fermentation

MDPI

Article

Exploring the Phytochemical Profiles, and Antioxidant and Antimicrobial Activities of the Hydroethanolic Grape Pomace Extracts from Two Romanian Indigenous Varieties

Alexandru Cristian Grosu [1], Filofteia Camelia Diguță [1,*], Mircea-Cosmin Pristavu [1], Aglaia Popa [1], Florentina Badea [1], Mihaela Dragoi Cudalbeanu [2,*], Alina Orțan [2], Ioan Dopcea [3] and Narcisa Băbeanu [1]

[1] Faculty of Biotechnologies, University of Agronomic Sciences and Veterinary Medicine Bucharest, 59 Marasti Blvd., 011464 Bucharest, Romania; alex_dinescu@yahoo.co.uk (A.C.G.); cosmin_mircea96@yahoo.com (M.-C.P.); aglaia_popa@yahoo.com (A.P.); badea_florentina2003@yahoo.com (F.B.); narcisa.babeanu@biotehnologii.usamv.ro (N.B.)
[2] Faculty of Land Reclamation and Environmental Engineering, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd., 011464 Bucharest, Romania; alina.ortan@fifim.ro
[3] CEBIS International, 47 Bd. Theodor Pallady, 032275 Bucharest, Romania; ioan.dopcea@gmail.com
* Correspondence: camelia.diguta@biotehnologii.usamv.ro (F.C.D.); mcudalbeanu@gmail.com (M.D.C.)

Abstract: In this study, the potential value of dried grape pomace (whole, seed, and skin) obtained from Fetească Neagră (FN) and Tămâioasă Românească (TR) as a source of secondary metabolites was evaluated following hydroethanolic extraction. The total polyphenol, flavonoid, and anthocyanin contents of FN and TR extracts have been determined, along with their antioxidant and antimicrobial activities. The investigation of seeds and the whole pomace FN extracts revealed higher levels of polyphenol, flavonoid, and anthocyanin content in comparison to those extracted from TR. Fifteen polyphenolic compounds were identified through ultra-high-performance liquid chromatography (UHPLC) analysis. The most abundant concentrations of catechin and epicatechin were detected in seed and whole pomace extracts derived from both Romanian grape varieties. The antioxidant activity was higher in the whole pomace and skin extracts derived from FN than those derived from TR. The antimicrobial evaluation demonstrated that 15 out of 18 reference pathogenic bacteria exhibited low MIC and MBC values, indicating a strong antibacterial activity of FN and TR extracts. No anti-*Candida* activity was observed. It can be reasonably deduced that the Fetească Neagră and Tămâioasă Românească by-products represent a sustainable resource for the development of new functional ingredients for the pharmaceutical and food industries, in alignment with the principles of the circular bioeconomy.

Keywords: grape by-products; bioactive compounds; antioxidants; antimicrobial activity; circular economy

Citation: Grosu, A.C.; Diguță, F.C.; Pristavu, M.-C.; Popa, A.; Badea, F.; Dragoi Cudalbeanu, M.; Orțan, A.; Dopcea, I.; Băbeanu, N. Exploring the Phytochemical Profiles, and Antioxidant and Antimicrobial Activities of the Hydroethanolic Grape Pomace Extracts from Two Romanian Indigenous Varieties. *Fermentation* **2024**, *10*, 470. https://doi.org/10.3390/fermentation10090470

Academic Editor: Maurizio Ciani

Received: 8 August 2024
Revised: 23 August 2024
Accepted: 6 September 2024
Published: 11 September 2024

1. Introduction

The wine sector represents a considerable component of the Romanian economy, with an extensive vine area of 159,740 hectares, grape production of 804,800 tons, and wine production of 4.8 million hectoliters in 2022 [1]. The most recent data published by the OIV (International Organization of Vine and Wine), in 2023, indicate that Romania occupies a position among the ten most significant wine producers within the European Union [2]. Concerning the grape varieties, international varieties (such as Cabernet Sauvignon, Chardonnay, Merlot, Sauvignon Blanc, etc.), as well as local varieties (such as Băbească neagră, Busuioacă de Bohotin, Cadarcă, Crâmpoșie, Fetească Regală, Fetească Albă, Fetească Neagră, Grasă de Cotnari, Tămâioasă Românească, etc.), are cultivated in the Romanian wine-growing areas. Among these, two iconic and oldest grape varieties are Fetească Neagră (cultivated on approximately 3028 hectares) and Tămâioasă

