

Article

Potential of Portuguese Viticulture By-Products as Natural Resources of Bioactive Compounds – Antioxidant and Antimicrobial Activities

Ana F. Vinha ^{1,2,3}, Carla Sousa ^{1,2,3}, Andreia Vilela ^{1,†}, Joana Ferreira ^{1,†}, Rui Medeiros ^{1,2,4} and Fátima Cerqueira ^{1,2,4,*}

¹ Faculty of Health Sciences, University Fernando Pessoa, Rua Carlos da Maia, 296, 4200-150 Porto, Portugal; acvinha@ufp.edu.pt (A.F.V.); sousasil@ufp.edu.pt (C.S.); afvilela29@gmail.com (A.V.); joanacferreira2899@gmail.com (J.F.); ruimedei@ipporto.min-saude.pt (R.M.)

² FP-I3ID, FP-BHS, GIT-LoSa, University Fernando Pessoa, Praça 9 de Abril, 349, 4249-004 Porto, Portugal

³ LAQV/REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira, 228, 4050-313 Porto, Portugal

⁴ Molecular Oncology and Viral Pathology GRP—IC, Portuguese Institute of Oncology of Porto (IPO Porto), Rua António Bernardino de Almeida, 4200-072 Porto, Portugal

* Correspondence: fatimaf@ufp.edu.pt

† These authors contributed equally to this work.

Abstract: *Vitis vinifera* is the grape variety used in the production of wine and other products. In the wine production process, many of the vine's by-products are wasted, namely seeds and stems. Given the proportion of wine production worldwide, the quantity of by-products is beginning to be an environmental problem, making it urgent to take measures for their use to obtain bioactive compounds with health benefits. The aim of this work was to study the antioxidant and antimicrobial activities of extracts from the seeds and stems of four Portuguese grape varieties: Touriga Franca, Touriga Nacional, Viosinho, and Tinta Roriz. Total phenolic (TPC) and total flavonoids (TFC) contents present in the different extracts were evaluated, as well as the antioxidant activity, by DPPH and FRAP methods. TPC and TFC values of the stem's extracts are much higher than those of the seeds of the same grape variety in the same solvent. The antioxidant activity of aqueous and ethanolic stem extracts is higher than that obtained for the seeds, showing that antioxidant activity is related to the content of polyphenols. The antimicrobial activity of different stem and seed extracts was determined against yeasts and Gram-positive and Gram-negative bacteria, and the effect was determined based on the minimal inhibitory concentrations calculated (MIC). In general, the ethanol:water (1:1) extract of the seeds from the different varieties tested inhibited *C. albicans* ATCC10231 and *C. krusei* ATCC6258 growth even at 200 µg/mL, and the effect was fungicidal at 200 µg/mL. The same type of extract showed selective antimicrobial activity, inhibiting *S. aureus* ATCC29213 growth but having no effect against *E. coli* ATCC25922 even at 200 µg/mL. The effect against *S. aureus* was bactericidal (at 200 µg/mL) for Touriga Franca, Touriga Nacional, and Viosinho. Taking all these results into account, it can be concluded that the by-products of the grape varieties tested are important sources of bioactive products, particularly as antioxidants and antimicrobials.

Keywords: by-products; vine; Touriga Franca; Touriga Nacional; Viosinho; Tinta Roriz; antioxidants; phenolics compounds; antibacterial; antifungal

Citation: Vinha, A.F.; Sousa, C.; Vilela, A.; Ferreira, J.; Medeiros, R.; Cerqueira, F. Potential of Portuguese Viticulture By-Products as Natural Resources of Bioactive Compounds—Antioxidant and Antimicrobial Activities. *Appl. Sci.* **2024**, *14*, 6278. <https://doi.org/10.3390/app14146278>

Academic Editors: Vittorio Gentile and Francesca Silvagno

Received: 8 June 2024

Revised: 8 July 2024

Accepted: 15 July 2024

Published: 18 July 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Winemaking has a historical connection to Portugal as an agricultural activity of great economic and social importance. According to data reported by the International Organization of Vine and Wine (OIV), in 2022, total global wine production was estimated at 258 million hectoliters [1]. During winemaking, 30% of the total quantity of grapes

corresponds to wine by-products, representing approximately 20 million tons of which 50% corresponds to the European Union (EU), that is, 10 million of the residues are produced by countries that are part of the EU [2]. Therefore, for this purpose, the valorization of wine residues and by-products presents itself as an emerging need based on the concept of sustainability. In fact, the identification of good practices for the efficient use of resources and the recovery of waste and agri-food by-products constitutes one of the main objectives of a sustainable economy [3–5], and it is necessary that production and consumption change to a “circular economy” model, which defends the idea of reducing waste and reusing waste, recycling it, reducing economic expenses, and reducing environmental damage [4,6].

Products obtained from different parts of the vine, namely stems, leaves, skin, seeds, and pomace, have been the subject of studies that demonstrate the importance of their use and beneficial effects on health, mainly in the prevention of cardiovascular diseases, cancer, diabetes, intestinal disorders, inflammatory diseases, and hemorrhages [7–9]. The main phytochemical compounds present in grapevine by-products are stilbenoids, phenolic compounds (gallic acid, catechin, myricetin, and kaempferol-3-O-rutinoside), aromatic acids (hydroxycinnamic and hydroxybenzoic acids), flavonoids, and proanthocyanidins [10,11]. These compounds have the potential to delay or promote the inhibition of substrate oxidation [12].

Grapevine stems are made up of around 30% cellulose, 21% hemicellulose, around 17% lignin, 15–16% tannins, and approximately 6% proteins [13], also presenting a high content of essential minerals [14]. The stem, which corresponds to almost 25% of the total by-products, is the least valued of all the by-products generated but the richest in phenolic compounds [9,15]. Grape seeds have a very rich nutritional profile, consisting of around 40% dietary fiber, 16% essential oils, 11% proteins, and 7% phenolic compounds [16]. The main phenolic compounds present in seeds are flavan-3-ols [17]. The composition of minerals [14] and phenolic compounds in vineyard by-products is influenced by growing conditions, climatic factors, geographic location, cultivation practices, and the stage of maturation [17,18].

Phenolic compounds are known for their antioxidant, anti-inflammatory, and antimicrobial potential [16,19], and are abundant in grapes. Due to these beneficial properties, extracts obtained from grape residues, which are rich in phenolic compounds, can help reduce the growth of pathogenic microorganisms that cause degradation and diseases, in addition to inhibiting lipid oxidation [16], enhancing their use in the development of various products, like medical, cosmetic, and food applications [16,19,20]. Flavonoids have antioxidant, anti-inflammatory, antiviral, antibacterial, antidiabetic, neuroprotective, cardioprotective, and anticancer properties [21–23], being capable of inhibiting virulence factors of microorganisms and presenting a synergistic effect with conventional antibiotics [16]. The stems have biological activities such as antimicrobial, antioxidant, and even anti-inflammatory [15,18]. Due to the high content of phenolic compounds, the seeds have antioxidant and antimicrobial activity [16].

