for the food industry and human health and are distinguished by their antioxidant capacity [1]. Their chemical structure consists of at least one benzene ring to which one or more hydroxyl groups are attached. Other functional groups, such as ethers and carboxylic acids, may also be attached. They can be divided into flavonoids (containing polyphenols) and non-flavonoids (characterized by simple phenols or phenolic acids) [2]. These compounds present antioxidant activity and are of great interest to the food industry since they have numerous applications, such as retarding the phenomenon of lipid oxidation, which is related to the alteration of food through the production of toxic substances and/or substances that confer undesirable flavor and/or color [3]. This chemical phenomenon occurs naturally and has direct implications on the functionality and commercial value of lipid raw materials and all the products that are formulated from them [4].

Phenolic compounds and other types of natural substances are classified as secondary metabolites from plants and microorganisms. Although plants and fruits are the main and best-known sources of these substances [5,6], microorganisms such as bacteria and fungi are also a rich source for obtaining phenolic compounds. In this sense, endophytic fungi stand out for their high production of these and other active metabolites, especially those isolated from medicinal plants [7,8].

Endophytic microorganisms live inside plant tissues such as roots, branches, leaves, and fruits, and interact with the plant at complex levels. They present as a rich source of natural compounds with important biological activities, which is possibly due to these interactions with their hosts [9]. The secondary metabolites produced by endophytic fungi have antibacterial, antifungal, antioxidant, anti-inflammatory, and cytotoxic activities, among others, and can be of different chemical classes, such as terpenes, alkaloids, and phenolic compounds [10,11].

Despite their potential, endophytic fungi isolated from tropical plants are still poorly studied and represent a promising source of bioactive compounds. Thus, the biodiversity in Brazil stands out and much of it is concentrated in its Amazon region, which has numerous species of plants known for their medicinal benefits and whose endophytic microbiota should be studied [12].

It is well known that several species of fungi have the ability to produce active metabolites such as phenolic compounds, among them are those of the genus *Cophinforma* [13,14]. This genus belongs to the Botryosphaeriaceae family and comprises species isolated as endophytic and as opportunistic pathogens [13]. In a previous study, we investigated extracts obtained from endophytic fungi isolated from *Fridericia chica*, an Amazonian medicinal species, regionally known as crajiru. The isolate, identified as *C. mamane* CF2-13, produced an extract rich in phenolic compounds with pronounced antimicrobial and antioxidant activities when compared to 107 other isolated fungi [14], and was selected to be used in the present study.

The process of obtaining and extracting phenolic compounds from the fungal culture medium is a crucial step for preserving and enhancing the structure/activity of these substances. Extraction affects stability and may cause the potential degradation of bioactive substances. Thus, it is important to select an appropriate extraction method to recover the target phenolic compounds, as well as optimize the factors that influence this process, which will also help to achieve high yields [15]. In this context, the aim of this study was to validate the best conditions for the extraction of the phenolic metabolites produced by the fungus *C. mamane* CF2-13 isolated from *F. chica*, as well as evaluate the antioxidant activity and its protective capacity against lipid oxidation. The accomplishment of these objectives could be of significant value for assessing the utilization of the phenolic extracts derived from endophytic fungi as additives or adjuvants in the food industry.

2. Materials and Methods

2.1. Reagents and Chemicals

The materials consisted of culture media, solvents, and analytical-grade reagents. Potato dextrose agar (PDA) was purchased from Kasvi (São José dos Pinhais, Brazil). Yeast extract was supplied by Himedia (Thane, India). Methanol P.A, ethanol P.A, 1-butanol P.A, ethyl acetate P.A, sodium carbonate, and sodium acetate were purchased from Honeywell | Riedel-de Haën (Seelze, Germany). Formaldehyde P.A and hydrochloric acid P.A were purchased from Honeywell | Fluka (Seelze, Germany). Dextrose, Iron (III) chloride, dimethyl sulfoxide (DMSO) P.A, cyclohexane P.A, glacial acetic acid P.A, iodide potassium, sodium chloride, sulphuric acid, aluminum chloride, vanillin, dichloromethane P.A, Dragendorff's reagent, sodium thiosulfate, and Folin–Ciocalteau solution were purchased from Dinâmica (Indaiatuba, Brazil). Acetonitrile HPLC was purchased from J.T. Baker (Pennsylvania, USA). Formic acid HPLC was purchased from LGC Biotecnologia (Cotia, Brazil). Chloroform HPLC, 2,2-diphenyl-1-picrylhydrazyl (DPPH) ≥98%, 2,4,6-tripyridyl-s-triazine (TPTZ) ≥98%, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) ≥98%,

α-tocopherol ≥95.5%, Trolox 97%, gallic acid ≥99%, caffeic acid ≥98%, quinic acid 100%, and chlorogenic acid ≥95% were obtained from Sigma-Aldrich (Saint Louis, MO, USA).

2.2. Endophytic Fungi

The endophytic fungi *Conphinforma mamane* CF2-13 was previously isolated from the leaves of the Amazonian species *F. chica* by Pereira et al. [16]. The isolation was registered for scientific research purposes in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) under the code A0B4857. The specimen of the fungi is deposited in the Central Microbiological Collection of the Amazonas State University (CCM/UEA) and preserved using the Castellani method [17].

C. mamane CF2-13 was reactivated by inoculating a fragment of the stock culture in Petri dishes containing PDA (potato dextrose agar), with subsequent incubation in a microbiological chamber (BOD) at 28 °C for 5–7 days.

2.3. Production of Fungal Metabolites

To produce the fungal metabolites, *C. mamane* CF2-13 was cultivated in a liquid medium composed of white potato broth (200 g/L); dextrose (10 g/L); yeast extract (2.0 g/L); and NaCl (5.0 g/L); pH 5.0. The inoculum consisted of three fungal mycelium fragments (5 × 5 mm in diameter) that were removed from the PDA plates. Cultivation was performed in 250 mL Erlenmeyer flasks with 150 mL of liquid medium under static conditions at 30 °C for 14 days according to the basal methodology of Bose et al. [18]. Figure 1 schematically illustrates the process used to obtain the fungal metabolites.

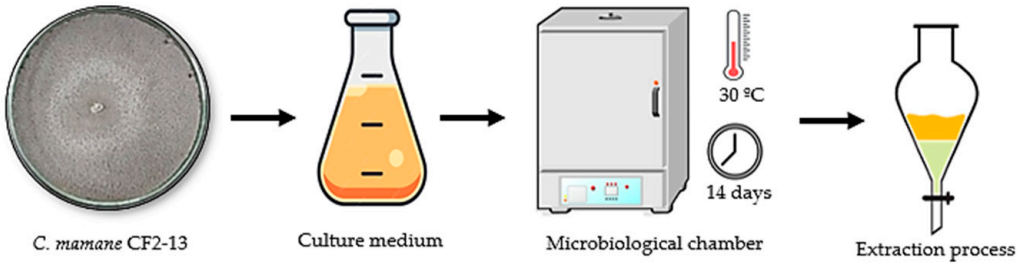

Figure 1. Schematic illustration of the process used to obtain the fungal metabolites.

2.4. Extraction of Fungal Metabolites

After the cultivation period, the fungal metabolites were extracted via liquid–liquid partition using the protocol described by Gurgel et al. [14]. As solvents, ethyl acetate (AcOEt), 1-butanol (ButOH), and the mixture ButOH:AcOEt (1:1, *v*/*v*) were used in the ratio of 1:2 in relation to the culture medium. The extractions with each solvent were performed in triplicate, under orbital agitation at 120 rpm in a shaker (KS-15, Edmund Bühler GmbH, Tübingen, Germany), at two different temperatures (20 °C and 30 °C ± 2), for periods of 30 min, 1, 2, 4, and 8 h. The extraction was also evaluated with the aid of an ultrasound (US) bath (Proclean 6.0 M-3, 60 W, Ulsonix, Berlin, Germany) at ambient temperature (22 °C ± 2) for periods of 7.5, 15, and 30 min.

After all these processes, the broth was separated from the mycelium by means of vacuum filtration with a Büchner funnel and filter paper. The solvent fractions were collected with the aid of a settling funnel and the solvent was removed by rotary evaporation (Rotavapor® R-300 system, Buchi, Flawil, Switzerland). The extracts were resuspended in ethanol at a concentration of 10 mg/mL and subsequently refrigerated until analysis.

