

Type of the Paper (Proceedings, Abstract, Extended Abstract, Editorial, etc.)

Antimicrobial properties of chestnut shell extract as an eco-friendly approach for food preservation

Maria Ciriaco ^{123*}, Luis Patarata ¹²³, Márcio Moura-Alves ¹²³, Fernando Nunes ⁴ and Cristina Saraiva ¹²³

¹ Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal

² Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal

³ Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences, ECAV University of Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal

⁴ CQ-VR-Food and Wine Chemistry Laboratory, Chemistry Research Centre-Vila Real, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal

* Correspondence: mariaciriaco@utad.pt

Abstract: The chestnut industry generates large quantities of by-products, including the chestnut shell, which is a source of phenolic compounds. In this study, MIC (minimum inhibitory concentration) of chestnut shell extract was determined by the disk diffusion method. The chestnut shell was freeze-dried and milled. The extract was obtained by ultrasound assisted technique using water 70% : etanol 30% (v/v) and subsequently lyophilized. Muller-Hinton plates were inoculated with $\sim 10^5$ CFU/mL of microorganisms. Sterile paper discs (6 mm) were placed on the inoculated culture medium and impregnated with 10 μ L of each extract. Seven concentrations of extract between 0.3% and 2.1% were tested. The plates were incubated for 24h at 37°C. The antibacterial efficacy of the extracts was indicated by a halo formed around the paper disk. The work was carried out in triplicate. Halos were found at 1.5%, 1.8% and 2.1% on *Listeria monocytogenes* ATCC 7973 (8.32 \pm 0.06 mm for 2.1%), *Enterococcus faecalis* 19433 (8.94 \pm 0.41 mm for 2.1%), and *Staphylococcus aureus* ATCC (10.26 \pm 0.19 mm at 2.1%). For the remaining microorganisms no halos were observed. The tested extract showed antimicrobial activity, demonstrating potential for the control of pathogens in the food industry.

Keywords: chestnut shell; antibacterial; minimum inhibitory concentration; antimicrobial activity; pathogens; *Enterococcus faecalis*; *Staphylococcus aureus*; *Listeria* spp.

1. Introduction

In Europe, the cultivation of chestnut trees (*Castanea sativa*, Mill.) has been increasing, as has the production of chestnuts [1]. Portugal is one of the European Union countries contributing most to the increase in production, with the Longal and Judia varieties being the most commonly cultivated [2]. Most chestnuts are eaten fresh, but their processing, whether to be sold frozen or in purees, for example, has been increasing [3].

The chestnut industry generates large quantities of by-products, including the chestnut shell, which is a source of phenolic compounds of great interest to the food and pharmaceutical industries [4,5]. The concept of the circular economy is increasingly being suggested and referred to, and the use of these by-products with added value can make a significant contribution to this concept and to the valorization of products [6,7].

Phenolic compounds play a fundamental role in plants, both in their reproduction and growth, as well as for their adaptation and survival in stressful situations, such as attack by pathogens [8]. Phenolic compounds also contribute to organoleptic properties of foods. Of all the classes of phenolic compounds, flavonoids are the most abundant. They are also essential for food production and preservation, as they play a fundamental

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Last-name

Published: date



Copyright: © 2023 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

role in oxidation processes [9]. These compounds have been studied for their antimicrobial, antioxidant, anti-inflammatory, antiviral, anti-hepatotoxic, among others [10].

The consumption of nuts is increasingly recommended, with studies reporting that they help reduce cholesterol levels and are thus associated with a lower incidence of cardiovascular disease, with these factors being associated with the antioxidant activity of the compounds present [11,12].

The search for natural sources of phenolic compounds with antimicrobial and antioxidant properties is therefore a current interest. This study is important due to the variability of the plant's origins, varieties and the variations of the technologies used for extraction that may influence the quantity of phenolic compounds and its activity. Also, the techniques used are constantly changing and there may be alterations when compared to previous studies. The aim of this study was to extract phenolic compounds from chestnut shell and evaluate their antimicrobial activity.

2. Materials and methods

2.1. Samples of chestnut shells

Chestnuts shells of the Judia variety were obtained from an industry in the north of Portugal. The samples were dried at 60°C, vacuum-packed and refrigerated (2°C) until extraction.

2.2. Extraction

The extract was obtained using ultrasound assisted technique at a controlled temperature (40°C) and time (30 minutes). The solvent used, was water 70%: ethanol 30%, (v/v). For this stage, a ratio of 1g of sample to 10mL of solvent was used. The extract obtained was centrifuged. The solvent was removed using a rotary evaporator at 38 °C under vacuum and lyophilized. For the analysis, the extract was reconstituted with water and filtered using a 0.20 µm syringe filter.