Portugal, which comprises 14 wine regions and 31 protected denominations of origin, is one of the countries with the largest number of national grape varieties [24]. In this work, three red wine varieties (Touriga Franca, Touriga Nacional, and Tinta Roriz) and a white wine variety (Viosinho) were studied. Seed oils from the Touriga Franca and Touriga Nacional varieties are rich in derivatives of the vitamin E family and fatty acids, presenting antioxidant activity [25]. These two varieties, as well as Tinta Roriz, being a red wine, have a higher concentration of resveratrol than white wine varieties [26]. According to Costa et al. [27], Tinta Roriz stems are richer in phenolic compounds than those of Touriga Franca, showing higher values of TPC, TFC, and antioxidant capacity. The stem of the Viosinho variety has high antioxidant activity and bioactivity against Gram-positive bacteria; these properties are associated with its composition of monomeric proanthocyanidins, catechins, flavonoids, and derivatives of hydroxycinnamic acids and anthocyanins [24,28].

For all these reasons, the by-products of the vineyard are potential sources of antimicrobial compounds and are therefore an important resource not only in terms of food and economics but also in terms of health [16,19,20,28].

This study evaluated the antimicrobial activities of extracts obtained from vine by-products, such as stems and seeds, from different Portuguese grape varieties: Touriga Franca, Touriga Nacional, Viosinho, and Tinta Roriz.

2. Materials and Methods

2.1. Samples

To perform this study, extracts of seeds and stems from four grape varieties (Touriga Nacional, Touriga Franca, Viosinho, and Tinta Roriz) were used. The seeds and stems were collected in October 2021 in Sociedade Agrícola Trigo de Negreiros Lda, Bragança, Portugal. A significant sample of approximately 10 kg of seeds and another sample of the same amount of stems were collected. The seeds and stems were dried at room temperature, protected from light. After confirming dehydration at <10% humidity, the seeds and stems were completely crushed separately with the help of a mill (GM GrinDomix 200, Retsh, Haan, Germany) for 120 s at 5000 rpm to obtain a fine powder. These powders were stored in hermetically sealed bottles, protected from light.

2.2. Extracts Preparation

To prepare the seed and stem extracts, 6.5 mg of each sample was pulverized in 100 mL of extracting solvent (pure water, ethanol, and an ethanol:water solution (50:50 *v/v*)). All samples were subjected to a solid/liquid extraction process for 1 h at 45 °C with constant stirring [29]. Then, filtration was carried out, collecting 10 mL of each of the extracts, which were stored at -20 °C until used. With these extracts, total phenolic compounds (TPC) and total flavonoids (TFC) were determined, and antioxidant activity was evaluated using two colorimetric methods (DPPH radical inhibition and ferric ion reduction (FRAP)). All assays were performed in triplicate.

2.3. Total Phenolic Content

The TPC determination followed the spectrophotometric methodology described by [30]. To 30 µL of extract, 150 µL of Folin–Ciocalteu reagent solution 1:10 *v/v* and 120 µL of sodium carbonate solution (7.5%) were added, homogenizing the resulting solution, which was incubated at 45 °C directly in the microplate reader for 15 min and protected from light. The absorbance values were read at 765 nm. Gallic acid was used as a standard. To obtain the correlation between the absorbance of the samples and the concentration of the standard, a calibration curve was performed ($r^2 = 0.9991$). The values obtained in the study were quantified in milligrams of gallic acid equivalents per 100 g of dry extract (mg GAE/100 g of dry extract).

2.4. Total Flavonoid Content

To determine this parameter, a colorimetric test was used, which involves the formation of a flavonoid–aluminum complex at 510 nm [30]. For every 30 µL of each extract, 75 µL of distilled water and 45 µL of 1% sodium nitrite solution were added. The mixture was left to react for 5 min and after this time, 45 µL of a 5% aluminum chloride solution was added. Subsequently, 60 µL of sodium hydroxide (1M) and 45 µL of distilled water were added. Catechin was used as a standard. To read the absorbances, a microplate reader was used. Different concentrations of catechin were used to obtain a calibration curve ($r^2 = 0.9994$). The results were presented as the amount of flavonoid found in the extract, expressed in milligrams of catechin equivalents per 100 g of dry extract (mg CE/100 g of dry extract).

2.5. Antioxidant Activity

2.5.1. DPPH Method

The free radical scavenging capacity (DPPH•) was evaluated using the method described by Costa et al. [29] with some modifications. 270 µL of DPPH• 6×10^{-5} M solution was added to 30 µL of Trolox standard, blank, or diluted extract (1:10). The decrease in DPPH• concentration was measured in a microplate reader at intervals of 10 min, monitoring the decrease in the corresponding absorbance at 515 nm. The end of the reaction occurred after 20 min. A calibration curve was plotted with Trolox ($r^2 = 0.9972$). DPPH• radical scavenging activity was expressed in micromoles of Trolox equivalents per 100 g of dry sample (µmol ET/100 g of dry sample).

2.5.2. FRAP Method

This study was carried out based on the work of Costa et al. [30], with minor modifications. Approximately 265 µL of FRAP reagent (0.3 M acetate buffer, 10 mM TPTZ solution, and 20 mM ferric chloride) was mixed with 35 µL of ferrous sulfate standard (5–600 µM), blank, or diluted extracts (1:10). The previous mixture was then kept at 37 °C for 30 min, protected from light. The absorbances were read at 595 nm on a microplate reader. A calibration curve was plotted with ferrous sulfate ($r^2 = 0.9998$), with the antioxidant power expressed in micromoles of ferrous sulfate equivalents per 100 g of dry sample (µmol FSE/100 g of dry sample).

2.6. Microorganisms

The antifungal activity of the strata was tested against *Candida albicans* ATCC10231 and *Candida krusei* ATCC6258, while the antibacterial activity was tested against *Staphylococcus aureus* ATCC29213 and *Escherichia coli* ATCC25922.

Yeast subcultures were performed in Sabouraud dextrose broth (SDB) (Sigma-Aldrich; St. Louis, MO, USA), to which agar (Liofilchem; Roseto Degli Abruzzi, Italy) was added. Bacteria were maintained in Mueller Hinton II broth (MHB-II) (Sigma-Aldrich; St. Louis, MO, USA), to which agar was also added.

2.7. Preparing Suspensions of Extracts to Antimicrobial Tests

The dried extracts were resuspended in dimethyl sulfoxide (DMSO; Merck KGaA; Darmstadt, Germany) to a concentration of 5 mg/mL and stored at -20 °C until testing.

2.8. Antifungal Activity

The antifungal activity of the compounds was assessed using a modified broth microdilution technique carried out in 96-well culture plates [31]. After 24 h of growth on SDA, the yeasts were suspended in saline solution (Merck KGaA; Darmstadt, Germany) at a density of 0.5 MacFarland, and two dilutions were performed in SDB (a 1:50 dilution followed by a 1:20 dilution). The concentration of the extracts was adjusted in SDB and added (1:1) to the yeast suspension, with the maximum concentration of extract tested being 200 µg/mL. In all the experiments, a positive growth control (yeast only) and a sterility control of the medium, or negative control (medium only), were carried out. After 48 h of incubation in an incubator at 35 °C, the plates were observed, and the minimum inhibitory concentration (MIC) for yeasts was defined as the lowest concentration that caused a total reduction in fungal growth by visual observation. In order to determine if the extracts had a fungicidal or fungistatic effect, they were tested at 200 µg/mL, as performed for MIC determination. After 48 h incubation, 10 µL of suspension was transferred to an SDA plate. The plates were placed in the incubator at 35 °C, and yeast growth was observed after 24 h of incubation. The effect was defined as fungicidal or fungistatic, depending on the absence or presence of yeast growth, respectively.