2.5. Global Phenolic Content

The phenolic component of the extracts was evaluated using the Folin–Ciocalteau method based on the protocol described by Singleton and Rossi [19]. The assay was performed in triplicate, in test tubes into which 0.125 mL of the sample, 1 mL of distilled water, and 0.125 mL of the Folin–Ciocalteau reagent were inserted; then mixed rapidly in a

vortex; and allowed to stand for 6 min in the absence of light. Subsequently, 2 mL of 10% Na_2CO_3 were inserted, the tubes shaken again, and allowed to stand for 1 h in the dark.

Afterwards, the absorbances were read at 760 nm in a spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan). The results were calculated from the gallic acid curve, obtained under the same conditions described for the samples, which generated the equation y = 3.8853x + 0.0179 (R^2 = 0.9992). The results were expressed in equivalent milligrams of gallic acid per gram of extract (mg GAEq/g).

For the fungal extract that had the best extraction condition, analyses were carried out regarding the global content of phenols and the quantification of these phenols regarding flavonoid and non-flavonoid phenols. Total phenols were then determined by measuring the absorbance at 280 nm [20]. The non-flavonoid phenols were determined after an elimination reaction of the flavonoid phenols, which consisted of mixing 0.5 mL of the sample with 0.5 mL of 25% HCl and 0.25 mL of formaldehyde at 2%. After 24 h of reaction away from light sources, the absorbances were read at 280 nm after adequate dilution with distilled water [21]. The values of the total phenols and non-flavonoid phenols were calculated using the equation y = 0.0357x − 0.0396 (R^2 = 0.9970), which was obtained via the gallic acid calibration curve and expressed in equivalent milligrams of gallic acid per g of extract (mg GAEq/g). The value of the flavonoid phenols was then calculated by the difference between the amount of the total phenols and non-flavonoid phenols [21]. All analyses were performed in triplicate.

2.6. Antioxidant Assays

The antioxidant activity of the extracts was determined using three different methodologies: the sequestration of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•); capture of the radiocarbon 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+); and ferric-reducing antioxidant power (FRAP).

To evaluate the sequestration capacity of DPPH•, 1.9 mL of the DPPH solution at 0.06 mmol/L was left in contact with 0.1 mL of the sample for 30 min, away from light sources. Subsequently, the absorbances were measured at 517 nm [22]. For the ABTS• + assay, a 7 mM radical solution was prepared and diluted in ethanol to reach an absorbance of 0.700 at 734 nm. Subsequently, 2 mL of the ABTS solution was in contact with 0.1 mL of the sample for 15 min, away from light sources, followed by the reading of the absorbances at 734 nm [23]. The ferric-reducing antioxidant power (FRAP) of the extracts was also evaluated based on the method described by Benzie and Strain [24]. To test the antioxidant activity, 2.45 mL of the FRAP reagent (100 mL of acetate buffer 0.3 mM; 10 mL of TPTZ 10 mM; and 10 mL of ferric chloride 20 mM) was incubated with 0.35 mL of the sample for 30 min, at 37 °C, under protection from light. After the reaction period, the absorbances were read at 595 nm. All the analyses were carried out in triplicate.

The results were expressed in millimol trolox equivalents per gram of extract (mmol TEq/g), calculated using Equations (1)–(3), generated from the calibration curves of this standard, obtained under the same conditions described for the samples in each method (three distinct curves).

$$\boldsymbol{DPPH}y = 169.09x - 7.5164\ (R^2 = 0.9968) \quad (1)$$

$$\boldsymbol{ABTS}\ y = 189.39x + 0.2343\ (R^2 = 0.9940) \quad (2)$$

$$\boldsymbol{ABTS}\ y = 189.39x + 0.2343\ (R^2 = 0.9940) \quad (3)$$

2.7. Chemical Profile of the Fungal Extract

For the chemical characterization of the extract obtained in the best extraction condition, an analysis using thin-layer chromatography (TLC) was initially performed in order to identify the main classes of metabolites present in the sample. An aliquot of 4 µL of the fungal extract solution at 20 mg/mL in methanol was applied with the aid of a micropipette on a silica gel chromatographic plate (TLC aluminum sheets, Macherey—Nagel, 20 × 20 cm, silica gel 60 matrix, fluorescent indicator). The mixture dichloromethane/methanol/formic

acid (90:8:2) was used as the eluent. After the chromatographic run in a vat saturated with the eluent, the chemical classes were detected via visualization under ultraviolet light, at 254 and 365 nm, and with the dying reagents aluminum chloride ($AlCl_3$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric chloride ($FeCl_3$), vanillin/H_2SO_4, and Dragendorff's reagent [25].

2.8. Individual Phenolic Acid Profile of the Fungal Extract

Phenolic acids were analyzed using high-performance liquid chromatography (HPLC) based on the methodology described by Whelan et al. [26]. Thus, an ultra-high performance liquid chromatograph (UHPLC) (Nexera XR, Shimadzu, Kyoto, Japan) coupled with a diode array detector (DAD) (SPD-M20A) was used. The stationary phase consisted of the spherisorb ODS2 column (80 Å, 5 µm, 4.6 mm × 150 mm, Waters, Milford, CT, USA). The mobile phase consisted of 20% acetonitrile and 80% of a 0.1% aqueous solution of formic acid with a constant flow of 0.7 mL/min. The oven temperature was maintained at 40 °C. All the samples, including the commercially purchased standards, were previously solubilized in methanol and filtered with a 25 mm diameter polyvinylidene fluoride (PVDF) signal filter with a 0.45 µm pore size. The injection volume was 4 µL and all analyses were carried out in triplicate. To confirm the presence of some of the phenolic acids (caffeic, quinic, chlorogenic, and gallic acids) in the extract, 30 µL of a solution of each of the standards at a concentration of 250 µg/mL in 600 µL of extract at 1 mg/mL were added and injected in the HPLC following the conditions previously described.

2.9. Lipid Oxidation Protection Assay

To analyze the protection against lipid oxidation, the peroxide values were measured over time following the method recommended in CEE law No. 2568/91 [27]. The fungal extract (20 mg/mL in ethanol) was in contact with extra-virgin olive oil (Gallo, Abrantes, Portugal) in open test tubes at a concentration of 2 mg of extract per gram of olive oil under light exposure and at a temperature of 30 °C ± 2 for 72 h. For control purposes, the oxidation products of the olive oil, olive oil with ethanol, and olive oil with the addition of α-tocopherol (0.2 mg/g) were also verified. The peroxide index (PI) was measured by titration with 0.01 N sodium thiosulfate after every 12 h and calculated according to Equation (4):

$$PI = (Vm \times N \times 1000)/P \quad (4)$$

where Vm = volume in milliliters of the titrant solution; N = normality of the titrant solution; and P = weight in grams of olive oil. The results were expressed in milli equivalents of O_2 per kilogram of olive oil ($meqO_2/kg$) and all analyses were carried out in triplicate.

2.10. Statistical Analysis

The data are presented as mean and standard deviation. To determine whether there was a statistically significant difference between the data obtained, an analysis of variance (ANOVA) and a comparison of treatment means were carried out. Tukey's honestly significant difference ($p < 0.05$) test was applied to the data to determine significant differences between the treatments (solvent and time variables at the two temperatures tested for the extraction conditions of fungal metabolites) and the data obtained from the lipid oxidation protection assay. All the analyses were performed using the software IBM SPSS Statistics version 29.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Total Phenolic Content

The total phenolic content was evaluated in the fungal extracts obtained via the different extraction conditions used. The best results were obtained with the solvent AcOEt at a temperature of 20 °C (Figure 2a), with no significant difference between the values of the total phenols extracted after 30 min, 1 h, and 2 h with this solvent (63.7, 64.6, and 61.6 mg GAEq/g, respectively).