2.3. Antimicrobial activity

According to the method of Garcia et al. [13], we carried out the disk diffusion method. Eleven microorganisms were tested in this study to determine the antimicrobial properties of the chestnut shell extract at different concentrations. The microorganisms used are shown in Table 1. In order to prepare the inoculums, the microorganisms were cultivated in the respective enrichment medium (table 1). Each isolated was then prepared in 0.1% tryptone salt and the concentration of the inoculum was obtained using the McFarland method to a standard of 0.5 (approximately 10⁸ colonizing forming units (CFU) per mL). Each preparation was inoculated (0.1mL) onto Mueller-Hinton agar. The inoculum was allowed to dry, and then the sterilized discs (6mm) were placed with 10µL of extract added to each one. Seven concentrations of extract were tested. The plates were then incubated for 37°C/24h for subsequent visual analysis. The plates were checked whether an inhibitory halo had formed, and when it did, it was measured (mm) and the values recorded.

Table 1. Microorganisms likely to occur in meat products and their culture conditions.

Microorganism	Gram	Liquid culture medium, temperature and incubation time	Selective culture medium, temperature and incubation time
<i>Escherichia coli</i> O157:H7 9001	-	BHI, 37°C, 24h/48h (610495)	TBX (Tryptone Bile X-Glucuronide-84637), 44°C, 24h
<i>Yersinia enterocolitica</i> ATCC 9018	-	BHI, 37°C, 24h/48h (610495)	Yersinia Selective Agar Base-VWR, 30°C, 24h
<i>Staphylococcus aureus</i> ATCC	+	BHI, 37°C, 24h/48h (610495)	BP (Baird Parker-VWR), 37°C, 24h

<i>Salmonella</i> ATCC	-	BHI, 37°C, 24h/48h (610495)	Chromagar <i>Salmonella</i> , 37°C, 24h
<i>Salmonella</i> Typhimurium 14028	-	BHI, 37°C, 24h/48h (610495)	Chromagar <i>Salmonella</i> , 37°C, 24h
<i>Enterococcus faecalis</i> 19433	+	BHI, 37°C, 24h/48h (610495)	VRBG (Violet Red Bile Glucose-VWR), 30°C 24h/48h
<i>Enterococcus faecium</i> 20477	+	BHI, 37°C, 24h/48h (610495)	VRBG (Violet Red Bile Glucose-VWR), 30°C 24h/48h
<i>Listeria monocytogenes</i> ATCC 7973	+	BHI, 37°C, 24h/48h (610495)	Chromagar <i>Listeria</i> , 37°C, 24h
<i>Listeria</i> ATCC 7644	+	BHI, 37°C, 24h/48h (610495)	Chromagar <i>Listeria</i> , 37°C, 24h
<i>Pseudomonas</i> JI-Me-LM03	-	BHI, 37°C, 24h/48h (610495)	CFC (<i>Pseudomonas</i> selective agar), 30°C, 48h
<i>Escherichia coli</i> ATCC 1175	-	BHI, 37°C, 24h/48h (610495)	TBX (Tryptone Bile X-Glucuronide-84637), 44°C, 24h

3. Results

The minimum inhibitory concentration of the chestnut shell extract is shown in Table 2.

Table 2. Antimicrobial susceptibility, diameter (mean ± standard deviation) of inhibition halos (mm).

Microorganism	1.2%	1.5%	1.8%	2.1%
	DDA	DDA	DDA	DDA
<i>Escherichia coli</i> O157:H7 9001	-	-	-	-
<i>Yersinia enterocolitica</i> ATCC 9018	-	-	-	-
<i>Staphylococcus aureus</i> ATCC	-	-	-	10.26±0.19
<i>Salmonella</i> ATCC	-	-	-	-
<i>Salmonella</i> Typhimurium 14028	-	-	-	-
<i>Enterococcus faecalis</i> 19433	-	7±0.25	7.74±0.25	8.94±0.41
<i>Enterococcus faecium</i> 20477	-	-	-	-
<i>Listeria monocytogenes</i> ATCC 7973	-	6.22±0.18	6.99±0.11	8.32±0.06
<i>Listeria</i> ATCC 7644	-	-	-	-
<i>Pseudomonas</i> JI-Me-LM03	-	-	-	-
<i>Escherichia coli</i> ATCC 1175	-	-	-	-

-not determined.

The chestnut shell extract showed antimicrobial capacity against 3/11 of the microorganisms studied (27.27%), at the 3 highest concentrations studied (1.5%, 1.8% and 2.1%).

The microorganisms where an inhibitory halo was found are gram+, *Staphylococcus aureus* ATCC (7.00 mm-9.47 mm), *Enterococcus faecalis* 19433 (6.65 mm – 8.52 mm), *Listeria monocytogenes* ATCC 7973 (6.04 mm- 7.37 mm). The results agree with other studies. J.-Y. An et al., [14] presented different extraction conditions, which allowed specific phenolic compounds to be isolated, however, they found an inhibitory halo for *S. aureus* and *E. Faecium*, as in our study. Silvia et al. [2], used a higher concentration of extract than this study, which resulted in slightly higher halos, however, the microorganisms inhibited were similar. For *Staphylococcus aureus* and *Enterococcus faecalis* they obtained an inhibitory halo of 12 mm and 11 mm, respectively.