Românească (cultivated on almost 1000 hectares), used for producing red wines and white wines characterized by a complex aromatic profile [3]. The winemaking process generates a considerable volume of by-products that, in the absence of appropriate treatment, could cause environmental pollution in several ways, including soil and water contamination, greenhouse gas emissions, and nuisances like odors and pests [4–6]. Consequently, the winemaking industry has been compelled to adopt a circular economic model, which entails zero waste and is intended to facilitate the development of eco-friendly methods for converting by-products into value-added products [7–11]. The main by-product of the winemaking process is grape pomace, which comprises grape seeds, skins, pulp, and dead yeast cells. The precise annual volume of by-products generated by the Romanian wine industry is difficult to ascertain due to fluctuations in wine production, the types of crushed grapes, and the specific production techniques used by different wineries. On average, for every ton of grapes processed, about 20–30% becomes waste, primarily as grape pomace. To illustrate, a work of research was conducted in the climatic conditions of the year 2020 to determine the percentage of grape pomace produced throughout the processing of white and red grapes derived from 25 different grapevine varieties, hybrids, and clones cultivated in the SCDVV Blaj vineyards, situated in Blaj, Craciunelu de Jos, and Ciumbrud. The lowest percentage of pomace was recorded for the Pinot gris 34 Bl clone, at 18.09%. In contrast, the white cultivar Hibernal and the white cultivar Pinot gris 18-5 exhibited the highest quantities of pomace with percentages exceeding 33%. The percentage of pomace obtained for the Fetească Neagră was 19.48% [12]. The grape pomace composition is influenced by several factors, including environmental factors such as terroir, grape variety, the ripening stage, and the manufacturing process itself [5,6,11–15]. The chemical composition of grape pomace is complex, comprising a range of compounds including cellulose, lignin, and protein, representing 27–37%, 16.8–24.2%, and, respectively, $\leq$4% of the total grape pomace compounds [7,8,13,16]. Overall, all grape by-products have been reported to be a valuable source of bioactive compounds, including phenolic acids, anthocyanins, catechins, flavones, procyanidins, tannins, trans-resveratrol, and more [16–20]. As indicated by the findings of Frîncu et al. [9], grape pomace samples derived from six distinct grapevine varieties (also including Tămâioasă Românească and Fetească Neagră) demonstrated elevated concentrations of the relevant compounds. The protein content was found to be 13.93% for the Tămâioasă Românească grape cultivar and 14.80% for Fetească Neagră, while the carbohydrate content was determined to be 15.59 g% for Tămâioasă Românească and 10.42 g% for Fetească Neagră. Additionally, the polyphenol content was quantified at 24.94 mg GAE/g DM for Tămâioasă Românească and 27.41 mg GAE/g DM for Fetească Neagră. Moreover, the antioxidant activity was determined to be 27.65 mg Trolox equivalent/g DM for the Tămâioasă Românească variety and 33.42 mg Trolox equivalent/g DM for the Fetească Neagră variety [9].

A review of the existing literature reveals a variety of conventional techniques for the extraction of biologically active compounds from grape pomace, including maceration, Soxhlet extraction, hydroxide distillation, and solid–liquid extraction [21–25]. The choice of solvents represents a critical parameter that influences the efficacy of extraction processes. Among the solvents employed for polyphenolic compounds extraction, acetone, ethanol, methanol, and water are the most used [26–30]. Moreover, it has been shown that the addition of water as a co-solvent enhances the efficacy of the extraction of phenolic compounds [30,31]. Additionally, more advanced techniques, such as supercritical fluid extraction, pulsed electric field, microwave-assisted extraction, and extrusion technology have the potential to deliver efficient and sustainable results [30–34].

A considerable proportion of documented cases of foodborne illnesses has been identified as being caused by known pathogens, such as *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter*, and *Salmonella*, as well as *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium* species [35–40]. The most frequently reported symptoms associated with foodborne illnesses are abdominal discomfort, vomiting, diarrhea, fever, and nausea. Moreover, the emergence of high-risk multidrug-resistant (MDR) pathogens represents

a significant challenge to global public health, given the growing concern over the declining efficacy of therapies due to prolonged and repeated usage of the antibiotics, as well as the increased likelihood of disease spread and severity of illness, and, in certain instances, elevated mortality rates [36,38–40]. Furthermore, five pathogens were identified as the underlying cause of over 50% of all bacterial deaths, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* [40]. As reported in the latest study by Naghavi et al. [40], *E. coli* and *S. aureus* were identified as the most significant contributors to Romania's disability-adjusted life-years (DALYs). The presence of these pathogenic bacteria represents a considerable challenge for stakeholders within the pharmaceutical and food industries, due to factors including sporulation, the secretion of toxins, and biofilm formation [35–37]. Consequently, the efficacy of probiotics [41–43], postbiotics [44–46], and plant-derived compounds [47–55] as potential alternative antimicrobial agents has attracted the attention of the scientific community. A growing amount of scientific evidence suggests that polyphenolic compounds derived from grapes and grape pomace [16–18,56] have the potential to exhibit significant bioactive properties with multiple health benefits such as antioxidant potential [57–65], antimicrobial activity against a wide spectrum of microbial species [66–73], anticancer and anti-inflammatory activities [57,59,61], and properties that act against skin disorders [74]. These valuable compounds have attracted considerable interest from companies and researchers, to develop eco-friendly and renewable products with applications in a wide range of domains, including pharmaceuticals [16,59,60,75–78], agro-food [19,20,23,79], animal feeds [80,81], cosmetics [74,82–84], vermicomposting as biofertilizers [85,86], and other potential applications.