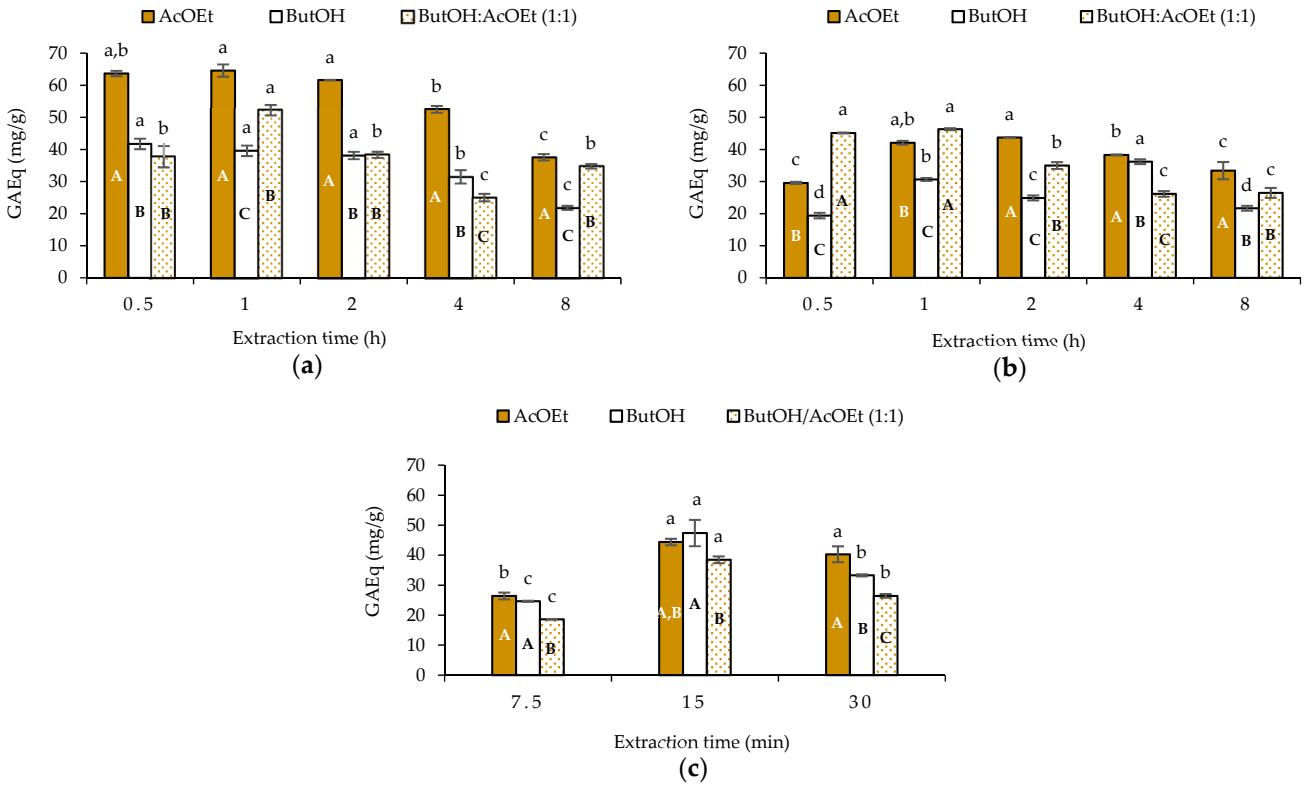

Figure 2. Quantification of the total phenolic compounds in the fungal extracts obtained using the different extraction conditions (solvents/times). (**a**) Extraction at 20 °C. (**b**) Extraction at 30 °C. (**c**) Extraction using an ultrasound bath. The values that share the same lowercase letters for the same solvent do not present a statistically significant difference between them over time and the values that share the same uppercase letters between the same time and for the different solvents do not present a statistically significant difference between them according to the Tukey test ($p < 0.05$).

For the temperature of 30 °C (Figure 2b), the combination of ButOH:AcOEt solvents (1:1, *v*/*v*) produced a better extraction of the total phenolic compounds at the times of 30 min and 1 h (45.1 and 46.3 mg GAEq/g, respectively) without statistical difference between these times. In addition, there was no statistical difference when ButOH:AcOEt solvents were used when compared to the phenolic content obtained with the solvent AcOEt, at 30 °C, after 1 h and 2 h of extraction (42.1 and 43.7 mg/g GAEq, respectively). The results obtained after 15 min of extraction with the use of the US bath (Figure 2c) for the solvents AcOEt and ButOH (42.10 and 43.72 mg/g GAEq, respectively) did not show significant differences when compared to the results for extraction at 30 °C (Figure 2b). However, the total phenol values were significantly lower when compared to those obtained with AcOEt at 20 °C after 30 min, 1, and 2 h.

3.2. Antioxidant Capacity

For the sequestration of the DPPH radical, the best antioxidant capacity values were found for the fungal extracts obtained via the use of the AcOEt solvent at both temperatures tested (Figure 3a,b). However, the results obtained for this solvent in the extraction times of 30 min and 2 h at 20 °C showed the highest antioxidant capacity values (0.45 and 0.47 mmol TEq/g, respectively), though, without statistical difference between them. In addition, extraction using US (Figure 3c) did not induce high antioxidant activity values (all the values were below 0.20 mmol TEq/g) when compared with the other assays.

For the analysis of the antioxidant capacity of the extracts against the ABTS radical, the best results were obtained using AcOEt at 20 °C after 2 and 4 h of extraction (0.45 and 0.47 mmol TEq/g, respectively) and at 30 °C, with the same solvent, after 2 h (0.45 mmol TEq/g), without statistical differences (Figure 4a,b). For the extraction using

US (Figure 4c), the extracts obtained with the three solvents after 7.5 min achieved results without statistical difference from those mentioned above (0.43, 0.47, and 0.53 mmol TEq/g, respectively). It is, therefore, observed that for the sequestration of the ABTS radical, the use of US in the extraction of fungal metabolites was positive, since we obtained similar results in a much shorter extraction time when compared to the extraction without US.

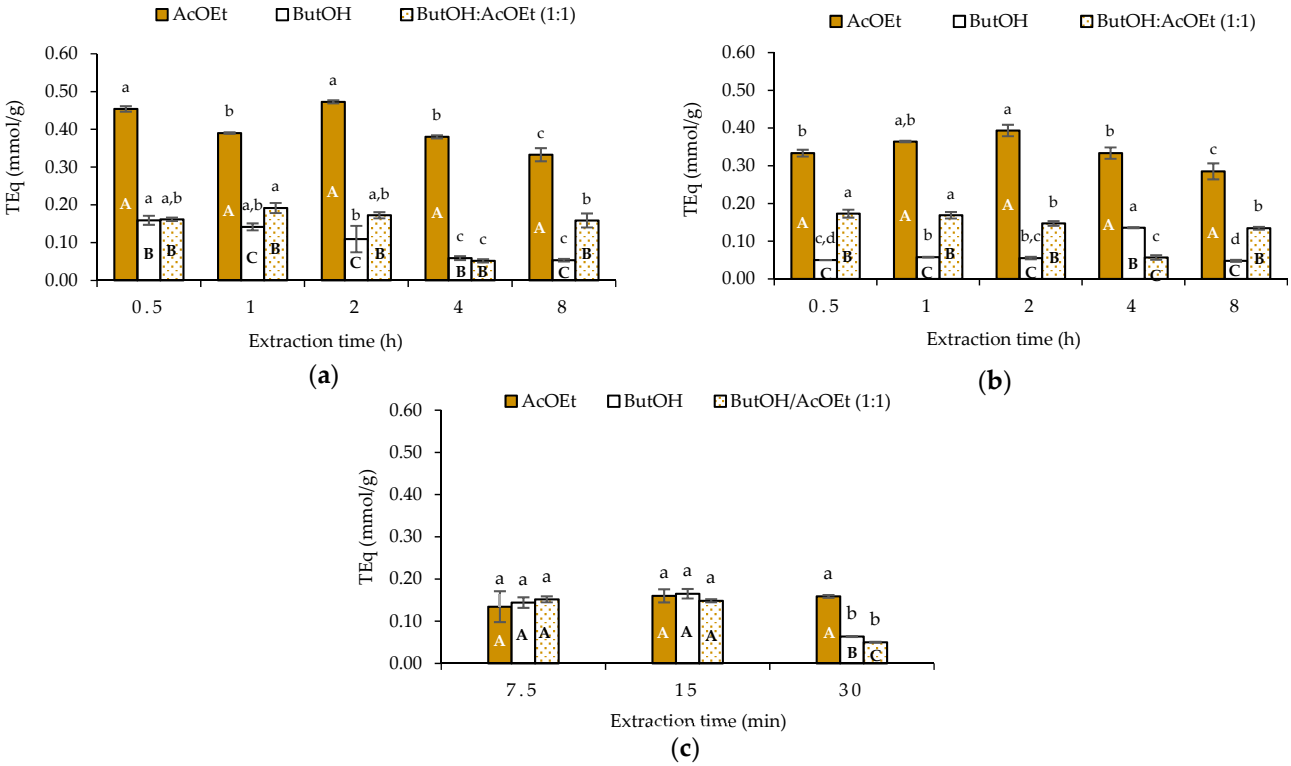

Figure 3. DPPH radical sequestration capacity of the fungal extracts obtained using the different extraction conditions (solvents/times). (**a**) Extraction at 20 °C. (**b**) Extraction at 30 °C. (**c**) Extraction using an ultrasound bath. The values that share the same lowercase letters for the same solvent do not present a statistically significant difference between them over time and the values that share the same uppercase letters between the same time and for the different solvents do not present a statistically significant difference between them according to the Tukey test ($p < 0.05$).