2.9. Antibacterial Activity

The bacteria, which had been cultured 24 h before the experiment on MH-II agar, were suspended in saline solution until an optical density of 0.5 MacFarland was obtained, and two dilutions were performed in MH-II broth (a 1:50 dilution followed by a 1:20 dilution) [32]. To evaluate the antibacterial activity of the extracts, dilutions were made in MH-II broth, which was added (1:1) to the previously prepared suspension of bacteria. The maximum concentration of extract tested was 200 µg/mL, and serial dilutions of 1:2 were carried out in 96-well plates. The plates were incubated for 24 h at 35 °C, and the MIC was defined as the lowest concentration that inhibited the growth of the bacteria, and the results were read visually. In order to determine if the extracts had a bactericidal or bacteriostatic effect, they were tested at 200 µg/mL, as performed for MIC determination. After 24 h incubation, 10 µL of suspension was transferred to an MH-II agar plate. The plates were placed in the incubator at 35 °C, and bacterial growth was observed after 24 h of incubation. The effect was defined as bactericidal or bacteriostatic depending on the absence or presence of bacterial growth, respectively.

3. Results

3.1. Total Phenolic and Flavonoid Compounds Content

Considering the chemical characterization and polarity of the different bioactive compounds present in the seeds and stems of the studied samples, extracts for bioactive compound determination were prepared in different solvents (aqueous, ethanolic, and hydroalcoholic).

According to Table 1, regarding the determination of TPC and TFC of seed extracts from the four varieties studied, ethanol is the solvent with the greatest extractive power. According to Table 2, the same pattern of behavior can be seen for the extracts from the stems of the previously mentioned varieties, apart from the TPC of the Tinta Roriz variety. In a general way, water is the solvent that has the lowest extractive power in all tests for determining TPC and TFC in seeds, and this comportment was also verified for the TPC of the stems. For the quantification of TFC from stems, the hydroalcoholic mixture resulted in the lowest concentration values, therefore having a lesser extractive capacity.

Table 1. Content of phenolic compounds, flavonoids, and antioxidant capacity of the seeds of the studied varieties.

Variety	Extractor Solvent	TPC (mg GAE/100 g)	TFC (mg CE/100 g)	DPPH (µmol TE/100 g)	FRAP (µmol FSE/100 g)
Touriga Franca	W	61.3 ± 0.7	37.1 ± 1.4	974.8 ± 1.2	2037.7 ± 1.2
	E	210.8 ± 0.4	70.3 ± 1.1	2096.3 ± 0.5	3756.8 ± 10.0
	E:W (50:50 v/v)	187.6 ± 1.4	61.4 ± 1.0	1749.8 ± 1.1	3019.0 ± 1.0
Touriga Nacional	W	74.8 ± 1.0	93.9 ± 1.0	1041.7 ± 1.4	2984.7 ± 0.3
	E	393.1 ± 1.8	195.0 ± 6.4	3323.6 ± 3.5	3780.9 ± 1.6
	E:W (50:50 v/v)	264.4 ± 4.4	102.0 ± 1.1	2791.5 ± 1.1	3064.7 ± 0.6
Viosinho	W	60.6 ± 0.8	37.5 ± 2.3	400.0 ± 1.9	791.0 ± 1.8
	E	80.2 ± 1.3	41.2 ± 0.8	792.1 ± 2.7	1490.7 ± 8.9
	E:W (50:50 v/v)	66.0 ± 2.0	27.7 ± 0.5	601.8 ± 0.8	1022.5 ± 1.3
Tinta Roriz	W	72.4 ± 0.7	40.2 ± 1.1	494.4 ± 1.1	1040.6 ± 0.9
	E	106.3 ± 6.7	62.1 ± 0.5	1005.3 ± 1.5	2032.1 ± 0.9
	E:W (50:50 v/v)	99.1 ± 0.8	54.4 ± 0.6	949.4 ± 1.0	1981.1 ± 1.1

W, water; E, ethanol; E:W, ethanol:water. TPC, total phenolic content; TFC, total flavonoids content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reduction activity potential. Values presented as mean ± standard deviation ($n = 3$).

Table 2. Content of phenolic compounds, flavonoids, and antioxidant capacity of the stems of the studied varieties.

Variety	Extractor Solvent	TPC (mg GAE/100 g)	TFC (mg CE/100 g)	DPPH ($\mu\text{mol TE}/100\text{ g}$)	FRAP ($\mu\text{mol FSE}/100\text{ g}$)
Touriga Franca	W	4306.4 \pm 328.1	969.8 \pm 118.0	2603.1 \pm 140.2	40,991.6 \pm 3633.2
	E	58,115.8 \pm 563.9	2051.9 \pm 107.9	4329.8 \pm 412.9	62,633.7 \pm 4390.6
	E:W (50:50 v/v)	14,184.7 \pm 749.8	311.3 \pm 28.0	930.6 \pm 85.7	954.0 \pm 203.5
Touriga Nacional	W	2885.1 \pm 209.3	1144.8 \pm 26.4	2824.5 \pm 376.7	38,842.3 \pm 4356.8
	E	5528.0 \pm 447.5	2753.0 \pm 214.0	4842.5 \pm 562.7	68,822.8 \pm 5178.2
	E:W (50:50 v/v)	3943.8 \pm 328.3	850.4 \pm 33.3	1279.9 \pm 172.6	11,470.4 \pm 1927.6
Viosinho	W	3817.5 \pm 184.2	1361.0 \pm 105.6	3817.5 \pm 184.2	43,637.1 \pm 484.7
	E	5865.5 \pm 598.4	2460.0 \pm 368.7	5865.5 \pm 598.4	66,871.0 \pm 173.2
	E:W (50:50 v/v)	3954.6 \pm 392.4	361.3 \pm 59.2	3954.6 \pm 392.4	9379.7 \pm 150.1
Tinta Roriz	W	4873.2 \pm 319.2	1519.6 \pm 92.3	4873.2 \pm 319.2	54,544.3 \pm 4496.0
	E	6892.3 \pm 458.6	2776.7 \pm 192.8	6892.3 \pm 458.6	71,024.5 \pm 2752.3
	E:W (50:50 v/v)	15,292.3 \pm 543.0	232.7 \pm 10.6	15,292.3 \pm 543.0	8662.0 \pm 853.7

W, water; E, ethanol; E:W, ethanol:water. TPC, total phenolic content; TFC, total flavonoids content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reduction activity potential. Values presented as mean \pm standard deviation ($n = 3$).

Comparing the TPC values of the stems of the four grape varieties under study (Table 2), the extracts from Tinta Roriz present higher values in water and in hydroalcoholic mixtures, showing Touriga Franca to have a higher content in ethanol. The Tinta Roriz variety presents the highest TFC values, while Touriga Franca shows the lowest content when in aqueous or ethanolic extract.