This study aims to evaluate the antioxidant and antibacterial activities of the compounds extracted from the grape pomace of the Fetească Neagră and Tămâioasă Românească varieties, as well as to determine the main polyphenolic compounds.

2. Materials and Methods

2.1. Grape Pomace Samples

The grape pomace was obtained from the winemaking (2023) of two indigenous grape varieties to România: Fetească Neagră (a red grape variety) and Tămâioasă Românească (a white grape variety) acquired from the "Pietroasa" viticulture and winemaking research and development facility (Buzău, România). The pomace samples were divided into skins and residual pulp (abbreviated as FNE and TRE), seeds (abbreviated as FNS and TRS), and whole pomace (abbreviated as FNT and TRT).

Subsequently, the samples were dried at 45 °C and milled with a laboratory blender, resulting in a particle size of less than 1 mm. The resulting material was then stored in paper bags in a dry and dark place until analysis.

2.2. Extraction Procedure

Pomace extracts were prepared by combining the dry and milled matter with 50% *v*/*v* ethanol/water, in a ratio of 1:10. The extraction procedure involved sonication of the samples in an ultrasonic homogenizer bath (HD 2070 Bandelin Sonopuls, Berlin, Germany) for 2 min at room temperature, followed by maceration at ambient temperature over 24 h with continuous agitation (150 rpm) using a shaking incubator (LSI-3016R, Lab Tech, Jeloud, Tunisia). Subsequently, the extracts were centrifuged for 10 min at 6000 rpm using a centrifuge (Universal 320 R, Hettich, Sérézin du Rhône, France) to separate the extract from the residue and filtered through sterile Millipore 0.22 µm filters (Sartorius, Goettingen, Germany). Finally, the alcoholic fraction was removed under vacuum at 40 °C using a rotary evaporator (Büchi R-210, Flawil, Switzerland) and freeze-dried using a FreeZone 6 (Labconco, Kansas City, MO, USA). The freeze-dried extracts were then stored at a temperature of +4 °C until further analysis.

2.3. Evaluation of Phenolic Profile and Content

2.3.1. The Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined using a modified Folin–Ciocâlteu assay, as previously described by Arlet et al. [53]. The diluted samples were combined with 500 µL of Folin–Ciocâlteu reagent and incubated for 8 min in the dark. Subsequently, a solution of sodium carbonate (7.5%) was added and the samples were incubated for an additional two hours in the dark. Then, the absorbances were measured at 765 nm. A standard curve was obtained by reading the absorbances of gallic acid solution displaying concentrations ranging from 50 to 500 µg/mL. The total phenolic content was expressed in terms of milligram-equivalent gallic acid (mg GAE) per gram of the analyzed sample.

2.3.2. Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was quantified using an aluminium chloride complexation assay, as described by Pękal and Pyrzynska [87] and Giurescu et al. [88]. The samples were diluted with sodium acetate (10%) and, following centrifugation and filtration, were mixed with 2 mL of aluminium chloride (2.5%). Incubation for 45 min in the dark was subsequently performed. Absorbance readings were recorded at a wavelength of 420 nm. A standard curve was obtained by reading the absorbances of rutin solution exhibiting a concentration range of 10–160 µg/mL. The total flavonoid content was quantified in milligram-equivalent rutin per gram of the sample, expressed as mg RE/g.

2.3.3. Total Anthocyanin Content (TAC)

The total anthocyanin content (TAC) was estimated using a method proposed by Teng et al. [89]. Consequently, the samples were diluted with a potassium chloride solution (25 mM) with a pH of 1.00. The anthocyanin content was expressed as milligram cyanidin-3-glucoside (cyd-3-glu) equivalents per gram of sample (mg cyd-3-gluE/g) using the following equation:

$$\text{TAC (mg cyd-3-gluE/g DM)} = \frac{\text{A} * \text{MW} * \text{DF} * 1000}{\varepsilon * \text{l}} \quad (1)$$

where:

A = absorbance at 520 nm; MW (molecular weight) = 449.2 g/mol for cyd-3-glu; DF = dilution factor; ε = 26 900 M extinction coefficients (L $\times$ mol^{-1} $\times$ cm^{-1}) for cyd-3-glu; 1 = path length in cm.