For antioxidant activity using the FRAP method, the values were significantly lower than those obtained for the sequestration of DPPH and ABTS radicals. The highest ferric reduction powers were observed for the extracts obtained at 20 °C via the use of AcOEt after 30 min, 2, and 4 h (0.28, 0.29, and 0.29 mmol/g, respectively) (Figure 5a,b). In addition, the application of the US (Figure 5c) did not yield favorable results regarding antioxidant activity when the FRAP method was employed. This is particularly evident when the results obtained with the extraction at 20 °C are considered.

3.3. Global Phenol Content

By jointly evaluating the amount of total phenols and the antioxidant activity of the extracts, an optimized condition was obtained for the extraction of bioactive phenolic compounds with the solvent AcOEt, for 2 h, at 20 °C ± 2 (AE-2h-20C). The fungal extract obtained under these conditions was evaluated for the total polyphenol index and presented 190.92 mg GAEq/g, being 71.22 mg GAEq/g of non-flavonoids and 119.70 mg GAEq/g of flavonoids. The yield of the AE-2h-20C extract was 108 mg per L of the fungal culture medium produced.

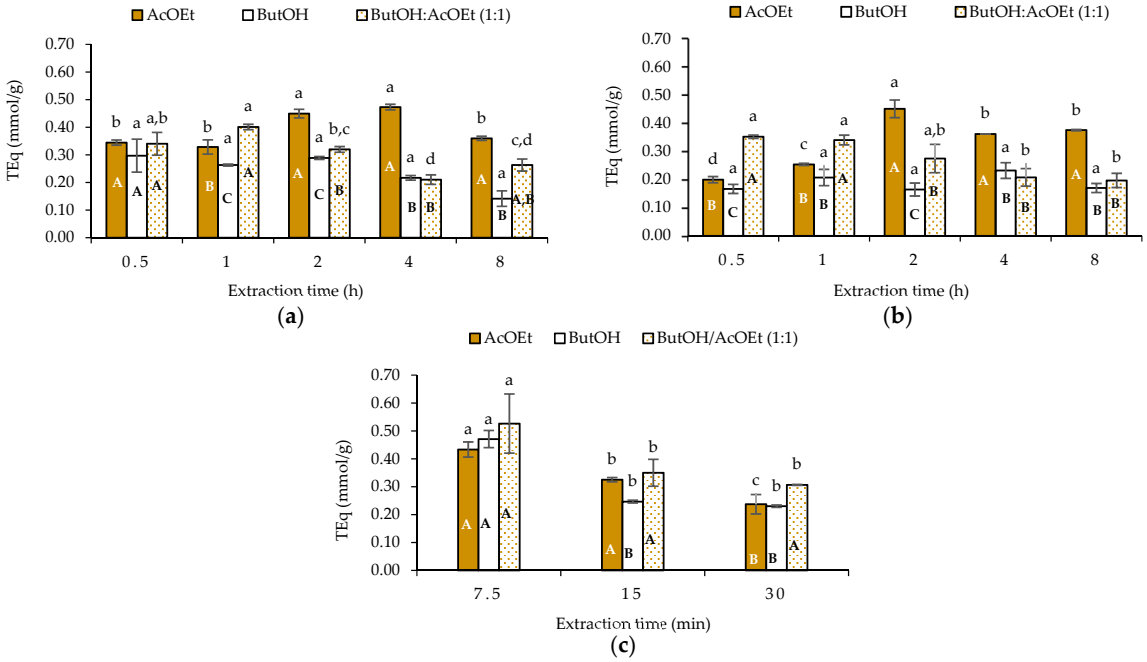

Figure 4. ABTS radical sequestration capacity of the fungal extracts obtained using the different extraction conditions (solvents/times). (**a**) Extraction at 20 °C. (**b**) Extraction at 30 °C. (**c**) Extraction using an ultrasound bath. The values that share the same lowercase letters for the same solvent do not present a statistically significant difference between them over time and the values that share the same uppercase letters between the same time and for the different solvents do not present a statistically significant difference between them according to the Tukey test ($p < 0.05$).

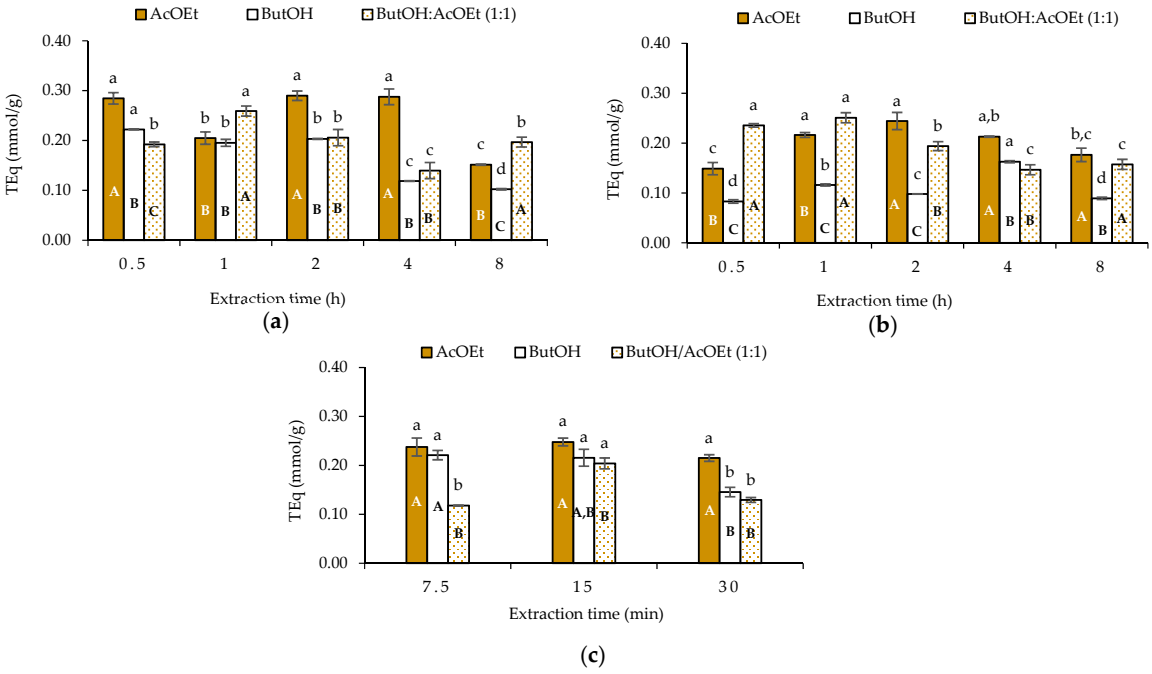

Figure 5. Ferric-reducing antioxidant power of the fungal extracts obtained using the different extraction conditions (solvents/times). (**a**) Extraction at 20 °C. (**b**) Extraction at 30 °C. (**c**) Extraction using an ultrasound bath. The values that share the same lowercase letters for the same solvent do not present a statistically significant difference between them over time and the values that share the same uppercase letters between the same time and for different solvents do not present a statistically significant difference between them according to the Tukey test ($p < 0.05$).