It can also be seen that the TPC and TFC values of the seeds are much lower than those of the stems of the same grape variety when the extracts are prepared with the same solvent.

3.2. Antioxidant Activity by FRAP and DPPH Methods

The analysis of the antioxidant capacity of different extracts constitutes a correlation tool with the content of total phenolics and flavonoids and also allows the assessment of the specific radical scavenging capacity attributable to the extracts under study. The results were expressed by carrying out two methods: FRAP, which detects the presence of antioxidants through the reduction of iron, and DPPH, which consists of the reaction of the radical of the molecule with the antioxidant substances in the extracts of the four varieties in the three solvents.

Regarding the antioxidant activity of seed extracts (Table 1), determined both by the DPPH and FRAP tests, Touriga Nacional presents higher values in any of the solvents, with the ethanolic extract being the one with higher concentrations for all the varieties. Comparing the antioxidant activity of the four stem varieties (Table 2), Tinta Roriz extracts show greater antioxidant potential in both analytical methods when the solvent is water or ethanol. For this grape variety, the hydroalcoholic mixture obtained greater activity in the DPPH method, with the ethanolic extract being superior in the FRAP method. In general, the antioxidant activity determined by both methods is higher for stems, except for Touriga Nacional (DPPH) and Touriga Franca (DPPH and FRAP) grape varieties hydroalcoholic extracts.

3.3. Antifungal Activity

The antifungal activity of the extracts of seeds and stems obtained from the different vine varieties was tested against *C. albicans* and *C. krusei*. The inhibitory capacity of the extracts to inhibit yeast growth (MIC) was evaluated (Table 3).

Table 3. Antifungal activity of the extracts prepared from the seeds of the different varieties tested.

Variety	Yeast	Extract	MIC ($\mu\text{g/mL}$)
Touriga Franca	<i>C. albicans</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	>200.0
	<i>C. krusei</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	17.5 \pm 6.9
Touriga Nacional	<i>C. albicans</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	100.0 \pm 0.0
	<i>C. krusei</i>	W	>200.0
		E	>200.0
		E:W	10.0 \pm 3.4
Viosinho	<i>C. albicans</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	200.0 \pm 0.0
	<i>C. krusei</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	8.8 \pm 3.4
Tinta Roriz	<i>C. albicans</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	>200.0
	<i>C. krusei</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	35.0 \pm 13.7

MIC, minimal inhibitory concentration; W, water; E, ethanol; E:W, ethanol:water. Values are mean \pm SD ($n = 3\text{--}5$).

Stem extracts from the different varieties tested, independently of the solvent used for the preparation, were not able to inhibit the yeast growth even when tested at concentrations as high as 200.0 $\mu\text{g/mL}$.

Considering the extracts obtained from the seeds, the aqueous and ethanolic extracts showed a high degree of antifungal activity (maximum tested concentration: 200.0 mg/mL). With regard to yeasts, the E:W extract of the different varieties inhibited the growth of *C. krusei*. *C. albicans* growth was only inhibited by the E:W extract prepared from the seeds of Touriga Nacional and Viosinho.

At 200.0 $\mu\text{g/mL}$, the E:W extract of the seeds of all the varieties showed fungicidal activity against *C. krusei*. With the exception of the E:W extract from Tinta Roriz, the same fungicidal effect was registered for *C. albicans* (200.0 $\mu\text{g/mL}$).

3.4. Antibacterial Activity

The antibacterial effect of the extracts of seeds and stems was also tested against a Gram-positive bacterium, *S. aureus*, and a Gram-negative bacterium, *E. coli* (Table 4).

The extracts obtained from the stems of the different vine varieties showed no antibacterial activity, even at the maximum concentration tested of 200 µg/mL.

In the case of the E:W extracts obtained from the seeds, they proved to be active against *S. aureus* but had no effect on the growth of *E. coli* even at 200 µg/mL.

Table 4. Antibacterial activity of the extracts prepared from the seeds of the different varieties tested.

Variety	Yeast	Extract	MIC (µg/mL)
Touriga Franca	<i>S. aureus</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	100.0 ± 0.0
	<i>E. coli</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	>200.0
Touriga Nacional	<i>S. aureus</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	40.0 ± 13.7
	<i>E. coli</i>	W	>200.0
		E	>200.0
		E:W	>200.0
Viosinho	<i>S. aureus</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	50.0 ± 35.4
	<i>E. coli</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	>200.0
Tinta Roriz	<i>S. aureus</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	100.0 ± 0.0
	<i>E. coli</i>	W	>200.0
		E	>200.0
		E:W (50:50 v/v)	>200.0

MIC, minimal inhibitory concentration; W, water; E, ethanol; E:W, ethanol:water. Values are mean ± SD ($n = 3-5$).

In terms of mechanism of action, and when tested at 200 µg/mL, the E:W extract of Touriga Nacional, Touriga Franca, and Viosinho seeds is bactericidal for *S. aureus*. For Tinta Roriz, at 200 µg/mL, the effect of the E:W extract varied between bacteriostatic and bactericidal.

4. Discussion

According to the TPC and TFC values, in general, ethanol is the solvent with the greatest extractive capacity, both for the seeds and the stems. The choice of extracting solvent influences the extraction process and the determination of phenolic compounds [33]. Methanol is frequently used as an extracting solvent, but due to its toxicity [34], we decided not to use it, which may have affected the effectiveness of the extraction process and the concentration of phenolic compounds, as reported in several studies [19,35,36]. In this work, in addition to water, ethanol was used, since the latter occurs naturally in wine, has low toxicity, and is therefore permitted by the food industry [27]. Comparing the TPC

and TFC values of seeds and stems of the same variety in the same solvent, it can be seen that the concentrations of these compounds are much higher in the stems. According to Simonetti et al. [17], this is another aspect to consider since each grape variety and each specific part of the vine have a different composition, and due to the polarity and affinity of the compounds present in each of them, the extraction of these compounds does not follow the same pattern, even when the solvents used in the extraction process are the same.

Dorosh et al. [37] compared TPC and TFC of ethanol:water 50:50 (*v/v*) stem extracts of Touriga Nacional and Tinta Roriz obtained by ultrasound-assisted extraction conditions. In a general way, the Tinta Roriz variety presented higher TPC and TFC values, which contradicts our results for flavonoid quantification. Costa et al. [27], in turn, compared the concentration of total phenolics and flavonoids in hydroalcoholic 50:50 (*v/v*) extracts of stems of Touriga Franca (56.17 ± 1.16 mg GAE/g DW and 40.52 ± 0.05 mg CE/g DW, respectively) and Tinta Roriz (64.73 ± 1.40 mg GAE/g DW and 43.76 ± 3.92 mg CE/g DW, respectively) concluded that the last variety has a higher content of the referred biocompounds, what is not in agreement with the results of this work. It is also possible to verify that our TPC values are higher, while our TFC values are lower than those presented by Costa et al. [27]. These divergences can be related to the different extractive conditions, growing conditions, cultivation practices, and the stage of grape maturation [17].