2.3.4. UHPLC Analysis

The UHPLC analysis was performed using an Acquity UHPLC I Class system equipped with a photodiode array (PDA) detector (Waters Corporation, Milford, MA, USA). The column used was Zorbax Eclipse Plus C18, 4.6 $\times$ 150 mm, 5 µm. The 10 µL of samples were injected using a flow-through-needle autosampler, and two solvents, (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile, were used in a gradient state as the mobile phase. The elution conditions were optimized: 0–100% (B) for 30 min, and the flow rate was 0.8 mL/min. The column temperature was maintained at 35 °C and the autosampler at 20 °C. The detector was set at 280 nm, 320 nm, and 370 nm wavelengths. Fifteen reference compounds, gallic acid, tannic acid, chlorogenic acid, catechin, caffeic acid, epicatechin, p-coumaric acid, isoquercetin, ferulic acid, naringin, rosmarinic acid, myricetin, luteolin, quercetin, and naringenin, were used to identify and quantify the polyphenolic compounds in a linear range between 5–150 µg/mL. Therefore, 280 nm was used for gallic acid, tannic acid, catechin, epicatechin, naringin, and naringenin; 320 nm was used for chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, and rosmarinic acid; and 370 nm was used for isoquercetin, myricetin, luteolin, and quercetin. The reference compounds and grape pomace extracts were run through the column under the same conditions, and the concentration was calculated using the calibration curves. The results were expressed for each specific polyphenolic compound as µg/g of the grape samples.

2.4. DPPH Antioxidant Assay

The antioxidant activity was determined using the DPPH (1-Diphenyl-2-picrylhydrazine) assay, following Brand-Williams et al. [90] with some modifications. The process involved vigorously mixing the 0.05 mL extract samples with 2.950 mL of a 100 µM DPPH solution (dissolved in pure ethanol) (Aldrich, Merck KGaA, Darmstadt, Germany), followed by incubation in a dark environment for 30 min at room temperature. Subsequently, the mixture was centrifuged at 6000× *g* for 10 min to allow for the separation of the different components. To guarantee the accuracy and reliability of the findings, the control samples were replaced by an equivalent volume of distilled water. In the control sample, the DPPH was exchanged for an equivalent volume of absolute alcohol. An equal volume of distilled water and absolute ethanol was employed as a calibration standard to enable the assessment of the results' accuracy and precision. The absorbance of the supernatant at 515 nm (OD515) was measured using a UV-1800 spectrophotometer (ChromTech, Minneapolis, MN, USA). The percentage of radical scavenging activity was estimated using the following equation:

$$AA\% = \frac{ABSDPPH - ABSsample}{ABSDPPH} \times 100 \quad (2)$$

where: AA (%) = antioxidant activity (%); ABSDPPH = the absorbance of DPPH solution without any sample; ABSsample = the absorbance of the mixture of the DPPH solution and sample.

2.5. Antibacterial Activity

2.5.1. Microorganisms

The antimicrobial activity of the FN and TR extracts has been tested against 13 Gram-positive bacteria (*B. cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Ent. faecium* ATCC 6057, *Ent. hirae* ATCC 10541, *Listeria innocua* ATCC 33090, *L. ivanovii* ATCC 19119, *L. monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 33592 (MRSA), *S. epidermidis* ATCC 12228, *S. epidermidis* ATCC 51625 (MRSE), *Streptococcus pyogens* ATCC 19615, *Rhodococcus* equi ATCC 6939), 5 Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas. aeruginosa* ATCC 27853, *Salmonella enterica* serovar Enteretidis ATCC 14028, *Salmonella enterica* serovar Typhimurium ATCC 14028, and *Serratia marcescens* ATCC 14756) and 4 *Candida* spp. (*C. albicans* ATCC 10231, *C. parapsilosis* ATCC 20019, *C. glabrata* ATCC 2001, and *C. tropicalis* ATCC 44508). The reference bacteria were cultivated in trypticase soya broth (TSB; Alliance Bio Expertise, Guipry Messac, France), and *Candida* strains were cultivated in potato dextrose broth (PDB; Alliance Bio Expertise, Guipry Messac, France) at 30 °C for 24 h.

2.5.2. Plate Screening of Antimicrobial Activity of the Extract Samples

The antimicrobial spectrum of the FN and TR extracts was evaluated against a panel of 18 pathogenic bacteria and 4 *Candida* spp. using the agar well diffusion assay, as outlined by Balouiri et al. [91] with minor modifications. Briefly, a solution of 1 ml of an overnight pathogen culture (with an optical density (OD600) value of 0.3 ± 0.05, corresponding to approximately 10^7–10^8 cfu/mL) was added to a sterile Petri dish (of diameter 90 mm). Subsequently, the dishes were filled with 25 mL of Tryptic Soy Agar (TSA; Alliance Bio Expertise, Guipry Messac, France) or Potato Dextrose Agar (PDA; Alliance Bio Expertise, Guipry Messac, France), respectively, at a temperature of 42–45 °C. Next, the Petri dishes were mixed gently until the culture medium had solidified. Wells with a diameter of six millimeters were punctured with a sterile needle. Each well was filled with 100 µL of the FN and TR extracts. The negative control was represented by ethanol at a concentration of 50% (*v*/*v*). Following this step, the Petri dishes were incubated at the optimal temperature for 24 h. The antibacterial activity was defined as a clear zone of inhibition around the well, which was then measured in millimetres (mm) using a ruler.