3.4. Chemical Profile of the Fungal Extract

The analysis of the extract AE-2h-20C using TLC showed the presence of conjugated double bonds after exposure to UV light at 254 nm and 365 nm (Figure 6a). It also indicated the presence of flavonoids after staining with $AlCl_3$ and exposure to UV at 365 nm (Figure 6b); compounds with antioxidant activity after staining with DPPH (Figure 6c); the presence of phenolic compounds after staining with $FeCl_3$ (Figure 6d); and terpenes after staining with H_2SO_4 (Figure 6e). No alkaloid-like compounds were detected after staining with Dragendorff's reagent.

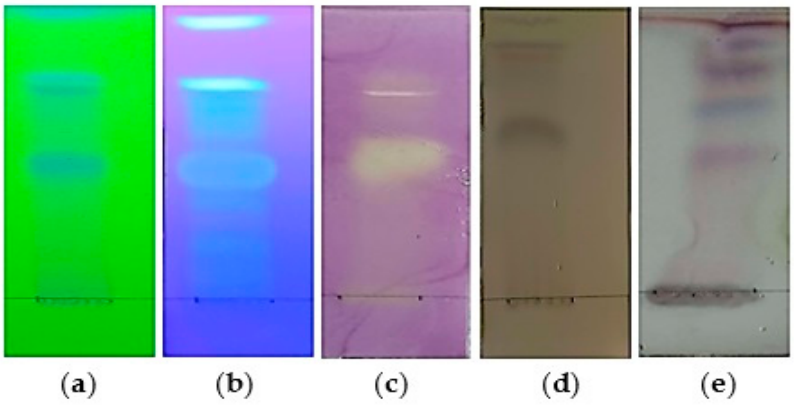

Figure 6. Thin-layer chromatography of the fungal extract AE-2h-20C. Exposure to UV light at 254 nm with bluish spots, indicative of conjugated double bonds (**a**); staining with $AlCl_3$ and exposure to UV light at 365 nm, in which the fluorescence intensification of the spots indicates the presence of flavonoids (**b**); staining with DPPH with yellowish spots, indicating compounds with antioxidant activity (**c**); staining with $FeCl_3$ with dark spots, indicating the presence of phenolic compounds (**d**); and with vanillin/H_2SO_4 with purple spots, indicative of the presence of terpenes (**e**).

3.5. Individual Phenolic Acids of the Fungal Extract

The fungal extract AE-2h-20C, which showed the highest levels of total phenolic content and antioxidant capacity values, was also analyzed using HPLC in order to identify the presence of any phenolic acids. For the analyzed individual phenolic acids (caffeic, quinic, chlorogenic, and gallic acids), only the presence of caffeic acid was detected at a concentration of 69.07 µg/mg of extract. This result was confirmed by the co-injection of this compound (Figure 7).

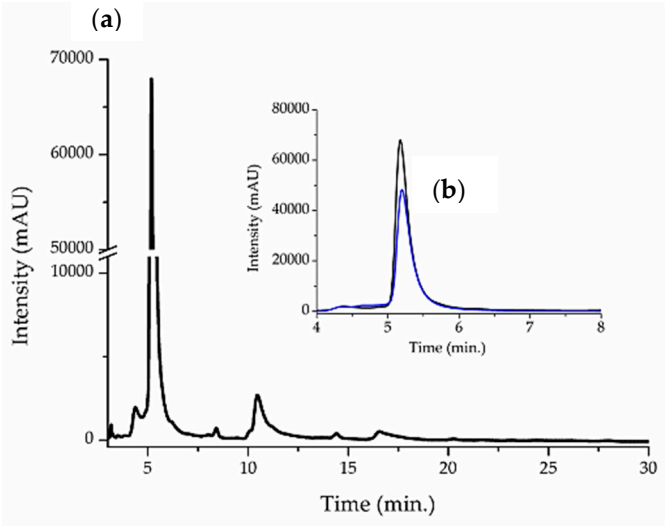

Figure 7. Chromatograms obtained using HPLC of fungal extract AE-2h-20C (**a**) and extract (blue line) and caffeic acid standard (black line) (**b**).

From the analysis of Figure 8, it is also possible to observe that the antioxidant activity previously observed may be related to the presence of caffeic acid in the extract AE-2h-20C. This result was obtained from the observation of the appearance of a band on the TLC plate stained with DPPH, and confirmed by the injection of this fraction, collected directly from the plate, in HPLC according to the analysis conditions previously described.

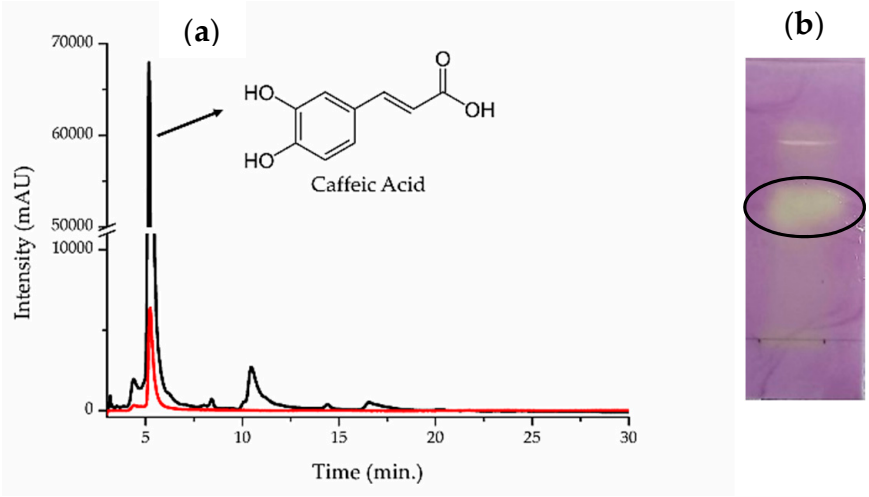

Figure 8. Chromatogram from the HPLC analysis of the fungal extract AE-2h-20C (black line), of the fraction of the extract recovered from the TLC (red line) (**a**), and the TLC plate with the DPPH-stained band (**b**).

3.6. Lipid Oxidation Protection

The AE-2h-20C extract was also evaluated for its ability to protect extra-virgin olive oil against lipid oxidation. The peroxide value (PI) was evaluated every 12 h for 72 h and the results are shown in Figure 9.

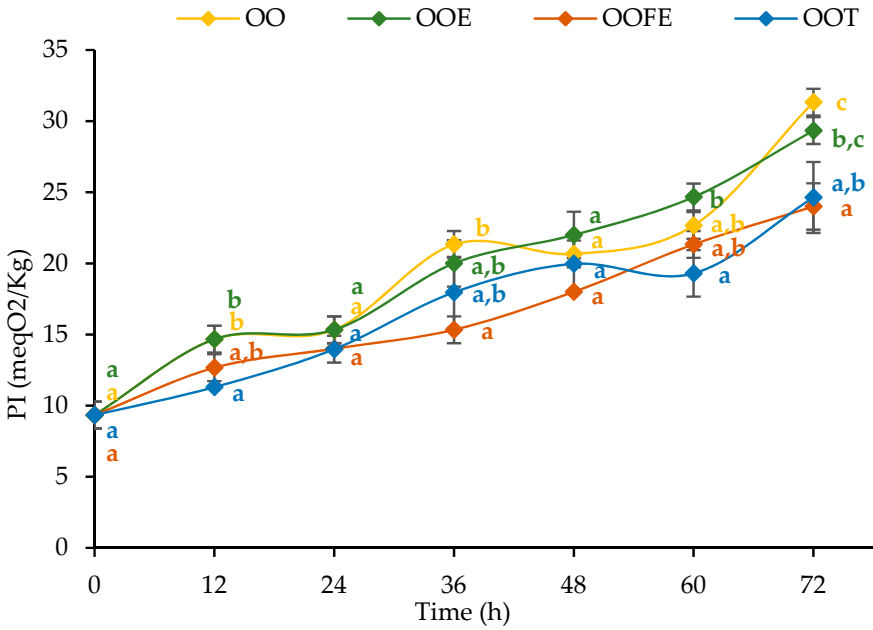

Figure 9. Evolution of the peroxide index used to evaluate the protective capacity of the fungal extract AE-2h-20C against lipid oxidation. OO = olive oil; OOE = olive oil and ethanol; OOFE = olive oil and fungal extract. OOT = olive oil and α-tocopherol. The values that share the same letters within the same time have no significant differences between them according to the Tukey test ($p < 0.05$).