Other studies have been carried out regarding the quantification of phenolic compounds and flavonoids from seeds in red varieties, such as Pinot Noir, Negro Amaro, Cabernet Sauvignon [38], and Marselan [39], among others, and the values of these compounds vary greatly from each other [38]. For example, studies carried out by Krasteva et al. [39] showed that extracts from Cabernet Sauvignon grape seeds, crushed to powder, in a 70% aqueous ethanol solution, had TPC values of 88.22 ± 0.72 mg GAE/g of dry extract. For the Marselan variety, the TPC values were 103.24 ± 1.28 mg GAE/g of dry extract and for Pinot Noir, 79.06 ± 0.65 mg EAG/g of dry extract. In the case of TFC, the values varied between 45.95 ± 0.14 mg CE/g of dry extract (Cabernet Sauvignon) and 52.01 ± 0.34 mg CE/g of dry extract (Marselan), lower than the respective values of TPC, which agrees with our results, except for the aqueous extract of Touriga Nacional. However, considering that the grape varieties and the method of extraction are different from those used in our work, it is not possible to make a direct comparison with the results we obtained.

Blackford et al. [40] compiled values for the content of phenolic compounds in stem extracts of several red grape varieties, namely Cabernet Sauvignon, Manto Negro, Mandilaria, and Tempranillo, among others. According to this review article, for example, TPC values for the Cabernet Sauvignon variety ranged between 348 and 7076 mg GAE/100 g of dry extract; for Mandilaria, between 1057 and 1434.3 mg GAE/100 g of dry extract; and for Tempranillo, between 4679 and 7622 mg GAE/100 g of dry extract, depending on the harvest year. In addition to the grape harvest year, which is very dependent on climatic factors, the composition of phenolic compounds depends on cultivation conditions and practices, geographic location, and stage of maturation [17,18], which is also reflected in the composition of the by-products. As mentioned above, for seeds, it is not possible to make a direct comparison with the results we obtained.

According to some studies, red wine varieties have higher levels of flavonoids and phenolic compounds [41–44]. This is in line with what was found by us for seeds, but not when comparing the Touriga Nacional (red) and Viosinho (white) grape varieties stems, which is in accordance with Costa et al. [27]. The quantitative differences found in the phenolic composition of the by-products, regardless of the extracting solvent, prove that each type of red or white grape by-product has a particular phenolic composition, which can be related to different winemaking practices [18,33,36,45]. For example, according to Mosele et al. [45], the by-products obtained from the carbonic maceration of grapes

contain smaller amounts of phenolic compounds than those obtained through conventional fermentation.

The breathing process and, in general, all oxidative reactions that occur in cells and use oxygen lead to the formation of free radicals, which are unstable molecules or ions that can cause cellular damage and consequently be harmful to the body. These damages are associated with various health problems, such as inflammation, tumors, Alzheimer's disease, and cardiovascular diseases. The antioxidants present in plants can act as reducing agents and scavengers of these free radicals, as well as enzyme inhibitors and metal chelators. The main antioxidants present in vegetables are phenolic compounds, especially flavonoids, as well as vitamins C and E and carotenoids [46,47]. Therefore, antioxidant activity can be related to the content of polyphenols, that is, the higher the concentration of these compounds, the greater the antioxidant activity [18,27,37]. In the present study, this correlation is verified in seed extracts, regardless of the variety and solvent used. As for the stem extracts, this pattern of behavior does not always occur for the hydroalcoholic solvent, but the amount of flavonoids seems to have a greater influence on the antioxidant activity of the Touriga Nacional and Touriga Franca varieties.

Blackford et al. [40] collected results from several studies on the antioxidant activity of stem extracts from various grape varieties, namely Touriga Nacional, Touriga Franca, Tinta Roriz, and Viosinho, among others. Using the FRAP method, Touriga Nacional and Tinta Roriz presented values of 257.8 and 235.3 mg TE/g of dry extract, correspondingly, while Viosinho and Touriga Franca exhibited 24 and 30 mM TE/100 g of dry extract, respectively. Since the extraction and quantification conditions are different from those of our study, it is not possible to compare the results. In the case of antioxidant activity, there is no specific analytical method widely used for its evaluation. However, there are several studies determining the antioxidant activity of extracts from stems and seeds of different red grape varieties, such as Pinot Noir, Negro Amaro, Castelão, and Tinto Cão, among others [38–40,48]. Krasteva et al. [39] evaluated the antioxidant activity of extracts from Pinot Noir grape seeds using the DPPH method, using a 70% aqueous ethanol solution as an extracting solvent, and obtained $579.33 \pm 4.15 \mu\text{M TE/g}$ of dry extract. According to Rockenbach et al. [38] the antioxidant activity of extracts from seeds of several varieties was evaluated using a mixture of methanol/water/acetic acid (80:20:5) as an extracting solvent, using the DPPH and FRAP methods. For the DPPH test, for example, the Pinot Noir variety had values of $16,925 \pm 189 \mu\text{mol TE/100 g}$ of dry extract, and Negro Amaro presented values of $7265 \pm 95 \mu\text{mol TE/100 g}$ of dry extract. For the FRAP test, the Pinot Noir variety presents values of $21,492 \pm 47 \mu\text{mol Fe}^{2+}/100 \text{ g}$ of dry extract, and the Negro Amaro of $9447 \pm 49 \mu\text{mol Fe}^{2+}/100 \text{ g}$ of dry extract. The values obtained by the FRAP method are higher than those of the DPPH, which is generally also verified in our study for seeds and stems. This was already expected since DPPH is a colorimetric method subject to various interferences.

Fungi and bacteria are responsible for most infections and the mortality associated with them [49,50], namely because of antimicrobial resistance [49–57].

The antimicrobial activity of parts of the vine has already been described in several scientific papers [17,18,26,33,44]. Some parts of the grapevine, such as grape seeds, grapes, and pomace, have been identified as having antifungal activity against yeasts and filamentous fungi, and the mechanisms of action described were diverse, such as altering ergosterol synthesis, interfering with fungal enzyme activity, reducing fungal cell adherence, or inducing fungus apoptosis [17]. For bacteria, the interference of compounds from grapes with Gram-positive and Gram-negative strains was also referred to [18], and one of the mechanisms of action already reported for seeds, pomace, stems, or leaves, among others, was the interference with bacterial biofilms [58]. In our work, and for all the varieties tested, the hydroalcoholic extract of the grains was the only one that showed antimicrobial activity, while the others showed no activity at the maximum concentration of $200 \mu\text{g/mL}$. However, when tested at the same concentration, none of the stalk extracts showed antimicrobial activity. According to our results, ethanol is the solvent that allows

the extraction of the greatest content of phenolic compounds and flavonoids. However, the extract that showed the best activity was hydroalcoholic. Furthermore, the phenolic and flavonoid contents were lower in seeds than in stems. It seems, based on our results, that the antimicrobial activity does not seem to depend on the total phenolic or flavonoid content of the extract. One explanation for these results could be that the type of phenols and flavonoids extracted will be more important than their total content.

Concerning the antimicrobial activity, our results showed a selectivity of the antimicrobial activity against the Gram-positive bacteria tested when compared to the Gram-negative. Our results are in accordance with others already available in the literature [59]. This fact can be due to the different structures and compositions of the bacterial cell walls of the two groups of bacteria [59].