2.5.3. Microdilution Technique

The sensitivity of pathogenic bacteria to the FN and TR extracts was established through the microdilution method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines, with minor modifications [92]. The pathogenic bacteria were inoculated in TSB and incubated overnight at 37 °C. The cells' viability was determined by a plate count agar. The average concentration of the FN extracts ranged from 37.11 to 0.29 mg/mL, while the average concentration of the TR extracts ranged from 57.83 to 0.45 mg/mL. A total volume of 40 µL of each extract was added to the initial Eppendorf tube. Subsequently, a series of tubes was prepared in which the concentration of the extract decreased in proportion to a twofold dilution factor in Mueller–Hinton broth (Tulip Diagnostics (P) LTD., Verna, India), and then 4 µL of cell suspension (10^5 cfu/mL) was added to each tube. The Mueller–Hinton broth inoculated with pathogenic bacteria served as the positive control, while the uninoculated MHB represented the negative control. The Eppendorf tubes were incubated for 24 h at a temperature of 37 °C. Thereafter, 20 µL of TTC (2,3,5-triphenyltetrazolium chloride, Merck KGaA, Darmstadt, Germany), a growth indicator, was added to each tube and incubated for 30 min to assess whether bacterial growth was inhibited. A visual observation was conducted to ascertain whether the unstained TTC had undergone a colour change, from its original state to a pink-red formazan, in the presence of bacteria. The lowest concentration of the extract that demonstrated the inhibition of bacterial growth was designated as the minimum inhibitory concentration (MIC). To ascertain the minimum bactericidal concentration (MBC), 10 µL was extracted from each dilution and inoculated onto a spot of Mueller–Hinton agar. Subsequently, the plates were incubated at 37 °C for 24 h. The lowest extract concentration capable of killing bacteria was designated the minimum bactericidal concentration (MBC).

2.6. Statistical Analysis

All experiments were conducted in triplicate. The results were presented as mean ± standard deviation (SD). The results for the phenolic composition, antioxidant activity, and antimicrobial activity were subjected to statistical analysis using the IBM SPSS Statistics software, version 29 (IBM Corp., Armonk, NY, USA). Comparisons between samples for all parameters were tested by ANOVA and post hoc Tukey test or Kruskal–Wallis test and Mann–Whitney U-tests, depending on the type of variables. All tests were two-sided and the level of significance was 5%.

3. Results

3.1. Phytochemical Profile

3.1.1. Determination of Total Phenolic, Flavonoid, and Anthocyanin Content

The phytochemical screening results revealed the presence of many secondary metabolites responsible for several biological activities. The analysis confirmed the presence of all classes of investigated phytoconstituents in all the samples taken into the study, regardless of the grape variety or the type of pomace component (Table 1).

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), and total anthocyanin content (TAC) values for grape hydroalcoholic extracts.

Grape Variety	Samples	TPC (mg GAE/g)	TFC (mg RE/g)	TAC (mg cyd-3-gluE/g)
Fetească Neagră	FNT	81.81 ± 3.86 ^{cd}	75.77 ± 5.02 ^{bc}	34.01 ± 2.21 ^{b}
	FNE	78.61 ± 3.83 ^{c}	75.09 ± 2.93 ^{bc}	36.75 ± 2.66 ^{b}
	FNS	93.52 ± 3.06 ^{f}	86.86 ± 7.24 ^{d}	11.18 ± 3.25 ^{a}
Tămâioasă Românească	TRT	45.34 ± 2.33 ^{b}	38.05 ± 3.5 ^{a}	1.69 ± 0.76 ^{a}
	TRE	27.72 ± 0.04 ^{a}	25.71 ± 0.71 ^{a}	1.06 ± 0.32 ^{a}
	TRS	90.43 ± 0.50 ^{ef}	61.01 ± 3.27 ^{b}	1.48 ± 0.56 ^{a}

The values are expressed as the mean ± the standard deviation from three independent experiments. Statistically significant differences ($p < 0.05$) were indicated by different lowercase letters.

Table 1 reveals a statistically significant variation ($p < 0.05$) in total phenolic content between the grape pomace samples (whole pomace, skin, and seeds) derived from both indigenous Romanian grape cultivars. The results of this study demonstrate that the yield of total phenolic content depends on both the specific grape variety and the type of grape pomace component.