It is observed that the fungal extract obtained under the best extraction conditions (AE-2h-20C extract) demonstrated the ability to protect olive oil against oxidation, presenting no significant difference when compared with the positive control, tocopherol. In addition, after 72 h, the protective effect becomes more evident, since the olive oil alone showed significantly higher PI values than the values for olive oil stored with the fungal extract or with α-tocopherol. These results indicate that the fungal extract AE-2h-20C has a potential application in the prevention of lipid oxidation in food products.

4. Discussion

Endophytic fungi are a rich reservoir of molecules with bioactive potential and comprise great chemical diversity in their secondary metabolites [28,29]. Due to the mutualistic relationship that they have with their hosts, the pharmacologically active natural compounds produced by endophytic fungi can be considered infinite and should be extensively studied [30]. Among the secondary metabolites, phenolic compounds are especially responsible for the antioxidant capacity, but they are also reported to have antimicrobial, anti-inflammatory, antiviral, and cytotoxic activity, among others [31].

The process of the extraction of bioactive compounds from the fungal culture medium, however, is a crucial step for obtaining the best quantity and quality of these molecules. Various solvents can be employed, alone or in combination, to extract fungal metabolites. Ethyl acetate, methanol, dichloromethane, hexane, and ethanol are routinely used to extract metabolites from culture broth; however, the solubility of the desired component will determine the extraction solvent [30]. In addition, safety concerns should also be considered. On the other hand, the extraction temperature is also a relevant factor for the process. Higher temperatures can increase solubility and diffusion, but it is also important to avoid overheating since if the extraction temperature is too high, the solvent will be lost, and the phenolic compounds will degrade [32]. Furthermore, the efficiency of the extraction depends on its duration. Longer extraction times increase efficiency, but when a solute equilibrium is achieved between the internal and external solid matter, the increased time will not affect the extraction [33].

In this study, the best extraction conditions for the phenolic compounds were evaluated, varying the solvent, temperature, and extraction time, correlating them with antioxidant activity, and analyzed using three different methodologies. The best extraction condition was defined by evaluating the binomial "total phenolic compounds x antioxidant activity" obtained with 2 h of extraction with AcOEt at 20 °C.

Different extraction methods have been used to obtain active phenolic compounds from the fungal culture medium [34]. In the study developed by Silva et al. [34], the phenolic metabolites produced by endophytic fungi isolated from *Passiflora incarnata* were extracted with AcOEt and ButOH (1:1). The authors found that the best phenolic extraction was obtained using AcOEt, which agrees with the results obtained in our study. Silva et al. [34] also evaluated the antioxidant activity of fungal extracts using the DPPH method; however, contrary to what we observed in our research, the antioxidant capacity was not directly proportional to the amount of total phenolics present in the extracts.

The effectiveness of an extraction solvent for phenolic compounds can vary according to its polarity and the fungal species producing such compounds. In the work developed by Tang et al. [35], the phenolic compounds of three endophytic fungal species were correlated with antioxidant activity (using the DPPH and ABTS methods) after the extraction of these metabolites with the solvents of medium (ethyl acetate and n-butanol) and low (oil ether and chloroform) polarity. For the strains of *Penicillium oxalicum* and *Simplicillium* sp., the largest amount of total phenols was extracted with AcOEt (94.58 and 58.64 mg GAEq/g, respectively), while for the strain of *Colletotrichum sp.*, the best result was obtained by the use of n-butanol (58.96 mg GAEq/g). These authors found that the medium-polarity solvents were the most efficient in the extraction of phenolic compounds and the antioxidant activity was directly proportional to the amount of total phenolics, corroborating the data found in the present study for the phenolic metabolites of *C. mamane* CF2-13.

When considering the temperatures and times for the extraction of phenolic compounds from fungal culture broths, a wide range is observed in the literature. Jamal et al. [36] performed their extraction with AcOEt from the metabolic broth of endophytic fungi isolated from *Gynura procumbens*, using a time of 10 min, directly in the separation funnel. The best results for the extraction of phenolic compounds from the culture medium were with the species *Colletotrichum glosporioides* SN11 (87 mg GAEq/g). In the same study, antioxidant activity was evaluated using the DPPH and FRAP methods, but the result was not directly proportional to the amount of total phenolics, and the extract produced by the species *Macrophomina phaseolina* SN6 presented the best result for the sequestration of the DPPH radical, while the extract produced by *Phomopsis* sp. SN2 showed the highest ferric reduction power.

In another study, Rocha et al. [37] carried out extractions from the culture media of endophytic fungi isolated from *Schinus terebinthifolius* Raddi using AcOEt, at 25 °C for 24 h, without agitation. The authors obtained 758.91 TEq mg/g (3.02 mmol TEq/g) in the extract produced by the species *Ochrocladosporium elatum* for antioxidant activity using the DPPH radical sequestration method. In this case, it is observed that the longer extraction time favored the obtaining of phenolic compounds, contrary to what we observed for the extraction of phenolic metabolites from the culture medium of *C. mamane* CF2-13, whose best result (0.47 mmol TEq/g) was obtained with 2 h of extraction. On the other hand, after 24 h of the extraction of metabolites produced by the species *Penicillium olsonii*, Rocha et al. [37] obtained 119.33 mg TEq/g (0.47 mmol TEq/g), which is the same value obtained in our study with only 2 h of extraction with ethyl acetate under agitation. Therefore, the influence of the fungal species on the phenolic metabolic extraction process is clear, and it is necessary to study the optimal conditions for each species of fungus.

Natural antioxidants produced by endophytic fungi, in general, are of the class of phenolic compounds, such as flavonoids and phenolic acids [38], which corroborates the results found in our study. The TLC results indicate the presence of these classes of molecules in the extract of *C. mamane* CF2-13 when obtained under the best extraction conditions (solvent AcOEt, at 20 °C for 2 h). Kaur et al. [39] also observed the presence of phenolic compounds and flavonoids in the metabolic extracts of the endophytic fungus *Aspergillus fumigatus* isolated from *Moringa oleifera*, which exhibited pronounced antioxidant activity against DPPH radicals. On the other hand, in a recent study, Vu et al. [29] identified not only phenolic compounds but also a new sesquiterpene in the extract of the endophytic fungi *Fusarium foetens* AQF6, which presented pronounced antioxidant activity. Thus, these results confirm the potential of endophytic fungi as a source of natural antioxidant compounds, which can be phenolic compounds, terpenes, and alkaloids, and consequently their possible use in the food industry.

Phenolic acids have been found in the culture medium of endophytic fungi and are directly associated with the antioxidant activity of several fungal extracts. In the study by Tang et al. [35], in which the composition of phenolic compounds in fungal extracts was evaluated using liquid chromatography coupled to mass spectrometry (LC-MS), caffeic acid was found in the extracts of *P. oxalicum* and *Simplicillium* sp., both obtained with AcOEt; and that of *Colletotrichum* sp., obtained with butanol, at concentrations of 3.01, 0.52, and 0.12 μmol/g, respectively. These results agree with those observed for the extract of *C. mamane* CF2-13, in which caffeic acid was found at a concentration of 12.07 μg/mL (0.22 μmol/g). In addition to caffeic acid, Tang et al. [35] also found chlorogenic acid in their fungal extracts, which was not observed in our research for *C. mamane* CF2-13.

Shen et al. [40] analyzed the extracts from the endophytic fungi isolated from *Alisma orientale* (Sam.) Juzep using LC-MS and found caffeic acid at concentrations of 10.12, 0.03, 0.02, and 0.01 μmol/g in the extracts obtained with AcOEt from the culture medium of the species *Chaetomium globosum*, *Alternaria compacta, Alternaria* sp., and *Alternaria* sp., respectively. Again, it is found that the fungal species plays a central role in order to optimize the extraction conditions of phenolic compounds produced by fungi.