Our work reinforces the potential of *Vitis vinifera* by-products as antioxidant and antimicrobial agents.

Author Contributions: Conceptualization, A.F.V., C.S. and F.C., investigation, A.F.V., C.S., A.V., J.F., R.M. and F.C., writing—original draft preparation, A.F.V., C.S., A.V. and J.F., writing—review and editing, A.F.V., C.S., A.V., J.F., R.M. and F.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded and sponsored by the national funds of FCT/MCTES—Foundation for Science and Technology I.P. from the Ministry of Science, Technology, and Higher Education (PIDDAC), the Research Center of the Portuguese Oncology Institute of Porto (project no. PI86-CI-IPOP-66-2019)] and Fundação Fernando Pessoa.

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: This work received support and help from FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020), through national funds.

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

References

1. OIV 2023. State of the World Vine and Wine Sector in 2022. Available online: https://www.oiv.int/sites/default/files/documents/OIV_State_of_the_world_Vine_and_Wine_sector_in_2022_2.pdf (accessed on 20 September 2023).
2. Ferrer-Gallego, R.; Silva, P. The Wine Industry By-Products: Applications for Food Industry and Health Benefits. *Antioxidants* **2022**, *11*, 2025. <https://doi.org/10.3390/antiox11102025>.
3. Kazakova, E.; Lee, J. Sustainable Manufacturing for a Circular Economy. *Sustainability* **2022**, *14*, 17010. <https://doi.org/10.3390/su142417010>.
4. Niculescu, V.-C.; Ionete, R.-E. An Overview on Management and Valorisation of Winery Wastes. *Appl. Sci.* **2023**, *13*, 5063. <https://doi.org/10.3390/app13085063>.
5. Chatzimitakos, T.; Athanasiadis, V.; Kalompatsios, D.; Mantiniotou, M.; Bozinou, E.; Lalas, S.I. Pulsed Electric Field Applications for the Extraction of Bioactive Compounds from Food Waste and By-Products: A Critical Review. *Biomass* **2023**, *3*, 367–401. <https://doi.org/10.3390/biomass3040022>.
6. Almulhim, A.I. Toward a Greener Future: Applying Circular Economy Principles to Saudi Arabia's Food Sector for Environmental Sustainability. *Sustainability* **2024**, *16*, 786. <https://doi.org/10.3390/su16020786>.
7. Constantin, O.E.; Stoica, F.; Ratu, R.N.; Stănciuc, N.; Bahrin, G.E.; Râpeanu, G. Bioactive Components, Applications, Extractions, and Health Benefits of Winery By-Products from a Circular Bioeconomy Perspective: A Review. *Antioxidants* **2024**, *13*, 100. <https://doi.org/10.3390/antiox13010100>.
8. Caldas, A.R.; Catita, J.; Machado, R.; Ribeiro, A.; Cerqueira, F.; Horta, B.; Medeiros, R.; Lúcio, M.; Lopes, C.M. Omega-3- and Resveratrol-Loaded Lipid Nanosystems for Potential Use as Topical Formulations in Autoimmune, Inflammatory, and Cancerous Skin Diseases. *Pharmaceutics* **2021**, *13*, 1202. <https://doi.org/10.3390/pharmaceutics13081202>.
9. Moldovan, M.L.; Carpa, R.; Fizes, I.; Vlase, L.; Bogdan, C.; Iurian, S.M.; Benedec, D.; Pop, A. Phytochemical Profile and Biological Activities of Tendrils and Leaves Extracts from a Variety of *Vitis vinifera* L. *Antioxidants* **2020**, *9*, 373. <https://doi.org/10.3390/antiox9050373>.
10. Prusova, B.; Licek, J.; Kumsta, M.; Baron, M.; Sochor, J. Polyphenolic composition of grape stems. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2020**, *48*, 1543–1559. <https://doi.org/10.15835/nbha48311936>.