The total phenolic content exhibited considerable variation, with values ranging between 27.72 ± 0.04 and 78.61 ± 3.83 mg GAE/g for the skin extracts and between 45.34 ± 2.33 and 81.81 ± 3.86 mg GAE/g for the whole grape pomace extracts derived from both Romanian grape cultivars (Table 1). The grape seed samples exhibited a markedly elevated level of total phenol content, with values of 90.43 ± 0.50 mg GAE/g for the Tămâioasă Românească cultivar and 93.52 ± 3.06 mg GAE/g for the Fetească Neagră cultivar.

In the case of seed extracts, it was observed that Fetească Neagră exhibited a higher total flavonoid content (86.86 ± 7.24 mg RE/g) than Tămâioasă Românească (61.01 ± 3.27 mg RE/g). Furthermore, the total flavonoid content of FNT and FNE extracts was found to be similar and notably higher than the corresponding values obtained for TR extracts (Table 1).

The total anthocyanin content was found to be significantly higher in the FNT and FNE extracts and lower in the FNS extract, respectively. Nevertheless, it is notable that all extracts derived from the Tămâioasă Românească variety exhibited a relatively low anthocyanin content, with values ranging between 1.06 ± 0.32 and 1.69 ± 0.76 mg cyd-3-gluE/g.

3.1.2. Bioactive Compounds Identification through UHPLC Analysis

Table 2 presents the findings of the UHPLC analysis of the polyphenolic compounds present in the extracts of whole pomaces, skins, and seeds (FN and TR extracts). This analysis identified and quantified 15 distinct polyphenolic compounds, including the following phenolic acids—gallic acid, tannic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, and rosmarinic acid—and the following flavonoids—catechin, epicatechin, isoquercetin, naringin, myricetin, luteolin, quercetin, and naringenin.

Table 2. UHPLC analysis of polyphenolic compounds present in grape pomace extracts.

No.	Compound Name	Compound Class	λ_{max} (nm)	TR (min)	Grape Pomace Extracts (μg/g)					
					FNT	FNE	FNS	TRT	TRE	TRS
1	GAL	Phenolic acids	280	3.10	7.65 ± 0.13 ^{a}	17.82 ± 0.42 ^{c}	26.32 ± 0.46 ^{d}	17.17 ± 0.92 ^{c}	10.96 ± 0.06 ^{b}	34.01 ± 0.02 ^{e}
2	TAN		280	3.44	50.04 ± 1.02 ^{d}	73.06 ± 0.28 ^{e}	20.87 ± 0.24 ^{a}	23.24 ± 57 ^{b}	19.99 ± 0.01 ^{a}	34.31 ± 0.36 ^{c}
3	CLO		320	6.65	0.13 ± 0.01 ^{d}	0.16 ± 0.00 ^{e}	0.05 ± 0.00 ^{b}	0.04 ± 0.00 ^{ab}	0.03 ± 0.00 ^{a}	0.07 ± 0.00 ^{c}
4	CAF		320	9.16	0.14 ± 0.01 ^{d}	0.14 ± 0.00 ^{d}	0.09 ± 0.00 ^{b}	0.09 ± 0.00 ^{b}	0.04 ± 0.00 ^{a}	0.11 ± 0.00 ^{c}
5	COU		320	12.71	0.12 ± 0.01 ^{bc}	0.14 ± 0.00 ^{c}	0.10 ± 0.01 ^{b}	0.05 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}	0.17 ± 0.01 ^{d}
6	FER		320	14.17	0.11 ± 0.00 ^{d}	0.11 ± 0.01 ^{d}	0.05 ± 0.00 ^{b}	0.04 ± 0.00 ^{b}	0.02 ± 0.00 ^{a}	0.07 ± 0.00 ^{c}
7	ROS		320	18.73	0.52 ± 0.04 ^{cd}	0.58 ± 0.00 ^{d}	0.29 ± 0.00 ^{b}	0.25 ± 0.01 ^{b}	0.17 ± 0.00 ^{a}	0.45 ± 0.02 ^{c}
8	CAT		280	7.09	103.38 ± 1.70 ^{c}	48.59 ± 0.58 ^{b}	298.78 ± 0.47 ^{e}	170.41 ± 0.92 ^{d}	19.48 ± 0.71 ^{a}	450.60 ± 0.85 ^{f}
9	EPI		280	9.65	197.82 ± 0.85 ^{d}	152.03 ± 0.35 ^{c}	226.63 ± 0.52 ^{e}	117.94 ± 0.57 ^{b}	23.06 ± 0.28 ^{a}	355.00 ± 7.07 ^{f}
10	NA	Flavonoid	280	16.38	31.69 ± 0.92 ^{c}	28.16 ± 0.23 ^{b}	48.22 ± 0.46 ^{d}	31.22 ± 0.31 ^{c}	17.32 ± 0.46 ^{a}	61.99 ± 0.01 ^{e}
11	NAR		280	23.32	2.66 ± 0.14 ^{c}	3.06 ± 0.09 ^{d}	0.30 ± 0.00 ^{a}	0.89 ± 0.01 ^{b}	2.60 ± 0.06 ^{c}	1.00 ± 0.01 ^{b}
12	ISO		370	13.84	75.11 ± 1.20 ^{d}	87.42 ± 0.60 ^{e}	10.53 ± 0.30 ^{a}	70.63 ± 0.52 ^{c}	103.22 ± 0.32 ^{f}	22.28 ± 0.40 ^{b}
13	MYR		370	18.96	12.09 ± 0.13 ^{d}	11.72 ± 0.23 ^{c}	1.36 ± 0.03 ^{a}	1.37 ± 0.01 ^{a}	1.04 ± 0.06 ^{a}	2.19 ± 0.04 ^{b}
14	LUT		370	21.66	5.43 ± 0.21 ^{d}	5.78 ± 0.03 ^{d}	1.38 ± 0.01 ^{a}	2.75 ± 0.07 ^{b}	2.63 ± 0.11 ^{b}	3.16 ± 0.14 ^{c}
15	QUE		370	21.83	28.89 ± 0.21 ^{e}	24.50 ± 0.03 ^{d}	3.06 ± 0.08 ^{a}	12.82 ± 0.14 ^{c}	13.34 ± 0.49 ^{c}	5.94 ± 0.08 ^{b}