4. Conclusions

The results obtained demonstrate the interesting antimicrobial potential of chestnut by-products such as the shell. The extract obtained is rich in phenolic compounds and could be interesting to use as an antimicrobial and antioxidant additive. It also offers a strong possibility of adding economic value to the region and the chestnut industry. It is necessary to complement this study with others, such as the identification of phenolic compounds present in the shell and their antioxidant capacity.

Funding: This work was supported by the project FCT AgriFood XXI (NORTE-01-0145-FEDER-000041). The authors would like to thank to CECAV by the projects UIDP/CVT/00772/2020 and LA/P/0059/2020 funded by the FCT.

Conflicts of Interest: The authors declare no conflict of interest.

References

- López-Sáez, J. A.; Glais, A.; Robles-López, S.; Alba-Sánchez, F.; Pérez-Díaz, S.; Abel-Schaad, D.; Luelmo-Lautenschlaeger, R. Unraveling the naturalness of sweet chestnut forests (*Castanea sativa* Mill.) in central Spain. *Vegetation History and Archaeobotany* **2017**, *26*, 167-182.
- Silva, V.; Falco, V.; Dias, M. I.; Barros, L.; Silva, A.; Capita, R.; Alonso-Calleja, C.; Amaral, J. S.; Igrejas, G.; CFR Ferreira, I. Evaluation of the phenolic profile of *Castanea sativa* Mill. by-products and their antioxidant and antimicrobial activity against multiresistant bacteria. *Antioxidants* **2020**, *9* (1), 87.
- Squillaci, G.; Apone, F.; Sena, L. M.; Carola, A.; Tito, A.; Bimonte, M.; De Lucia, A.; Colucci, G.; La Cara, F.; Morana, A. Chestnut (*Castanea sativa* Mill.) industrial wastes as a valued bioresource for the production of active ingredients. *Process Biochemistry* **2018**, *64*, 228-236.
- Esposito, T.; Celano, R.; Pane, C.; Piccinelli, A. L.; Sansone, F.; Picerno, P.; Zaccardelli, M.; Aquino, R. P.; Mencherini, T. Chestnut (*Castanea sativa* Miller.) burs extracts and functional compounds: UHPLC-UV-HRMS profiling, antioxidant activity, and inhibitory effects on phytopathogenic fungi. *Molecules* **2019**, *24* (2), 302.
- Fernandez-Agullo, A.; Freire, M. S.; Antorrena, G.; Pereira, J. A.; Gonzalez-Alvarez, J. Effect of the extraction technique and operational conditions on the recovery of bioactive compounds from chestnut (*Castanea sativa*) bur and shell. *Separation Science and Technology* **2014**, *49* (2), 267-277.
- Benincasa, C.; Santoro, I.; Nardi, M.; Cassano, A.; Sindona, G. Eco-friendly extraction and characterisation of nutraceuticals from olive leaves. *Molecules* **2019**, *24* (19), 3481.
- Tyskiewicz, K.; Konkol, M.; Rój, E. The application of supercritical fluid extraction in phenolic compounds isolation from natural plant materials. *Molecules* **2018**, *23* (10), 2625.
- Stefanovic, O.; Comic, L. Synergistic antibacterial interaction between *Melissa officinalis* extracts and antibiotics. *Journal of applied pharmaceutical science* **2012**, (Issue), 01-05.
- Oliveira, L. d. L. d.; Carvalho, M. V. d.; Melo, L. Health promoting and sensory properties of phenolic compounds in food. *Revista Ceres* **2014**, *61*, 764-779.
- Takshak, S. Bioactive compounds in medicinal plants: A condensed review. *SEJ Pharm. Nat. Med* **2018**, *1* (1), 1-35.
- Sabaté, J.; Oda, K.; Ros, E. Nut consumption and blood lipid levels: a pooled analysis of 25 intervention trials. *Archives of internal medicine* **2010**, *170* (9), 821-827.
- Shahidi, F.; Ambigaipalan, P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects—A review. *Journal of functional foods* **2015**, *18*, 820-897.
- Garcia, J.; Afonso, A.; Fernandes, C.; Nunes, F. M.; Marques, G.; Saavedra, M. J. Comparative antioxidant and antimicrobial properties of *Lentinula edodes* Donko and Koshin varieties against priority multidrug-resistant pathogens. *South African Journal of Chemical Engineering* **2021**, *35* (1), 98-106.

-
14. An, J.-Y.; Wang, L.-T.; Lv, M.-J.; Wang, J.-D.; Cai, Z.-H.; Wang, Y.-Q.; Zhang, S.; Yang, Q.; Fu, Y.-J. An efficiency strategy for extraction and recovery of ellagic acid from waste chestnut shell and its biological activity evaluation. *Microchemical Journal* **2021**, *160*, 105616.

1
2
3
4