Caffeic acid is classified as a hydroxycinnamic acid, a phenolic compound of the non-flavonoid type that belongs to the group of phenolic acids. These are characterized by a single phenyl group substituted by a carboxylic group and one or more hydroxyl (OH) groups. Not only does it have antioxidant activity, but also antimicrobial, anti-inflammatory, and anticancer activities, among others. It is present mainly in red fruits, coffee, wine, and vegetables [41]. According to Espíndola et al. [42], the consumption of foods rich in caffeic acid leads to a protective effect against carcinogenesis by preventing the formation of nitrosamines and nitrosamides. The anticancer action of caffeic acid is mainly associated with its antioxidant and prooxidant capabilities, which are attributed to its chemical structure [42].

Natural antioxidants have a wide application in the food industry. They can be used to slow down the oxidation process of various foods, especially lipid food matrices [43]. In our study, we evaluated the ability of the fungal extract obtained under the best extraction conditions to slow the lipid oxidation of extra-virgin olive oil. The results are promising, considering that the fungal extract did not present statistical differences when compared to the standard antioxidant tocopherol, and maintained a lower peroxide value than the other experiments. This is an important finding as there are very few studies that have evaluated the ability of fungal extracts to retard lipid oxidation. Generally, studies tend to examine fruit extracts, such as that of Correia and Jordão [44], which evaluated the protective effect of extracts from different types of grapes. In this case, using butter, the authors verified that after 14 days at a temperature of 20 °C, the control sample (butter only) presented a higher level of peroxides (56.6 meq O_2/kg), while the experiments using the grape extracts, rich in phenolic content, obtained from Touriga Franca grapes, presented a lower value of peroxides (38.1 meq O_2/kg), indicating a potential protective effect against the lipid oxidation.

The Brazilian National Health Surveillance Agency (ANVISA, RDC No. 740/2022) [45] authorizes the use of food additives and technological adjuvants in various food products, and the maximum amount of tocopherol allowed to be used as an antioxidant in olive oil is 200 mg/kg. In this study, we used this concentration of tocopherol (0.2 mg/g) as the positive control to evaluate the lipid oxidation of extra virgin olive oil. The fungal extract obtained under the best extraction conditions was able to protect the olive oil against lipid oxidation without significant difference when compared to tocopherol, which highlights once again the potential of this extract to be used in the food industry as an antioxidant, especially for food products rich in lipids.

5. Conclusions

The data obtained in this study show the potential of endophytic fungi in the production of phenolic compounds with antioxidant activity, which are widely used in the food industry. It also demonstrates the importance/influence of the extraction process on the structure–activity relationship of these compounds, in which the optimized condition was obtained with ethyl acetate at 20 °C during 2 h of extraction for the species *C. mamane* CF2-13, isolated from the Amazonian medicinal plant *F. chica*. This extraction condition presented good results in the binomial "phenolic compounds x antioxidant activity", including antioxidant action against the oxidation of olive oil, emphasizing its potential use in the food industry. In addition, the optimized extract can be considered a new source of caffeic acid, as this phenolic acid was found in the fungal metabolites produced. Consequently, the novelty of our results lies in the fact that the *C. mamane* extract, which was obtained under optimized conditions, can be used in food processes.

Author Contributions: Conceptualization, P.M.A. and A.M.J.; methodology, R.S.G., B.M., C.M.B.N., C.D.L. and L.S.F.; validation, C.M.B.N., A.F.P., C.D.L. and L.S.F.; formal analysis, P.M.A. and A.M.J.; investigation, R.S.G., D.I.M.P. and B.M.; resources, P.M.A., A.F.P. and A.M.J.; data curation, R.S.G.; writing—original draft preparation, R.S.G. and C.D.L.; writing—review and editing, P.M.A. and A.M.J.; supervision, P.M.A. and A.M.J.; project administration, P.M.A. and A.M.J.; funding acquisition, P.M.A. and A.M.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) (grants number 01.02.016301.00568/2021-05 and 01.02.016301.00101/2024-08), by POSGRAD/FAPEAM 2023, by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (finance code 001 and grants number 88881.510151/2020-01 and 88887.946446/2024-00), and by CERNAS (UIDB/00681/2020—DOI:10.54499/UIDP/00681/2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: Universidade do Estado do Amazonas—UEA, Polytechnic University of Viseu, Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for supporting this research.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Vuolo, M.M.; Lima, V.S.; Maróstica, M.R., Jr. Phenolic compounds: Structure, classification, and antioxidant power. In *Bioactive Compounds: Health Benefits and Potential Applications*; Campos, M.R.S., Ed.; Woodhead Publishing: Oxford, UK, 2019; pp. 33–50.
2. Abbas, O.; Compère, G.; Larondelle, Y.; Pompeu, D.; Rogez, H.; Baeten, V. Phenolic compound explorer: A mid-infrared spectroscopy database. *Vib. Spectrosc.* **2017**, *92*, 111–118. [CrossRef]
3. Wong, D.W.S.; Lorenzo, P.L. *Quimica de los Alimentos: Mecanismos y Teoria*; Acribia: Zaragoza, Spain, 1995; pp. 1–10.
4. Chorilli, M.; Leonardi, G.; Salgado, H.R.N. Free radicals and antioxidant agents: Concepts to applicationin pharmaceutical and cosmetic formulations. *Rev. Bras. Farm.* **2007**, *88*, 113–118.
5. Lin, D.; Xiao, M.; Zhao, J.; Li, Z.; Xing, B.; Li, X.; Kong, M.; Li, L.; Zhang, Q.; Yaowen, L.; et al. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules* **2016**, *21*, 1374. [CrossRef] [PubMed]
6. De la Rosa, L.A.; Moreno-Escamilla, J.O.; Rodrigo-García, J.; Alvarez-Parilla, E. Phenolic compounds. In *Postharvest Physiology and Biochemistry of Fruits and Vegetables*; Yahia, E.M., Ed.; Woodhead Publishing: Oxford, UK, 2019; pp. 253–271.
7. Zimowska, B.; Bielecka, M.; Abramczyk, B.; Nicoletti, R. Bioactive products from endophytic fungi of Sages (*Salvia* spp.). *Agriculture* **2020**, *10*, 543. [CrossRef]
8. Basappa, K.S.; Raghava, S.; Umesha, S. Potential of endophytic fungus untapped, including novel bioactive compounds. *Pharmacog. Com.* **2023**, *13*, 50–63. [CrossRef]
9. Fadiji, A.E.; Babalola, O.O. Elucidating mechanisms of endophytes used in plant protection and other bioactives with multifunctional prospects. *Front. Bioeng. Biotecnol.* **2020**, *8*, 467.
10. Zhang, J.; Zhu, Y.; Si, J.; Wu, L. Metabolites of medicine food homology-derived endophytic fungi and their activities. *Curr. Res. Food Sci.* **2022**, *5*, 1882–1896. [CrossRef]
11. Jha, P.; Kaur, T.; Chhabra, I.; Panja, A.; Paul, S.; Kumar, V.; Malik, T. Endophytic fungi: Hidden treasure chest of antimicrobial metabolites interrelationship of endophytes and metabolites. *Front. Microbiol.* **2023**, *14*, 1227830. [CrossRef]
12. Fearnside, M.P. The intrinsic value of Amazon biodiversity. *Biodivers. Conserv.* **2021**, *30*, 1199–1202. [CrossRef]
13. Phillips, A.J.L.; Alves, A.; Abdollahzadeh, J.; Slippers, B.; Wingfield, M.J.; Groenewald, J.Z.; Crous, P.W. The Botryosphaeriaceae: Genera and species known from culture. *Stud. Mycol.* **2013**, *76*, 51–167. [CrossRef]
14. Gurgel, R.S.; Pereira, D.I.M.; Garcia, A.V.F.; Souza, A.T.F.; Silva, T.M.; Andrade, C.P.; Silva, W.L.; Nunez, C.V.; Fantin, C.; Procópio, R.E.L.; et al. Antimicrobial and antioxidant activities of endophytic fungi associated with *Arrabidaea chica* (Bignoniaceae). *J. Fungi* **2023**, *9*, 864. [CrossRef] [PubMed]
15. Alara, O.R.; Abdurahman, N.H.; Ukaegbu, C.I. Extraction of phenolic compounds: A review. *Curr. Res. Food Sci.* **2021**, *4*, 200–214. [CrossRef] [PubMed]
16. Pereira, D.I.M.; Gurgel, R.S.; Souza, A.T.F.; Matias, R.R.; Falcão, L.S.; Chaves, F.C.M.; Silva, G.F.; Martínez, J.G.; Procópio, R.E.L.; Fantin, C.; et al. Isolation and identification of pigment-producing endophytic fungi from the Amazonian species *Fridericia chica*. *J. Fungi.* **2024**, *10*, 77. [CrossRef]
17. Castellani, A. Viability of some pathogenic fungi in distilled water. *J. Trop. Med. Hyg.* **1939**, *42*, 225–226.
18. Bose, P.; Gowrie, S.U.; Chathurdevi, G. Optimization of culture conditions of growth and production of bioactive metabolites by endophytic fungus—*Aspergillus tamarii*. *Int. J. Pharm. Biol. Sci.* **2019**, *9*, 469–478.

19. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.* **1965**, *16*, 144–158. [CrossRef]
20. Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. *Handbook of Enology—The Chemistry of Wine Stabilization and Treatments*, 2nd ed.; John Wiley & Sons: Hoboken, NJ, USA, 2007; Volume 2.
21. Kramling, T.E.; Singleton, V.L. An estimate of the nonflavonoid phenols in wines. *Am. J. Enol. Viticult.* **1969**, *20*, 86–92. [CrossRef]
22. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* **1995**, *28*, 25–30. [CrossRef]
23. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying and improved ABTS radical cátion decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
24. Benzie, I.F.F.; Strain, J.J. The Ferric Reducing Ability of Plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef]
25. Lopes, J.L.C. Cromatografia em Camada Delgada. In *Fundamentos de Cromatografia*; Collins, C.H., Braga, G.L., Bonato, P.S., Eds.; Editora da Unicamp: Campinas, Brazil, 2006; pp. 67–86.
26. Whelan, L.C.; Geary, M.; Healy, J. A novel, simple rapid reverse-phase HPLC-DAD analysis, for the simultaneous determination of phenolic compounds and abscisic acid commonly found in foodstuff and beverages. *J. Chromatogr. Sci.* **2022**, *60*, 648–654. [CrossRef]
27. *Commission Regulation (EEC) No. 2568 of 11 July 1991. Concerning the Characteristics of Olive Oils and Olive Pomace Oils, as Well as Related Methods of Analysis*; FAO: Rome, Italy, 1991.
28. Tousif, M.I.; Tauseef, S.; Nabeelah, S.; Sharmeen, J.; Zengin, G.; Legoabe, L.; Imran, M.; Mahomoodal, M.F. Phenolics from endophytic fungi as natural α-glucosidase inhibitors: A comprehensive review. *J. Mol. Struct.* **2023**, *1291*, 135852. [CrossRef]
29. Vu, T.H.N.; Pham, N.S.; Quach, N.T.; Le, P.C.; Pham, Q.A.; Ngo, C.C.; Nguyen, V.T.; Anh, D.H.; Quang, T.H.; Chu, H.H.; et al. *Fusarium foetens* AQF6 isolated from *Amentotaxus ynnanensis* H.L.Li as a prolific source of antioxidant compounds. *Appl. Sci.* **2024**, *14*, 2048. [CrossRef]
30. Singh, V.K.; Kumar, A. Secondary metabolites from endophytic fungi: Production, methods of analysis, and diverse pharmaceutical potential. *Symbiosis* **2023**, *90*, 111–125. [CrossRef] [PubMed]
31. Carvalho, P.L.N.; Silva, E.O.; Chagas-Paula, D.A.; Luiz, J.H.H.; Ikegaki, M. Importance and implications of the production of phenolic secondary metabolites by endophytic fungi: A mini-review. *Mini Rev. Med. Chem.* **2016**, *16*, 259–271. [CrossRef] [PubMed]
32. Zhang, Q.W.; Lin, L.G.; Ye, W.C. Techniques for extraction and isolation of natural products: A comprehensive review. *Chin. Med.* **2018**, *13*, 20. [CrossRef]
33. Shi, L.; Zhao, W.; Yang, Z.; Subbiah, V.; Suleria, H.A.R. Extraction and characterization of phenolic compounds and their potential antioxidant activities. *Environ. Sci. Pollut. Res. Int.* **2022**, *29*, 81112–81129. [CrossRef]
34. Silva, M.H.R.; Cueva-Yesquén, L.G.; Boaventura, S.B., Jr.; Garcia, V.L.; Sartoratto, A.; Angelis, D.F.; Angelis, D.A. Endophytic fungi from *Passiflora incarnata*: An antioxidant compound source. *Arch. Microbiol.* **2020**, *202*, 2779–2789. [CrossRef]
35. Tang, Z.; Qin, Y.; Chen, W.; Zhao, Z.; Lin, W.; Xiao, Y.; Chen, H.; Liu, Y.; Chen, H.; Bu, T.; et al. Diversity, chemical constituents, and biological activities of endophytic fungi isolated from *Ligusticum chuanxiong* Hort. *Front. Microbiol.* **2021**, *12*, 771000. [CrossRef]
36. Jamal, H.A.A.; Husaini, A.; Sing, N.N.; Roslan, H.A.; Zulkharnain, A.; Akinkunmi, W.A. Characterization of bioactive compounds produced by endophytic fungi isolated from *Gynura procumbens* (*Sambung Nyawa*). *Braz. J. Microbiol.* **2022**, *53*, 1857–1870. [CrossRef]
37. Rocha, P.S.; Paula, V.M.B.; Olinto, S.C.F.; Santos, E.L.; Souza, K.P.; Estevinho, L.M. Diversity, chemical constituents and biological activities of endophytic fungi isolated from *Schinus terebinthifolius* Raddi. *Microorganisms* **2020**, *8*, 859. [CrossRef] [PubMed]
38. Aguirre, J.J.; Toledo, H.L.G.; Cruz, A.Z.; Belmares, R.C.; Aguilar, N.C. The optimization of phenolic compounds extraction from cactus pear (*Opuntia ficus-indica*) skin in a reflux system using response surface methodology. *Asian Pac. J. Trop. Biomed.* **2013**, *3*, 436–442.
39. Kaur, N.; Arora, D.S.; Kalia, N.; Kaur, M. Antibiofilm, antiproliferative, antioxidant and antimutagenic activities of an endophytic fungus *Aspergillus fumigatus* from *Moringa oleifera*. *Mol. Biol. Rep.* **2020**, *47*, 2901–2911. [CrossRef] [PubMed]
40. Shen, N.; Chen, Z.; Cheng, G.; Lin, W.; Qin, Y.; Xiao, Y.; Chen, H.; Tang, Z.; Li, Q.; Yuan, M.; et al. Diversity, chemical constituents and biological activities of endophytic fungi from *Alisma orientale* (Sam.) Juzep. *Front. Microbiol.* **2023**, *14*, 1190624. [CrossRef]
41. Kadar, N.N.M.A.; Ahmad, F.; Teoh, S.L.; Yahaya, M.F. Caffeic acid on metabolic syndrome: A review. *Molecules* **2021**, *26*, 5490. [CrossRef]
42. Espíndola, K.M.M.; Ferreira, R.G.; Narvaez, L.E.M.; Rosario, A.C.R.S.; Silva, A.H.M.; Silva, A.G.B.; Vieira, A.P.O.; Monteiro, M.C. Chemical and pharmacological aspects of caffeic acid and its activity in hepatocarcinoma. *Front. Oncol.* **2019**, *9*, 541–551. [CrossRef]
43. Xu, D.P.; Li, Y.; Meng, X.; Zhou, T.; Zhou, Y.; Zheng, J.; Zhang, J.J.; Li, H.B. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *Int. J. Mol. Sci.* **2017**, *18*, 96. [CrossRef]

44. Correia, A.C.; Jordão, A.M. Antioxidant capacity, radical scavenger activity, lipid oxidation protection analysis and antimicrobial activity of red grape extracts from different varieties cultivated in Portugal. *Nat. Prod. Res.* **2015**, *29*, 438–440. [CrossRef]
45. ANVISA—Agência Nacional de Vigilância Sanitária. *Resolução da Diretoria Colegiada (RDC) nº 740 de 9 de Agosto de 2022. Autoriza o Uso de Aditivos Alimentares e Coadjuvantes de Tecnologia em Diversas Categorias de Alimentos*; ANVISA: Brasília, Brazil, 2022.