11. Moreira, M.M.; Barroso, M.F.; Porto, J.V.; Ramalhosa, M.J.; Švarc-Gajić, J.; Estevinho, L.; Morais, S.; Delerue-Matos, C. Potential of Portuguese vine shoot wastes as natural resources of bioactive compounds. *Sci. Total Environ.* **2018**, *634*, 831–842. <https://doi.org/10.1016/j.scitotenv.2018.04.035>.
12. Dani, C.; Oliboni, L.S.; Agostini, F.; Funchal, C.; Serafini, F.; Henriques, J.A.; Salvador, M. Phenolic content of grapevine leaves (*Vitis labrusca* var. Bordo) and its neuroprotective effect against peroxide damage. *Toxicol. Vitro.* **2010**, *24*, 148–153. <https://doi.org/10.1016/j.tiv.2009.08.006>.
13. Prozil, S.O.; Evtuguin, D.V.; Lopes, L.P.C. Chemical and structural characterization of grape stalks and evaluation of its potential as lignocellulosic raw materials. *Ind. Crops Prod.* **2013**, *35*, 178–184. <https://doi.org/10.1016/j.indcrop.2011.06.035>.
14. Leal, C.; Costa, C.M.; Barros, A.I.R.N.A.; Gouvinhas, I. Assessing the Relationship Between the Phenolic Content and Elemental Composition of Grape (*Vitis vinifera* L.) Stems. *Waste Biomass Valorization* **2021**, *12*, 1313–1325. <https://doi.org/10.1007/s12649-020-01090-6>.
15. Leal, C.; Gouvinhas, I.; Santos, R.A.; Rosa, E.; Silva, A.M.; Saavedra, M.J.; Barros, A.I.R.N.A. Potential application of grape (*Vitis vinifera* L.) stem extracts in the cosmetic and pharmaceutical industries: Valorization of a by-product. *Ind. Crops Prod.* **2020**, *154*, 112675. <https://doi.org/10.1016/j.indcrop.2020.112675>.
16. Mattos, G.N.; Tonon, R.V.; Furtado, A.A.L.; Cabral, L.M.C. Grape by-product extracts against microbial proliferation and lipid oxidation: A review. *J. Sci. Food Agric.* **2017**, *97*, 1055–1064. <https://doi.org/10.1002/jsfa.8062>.
17. Simonetti, G.; Brasili, E.; Pasqua, G. Antifungal Activity of Phenolic and Polyphenolic Compounds from Different Matrices of *Vitis vinifera* L. against Human Pathogens. *Molecules* **2020**, *25*, 3748. <https://doi.org/10.3390/molecules25163748>.
18. Leal, C.; Santos, R.A.; Pinto, R.; Queiroz, M.; Rodrigues, M.; Saavedra, M.J.; Barros, A.; Gouvinhas, I. Recovery of bioactive compounds from white grape (*Vitis vinifera* L.) stems as potential antimicrobial agents for human health. *Saudi J. Biol. Sci.* **2020**, *27*, 1009–1015. <https://doi.org/10.1016/j.sjbs.2020.02.013>.
19. Troilo, M.; Difonzo, G.; Paradiso, V.M.; Summo, C.; Caponio, F. Bioactive Compounds from Vine Shoots, Grape Stalks, and Wine Lees: Their Potential Use in Agro-Food Chains. *Foods* **2021**, *10*, 342. <https://doi.org/10.3390/foods10020342>.
20. Michailidis, D.; Angelis, A.; Nikolaou, P.E.; Mitakou, S.; Skaltsounis, A.L. Exploitation of *Vitis vinifera*, *Foeniculum vulgare*, *Cannabis sativa* and *Punica granatum* By-Product Seeds as Dermo-Cosmetic Agents. *Molecules* **2021**, *26*, 731. <https://doi.org/10.3390/molecules26030731>.
21. Nassiri-Asl, M.; Hosseinzadeh, H. Review of the Pharmacological Effects of *Vitis vinifera* (Grape) and its Bioactive Constituents: An Update. *Phytother. Res.* **2016**, *30*, 1392–1403. <https://doi.org/10.1002/ptr.5644>.
22. Ullah, A.; Munir, S.; Badshah, S.L.; Khan, N.; Ghani, L.; Poulson, B.G.; Emwas, A.-H.; Jaremko, M. Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules* **2020**, *25*, 5243. <https://doi.org/10.3390/molecules25225243>.
23. Shamsudin, N.F.; Ahmed, Q.U.; Mahmood, S.; Shah, S.A.A.; Sarian, M.N.; Khattak, M.M.A.K.; Khatib, A.; Sabere, A.S.M.; Yusoff, Y.M.; Latip, J. Flavonoids as Antidiabetic and Anti-Inflammatory Agents: A Review on Structural Activity Relationship-Based Studies and Meta-Analysis. *Int. J. Mol. Sci.* **2022**, *23*, 12605. <https://doi.org/10.3390/ijms232012605>.
24. Fraga, H.; Atauri, I.G.C.; Malheiro, A.C.; Moutinho-Pereira, J.; Santos, J. Viticulture in Portugal: A review of recent trends and climate change projections. *Oeno One* **2017**, *51*, 61–69. <https://doi.org/10.20870/oeno-one.2017.51.2.1621>.
25. Fernandes, L.; Casal, S.; Cruz, R.; Pereira, J.A.; Ramalhosa, E. Seed oils of ten traditional Portuguese grape varieties with interesting chemical and antioxidant properties. *Food Res. Int.* **2013**, *50*, 161–166. <https://doi.org/10.1016/j.foodres.2012.09.039>.
26. Vestergaard, M.; Ingmer, H. Antibacterial and antifungal properties of resveratrol. *Int. J. Antimicrob. Agents* **2019**, *53*, 716–723. <https://doi.org/10.1016/j.ijantimicag.2019.02.015>.
27. Costa, R.D.; Domínguez-Perles, R.; Abraão, A.; Gomes, V.; Gouvinhas, I.; Barros, A.N. Exploring the Antioxidant Potential of Phenolic Compounds from Winery By-Products by Hydroethanolic Extraction. *Molecules* **2023**, *28*, 6660. <https://doi.org/10.3390/molecules28186660>.
28. Schwartz, C.G.K.; de Jesus, J.L.L.; Ramos, F.A.P.; Mezalira, T.S.; Ferreira, R.G.; Otutumi, L.K.; Soares, A.A. Compostos bioativos do bagaço de uva (*Vitis vinifera*): Seus benefícios e perspectivas para o desenvolvimento sustentável. *Tecnologia de Alimentos: Tópicos Físicos, Químicos e Biológicos* **2020**, *1*, 485–500. <https://doi.org/10.37885/200700653>.
29. Costa, A.S.G.; Alves, R.C.; Vinha, A.F.; Barreira, S.V.P.; Nunes, M.A.; Cunha, L.M.; Oliveira, B.B.P.P. Optimization of antioxidants extraction from coffee silverskin, a roasting by-product, having in view a sustainable process. *Ind. Crops Prod.* **2014**, *53*, 350–357. <https://doi.org/10.1016/j.indcrop.2014.01.006>.
30. Costa, A.S.G.; Alves, R.C.; Vinha, A.F.; Costa, E.; Costa, C.S.G.; Nunes, M.A.; Almeida, A.A.; Santos-Silva, A.; Oliveira, M.B.P.P. Nutritional, chemical and antioxidant/pro-oxidant profiles of silverskin, a coffee roasting by-product. *Food Chem.* **2018**, *267*, 28–35. <https://doi.org/10.1016/j.foodchem.2017.03.106>.
31. Almada, S.; Maia, L.B.; Waerenborgh, J.C.; Vieira, B.J.C.; Mira, N.P.; Silva, E.R.; Cerqueira, F.; Pinto, E.; Alves, L.G. Cyclam-based iron(III) and copper(II) complexes: Synthesis, characterization and application as antifungal agents. *New J. Chem.* **2022**, *46*, 16764–16770.
32. Sousa, C.; Gabriel, C.; Cerqueira, F.; Manso, M.C.; Vinha, A. Coffee industrial waste as a natural source of bioactive compounds with antibacterial and antifungal activities. In *The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs*; Formatex Research Center: Barcelona, Spain, 2015; pp. 131–136.
33. Vázquez-Armenta, F.J.; Silva-Espinoza, B.A.; Cruz-Valenzuela, M.R.; González-Aguilar, M.R.; Nazzaro, F.; Fratianni, F.; Ayala-Zavala, J.F. Antibacterial and antioxidant properties of grape stem extract applied as disinfectant in fresh leafy vegetables. *J. Food Sci. Technol.* **2017**, *54*, 3192–3200. <https://doi.org/10.1007/s13197-017-2759-5>.