The mean retention time (TR) error for polyphenolic compounds was ± 0.0001–0.20 min. Gallic acid—GAL; Tannic acid—TAN; Chlorogenic acid—CLO; Catechin—CAT; Caffeic acid—CAF; Epicatechin—EPI; Coumaric acid—COU; Isoquercetin—ISO; Ferulic acid—FER; Naringin—NA; Rosmarinic acid—ROS; Myricetin—MYR; Luteolin—LUT; Quercetin—QUE; Naringenin—NAR. The values are expressed as the mean ± the standard deviation from three independent experiments. Statistically significant differences ($p < 0.05$) were indicated by different lowercase letter.

The identified phenolic acids present in the FN and TR extracts are divided into hydroxybenzoic acids and hydroxycinnamic acids. The hydroxybenzoic acids were identified in descending order as tannic acid, followed by gallic acid. The FNE extract contains the highest quantity of tannic acid (73.06 ± 0.28 μg/g), followed by the FNT extract (50.04 ± 1.02 μg/g). The FNS extract and TR extracts (TRT, TRE, and TRS) contain a lower content of tannic acid (<50 μg/g). The highest quantity of gallic acid was observed in the TRS extract (34.01 ± 0.02 μg/g), followed by the FNS extract (26.32 ± 0.46 μg/g).

The extracts of whole pomaces (FNT and TRT) and grape skins (FNE and TRE) contain gallic acid in the range of 7.65 ± 0.13–17.82 ± 0.42 µg/g. The hydroxycinnamic acids (chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, and rosmarinic acid) in the FN and TR extracts are found in lower quantity than hydroxybenzoic acids, in the range of 0.02 ± 0.00–0.58 ± 0.00 µg/g.

The identified flavonoids present in the FN and TR extracts are divided into flavonols, flavanols, flavanones, and flavones. As expected, the extracts of grape seeds contain the highest quantity of flavanols, catechin (TRS—450.60 ± 0.85 µg/g; FNS—298.78 ± 0.47 µg/g), and epicatechin (TRS—355.00 ± 7.07 µg/g; FNS—226.63 ± 0.52 µg/g), followed by the extracts of whole pomaces (FNT and TRT) and grape skins (FNE and TRE). Similar to flavanols, the naringin flavanone is also found in the highest amount in the extracts of grape seeds (TRS—61.99 ± 0.01 µg/g; FNS—48.22 ± 0.46 µg/g). Regarding the other types of flavonoids, for example, naringenin, isoquercetin, myricetin, luteolin, and quercetin, they are present in higher quantities in the extracts of whole pomaces (FNT and TRT) and grape skins (FNE and TRE) than in the grape seeds extracts (FNS and TRS).

