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

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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 ~10^5 CFU/mL of microorganisms. Sterile paper discs (6 mm) were placed on the inoculated culture medium and impregnated with 10 µL of each extract. Seven concentrations of extract between 0.3% and 2.1% were tested. The plates were incubated for 24h at 37ºC. 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* monocytogenesATCC 7973 (8.32±0.06 mm for 2.1%), *Enterococcus faecalis* 19433 (8.94 ±0.41 mm for 2.1%), and *Staphylococcus aureus* ATCC (10.26±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

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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 60ºC, vacuum-packed and refrigerated (2ºC) until extraction.

2.2. Extraction

The extract was obtained using ultrasound assisted technique at a controlled temperature (40ºC) 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 10^8 coloning 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 37ºC/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
Escherichia coli O157:H7 9001	-	BHI, 37ºC, 24h/48h (610495)	TBX (Tryptone Bile X-Glucuronide-84637), 44ºC, 24h
Yersinia enterocolitica ATCC 9018	-	BHI, 37ºC, 24h/48h (610495)	Yersinia Selective Agar Base-VWR, 30ºC, 24h
Staphylococcus aureus ATCC	+	BHI, 37ºC, 24h/48h (610495)	BP (Baird Parker-VWR), 37ºC, 24h

Salmonella ATCC	-	BHI, 37ºC, 24h/48h (610495)	Chromagar *Salmonella*, 37ºC, 24h
Salmonella Typhimurium 14028	-	BHI, 37ºC, 24h/48h (610495)	Chromagar *Salmonella*, 37ºC, 24h
Enterococcus faecalis 19433	+	BHI, 37ºC, 24h/48h (610495)	VRBG (Violet Red Bile Glucose-VWR), 30ºC 24h/48h
Enterococcus faecium 20477	+	BHI, 37ºC, 24h/48h (610495)	VRBG (Violet Red Bile Glucose-VWR), 30ºC 24h/48h
Listeria monocytogenes ATCC 7973	+	BHI, 37ºC, 24h/48h (610495)	Chromagar *Listeria*, 37ºC, 24h
Listeria ATCC 7644	+	BHI, 37ºC, 24h/48h (610495)	Chromagar *Listeria*, 37ºC, 24h
Pseudomonas JI-Me-LM03	-	BHI, 37ºC, 24h/48h (610495)	CFC (Pseudomonas selective agar), 30ºC, 48h
Escherichia coli ATCC 1175	-	BHI, 37ºC, 24h/48h (610495)	TBX (Tryptone Bile X-Glucuronide-84637), 44ºC, 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**
Escherichia coli O157:H7 9001	-	-	-	-
Yersinia enterocolitica ATCC 9018	-	-	-	-
Staphylococcus aureus ATCC	-	-	-	10.26±0.19
Salmonella ATCC	-	-	-	-
Salmonella Typhimurium 14028	-	-	-	-
Enterococcus faecalis 19433	-	7±0.25	7.74±0.25	8.94±0.41
Enterococcus faecium 20477	-	-	-	-
Listeria monocytogenes ATCC 7973	-	6.22±0.18	6.99±0.11	8.32±0.06
Listeria ATCC 7644	-	-	-	-
Pseudomonas JI-Me-LM03	-	-	-	-
Escherichia coli 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 (10.26 mm), *Enterococcus faecalis* 19433 (7 mm – 8.94 mm), *Listeria monocytogenes* ATCC 7973 (6.22 mm- 8.32 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.

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

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