34. Joshi, D.; Adhikari, N. An Overview on Common Organic Solvents and Their Toxicity. *J. Pharm. Res. Int.* **2019**, *28*, 1–18. <https://doi.org/10.9734/jpri/2019/v28i330203>.
35. Baiano, A. Recovery of Biomolecules from Food Wastes: A review. *Molecules* **2014**, *19*, 14821–14842. <https://doi.org/10.3390/molecules190914821>.
36. Barros, A.; Gironés-Vilaplana, A.; Teixeira, A.; Collado-González, J.; Moreno, D.A.; Gil-Izquierdo, A.; Rosa, E.; Domínguez-Perles, R. Evaluation of grape (*Vitis vinifera* L.) stems from Portuguese varieties as a resource of (poly)phenolic compounds: A comparative study. *Food Res. Int.* **2014**, *65 Part C*, 375–384. <https://doi.org/10.1016/j.foodres.2014.07.021>.
37. Dorosh, O.; Moreira, M.M.; Rodrigues, F.; Peixoto, A.F.; Freire, C.; Morais, S.; Delerue-Matos, C. Vine-Canes Valorisation: Ultrasound-Assisted Extraction from Lab to Pilot Scale. *Molecules* **2020**, *25*, 1739. <https://doi.org/10.3390/molecules25071739>.
38. Rockenbach, I.I.; Gonzaga, L.V.; Rizelio, V.M.; Gonçalves, A.E.S.S.; Genovese, M.I.; Fett, R. Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking. *Food Res. Int.* **2011**, *44*, 897–901. <https://doi.org/10.1016/j.foodres.2011.01.049>.
39. Krasteva, D.; Ivanov, Y.; Chengolova, Z.; Godjevargova, T. Antimicrobial Potential, Antioxidant Activity, and Phenolic Content of Grape Seed Extracts from Four Grape Varieties. *Microorganisms* **2023**, *11*, 395. <https://doi.org/10.3390/microorganisms11020395>.
40. Blackford, M.; Comby, M.; Zeng, L.; Dienes-Nagy, Á.; Bourdin, G.; Lorenzini, F.; Bach, B. A Review on Stems Composition and Their Impact on Wine Quality. *Molecules* **2021**, *26*, 1240. <https://doi.org/10.3390/molecules26051240>.
41. Georgiev, V.; Ananga, A.; Tsoleva, V. Recent Advances and Uses of Grape Flavonoids as Nutraceuticals. *Nutrients* **2014**, *6*, 391–415. <https://doi.org/10.3390/nu6010391>.
42. Sousa, C.; Vinha, A.F.; Nunes, A. *Desperdícios da Vinicultura: Potenciais Aplicações e Sustentabilidade*; Novas Edições Acadêmicas (OmniScriptum AraPers GmbH): Beau-Bassin, Mauritius, 2017; ISBN: 978-3-330-20104-0.
43. Li, L.; Sun, B. Grape and wine polymeric polyphenols: Their importance in enology. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 563–579. <https://doi.org/10.1080/10408398.2017.1381071>.
44. Radojević, I.D.; Mladenović, K.G.; Grujović, M.Ž.; Vasić, S.M. Grape stalks as a source of antioxidant and antimicrobial substances and their potential application. *Bot. Serbica* **2022**, *46*, 179–186. <https://doi.org/10.2298/BOTSERB2202179R>.
45. Mosele, J.; da Costa, B.S.; Bobadilla, S.; Motilva, M.-J. Phenolic Composition of Red and White Wine Byproducts from Different Grapevine Cultivars from La Rioja (Spain) and How This Is Affected by the Winemaking Process. *J. Agric. Food Chem.* **2023**, *71*, 18746–18757. <https://doi.org/10.1021/acs.jafc.3c04660>.
46. Cataneo, C.B.; Caliar, V.; Valdemiro Gonzaga, L.; Marta Kuskoski, E.; Fett, R. Antioxidant activity and phenolic content of agricultural by-products from wine production. *Semin. Ciências Agrárias* **2008**, *29*, 93–101.
47. Silva, M.L.C.; Costa, R.S.; Santana, A.d.S.; Koblit, M.G.B. Compostos fenólicos, carotenóides e atividade antioxidante em produtos vegetais. *Semin. Ciências Agrárias* **2010**, *31*, 669–682.
48. Costa, E.; Cosme, F.; Jordão, A.M.; Mendes-Faia, A. Anthocyanin profile and antioxidant activity from 24 grape varieties cultivated in two Portuguese wine regions. *Oeno One* **2014**, *48*, 51–62. <https://doi.org/10.20870/oeno-one.2014.48.1.1661>.
49. Denning, D.W. Global incidence and mortality of severe fungal disease. *Lancet Infect. Dis.* **2024**, *24*, e428–e438. [https://doi.org/10.1016/S1473-3099\(23\)00692-8](https://doi.org/10.1016/S1473-3099(23)00692-8). PMID: 38224705.
50. Bhattarai, S.; Sharma, B.K.; Subedi, N.; Ranabhat, S.; Baral, M.P. Burden of Serious Bacterial Infections and Multidrug-Resistant Organisms in an Adult Population of Nepal: A Comparative Analysis of Minimally Invasive Tissue Sampling Informed Mortality Surveillance of Community and Hospital Deaths. *Clin. Infect. Dis.* **2021**, *73* (Suppl. 5), S415–S421. <https://doi.org/10.1093/cid/ciab773>. PMID: 34910184; PMCID: PMC8672751.
51. Fisher, M.C.; Alastruey-Izquierdo, A.; Berman, J.; Bicanic, T.; Bignell, E.M.; Bowyer, P.; Bromley, M.; Brüggemann, R.; Garber, G.; Cornely, O.A.; et al. Tackling the emerging threat of antifungal resistance to human health. *Nat. Rev. Microbiol.* **2022**, *20*, 557–571. <https://doi.org/10.1038/s41579-022-00720-1>. PMID: 35352028; PMCID: PMC8962932.
52. GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet* **2022**, *400*, 2221–2248. [https://doi.org/10.1016/S0140-6736\(22\)02185-7](https://doi.org/10.1016/S0140-6736(22)02185-7). PMID: 36423648; PMCID: PMC9763654.
53. *WHO Fungal Priority Pathogens List to Guide Research, Development and Public Health Action*; World Health Organization: Geneva, Switzerland, 2022.
54. *WHO Bacterial Priority Pathogens List, 2024. Bacterial Pathogens of Public Health Importance to Guide Research, Development and Strategies to Prevent and Control Antimicrobial Resistance*; World Health Organization: Geneva, Switzerland, 2024.
55. Jamiu, A.T.; Albertyn, J.; Sebolai, O.M.; Pohl, C.H. Update on *Candida krusei*, a potential multidrug-resistant pathogen. *Med Mycol.* **2021**, *59*, 14–30. <https://doi.org/10.1093/mmy/myaa031>. PMID: 32400853.
56. Hornik, C.D.; Bondi, D.S.; Greene, N.M.; Cober, M.P.; John, B. Review of Fluconazole Treatment and Prophylaxis for Invasive Candidiasis in Neonates. *J. Pediatr. Pharmacol. Ther.* **2021**, *26*, 115–122. <https://doi.org/10.5863/1551-6776-26.2.115>. PMID: 33603574; PMCID: PMC7887891.

57. Lalloo, U.G.; Komarow, L.; Aberg, J.A.; Clifford, D.B.; Hogg, E.; McKhann, A.; Bukuru, A.; Lagat, D.; Pillay, S.; Mave, V.; et al. ACTG A5225 Team. Higher Dose Oral Fluconazole for the Treatment of AIDS-related Cryptococcal Meningitis (HIFLAC)-report of A5225, a multicentre, phase I/II, two-stage, dose-finding, safety, tolerability and efficacy randomised, amphotericin B-controlled trial of the AIDS Clinical Trials Group. *PLoS ONE* **2023**, *18*, e0281580. <https://doi.org/10.1371/journal.pone.0281580>. PMID: 36780493; PMCID: PMC9925064.
58. Bogdan, C.; Pop, A.; Iurian, S.M.; Benedec, D.; Moldovan, M.L. Research Advances in the Use of Bioactive Compounds from *Vitis vinifera* By-Products in Oral Care. *Antioxidants* **2020**, *9*, 502. <https://doi.org/10.3390/antiox9060502>. PMID: 32521718; PMCID: PMC7346141.
59. Pozzo, L.; Grande, T.; Raffaelli, A.; Longo, V.; Weidner, S.; Amarowicz, R.; Karamać, M. Characterization of Antioxidant and Antimicrobial Activity and Phenolic Compound Profile of Extracts from Seeds of Different Vitis Species. *Molecules* **2023**, *28*, 4924. <https://doi.org/10.3390/molecules28134924>.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.