The extracts of grape seeds (FNS and TRS) contain the highest quantity of polyphenolic compounds (phenolic acids and flavonoids) compared to the extracts of whole pomaces (FNT and TRT) and grape skins (FNE and TRE). The chromatogram of the 15 distinct identified polyphenolic compounds in the FNS extract is reported in Figure 1: gallic acid (compound #1–3.054 min); tannic acid (compound #2–3.607 min); chlorogenic acid (compound #3–6.246 min); catechin (compound #4–6.962 min); caffeic acid (compound #5–9.030 min); epicatechin (compound #6–9.543 min); p-coumaric acid (compound #7–12.680 min); isoquercetin (compound #8–13.637 min); ferulic acid (compound #9–14.111 min); naringin (compound #10–16.243 min); rosmarinic acid (compound #11–18.637 min); myricetin (compound #12–18.953 min); luteolin (compound #13–21.554 min); quercetin (compound #14–21.746 min); and naringenin (compound #10–23.268 min).

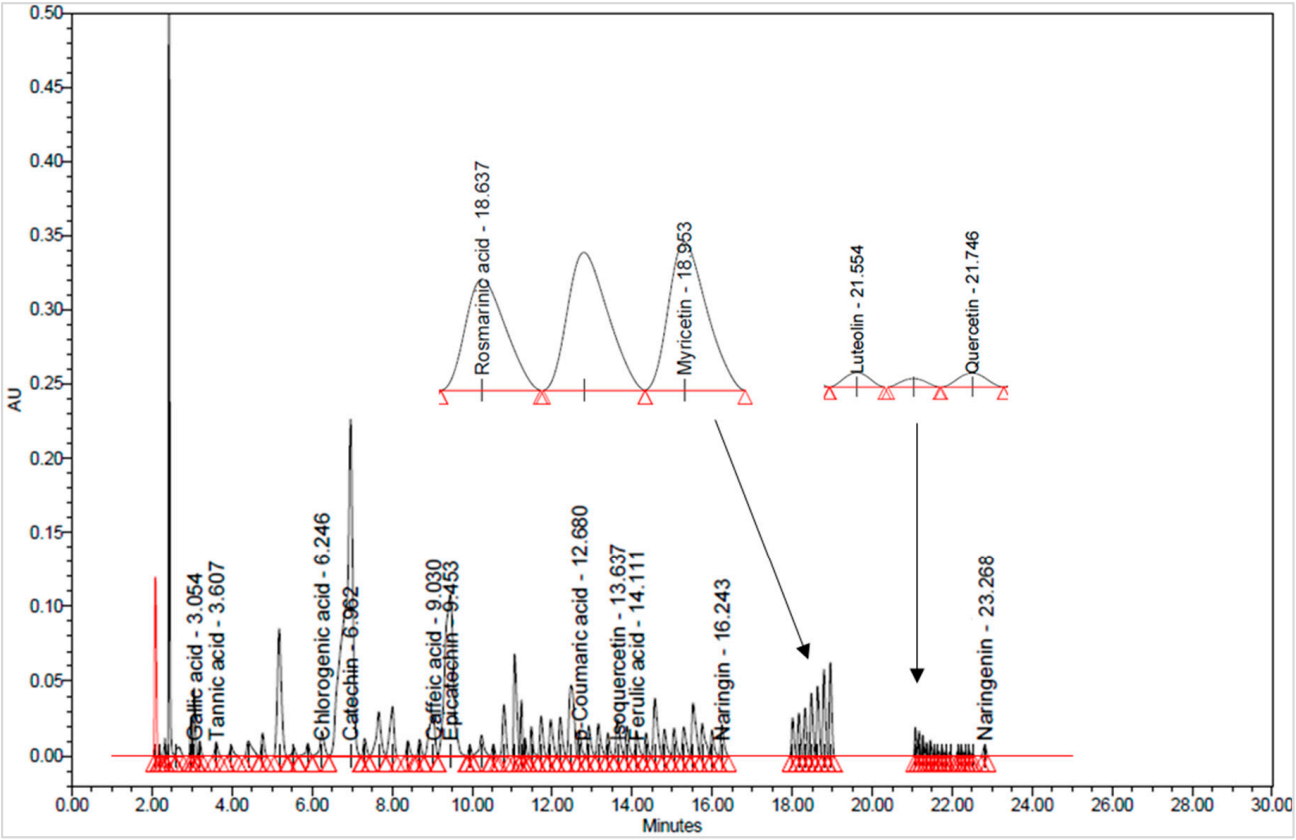

Figure 1. A chromatogram of FNS grape pomace extract at 280 nm in UHPLC.

The chromatogram of the 15 distinct identified polyphenolic compounds in the TRS extract is reported in Figure 2: gallic acid (compound #1–3.002 min); tannic acid (compound #2–3.594 min); chlorogenic acid (compound #3–6.230 min); catechin (compound #4–6.932 min); caffeic acid (compound #5–9.036 min); epicatechin (compound #6–9.638 min); p-coumaric acid (compound #7–12.665 min); isoquercetin (compound #8–13.777 min); ferulic acid (compound #9–14.098 min); naringin (compound #10–16.232 min); rosmarinic acid (compound #11–18.625 min); myricetin (compound #12–18.942 min); luteolin (compound #13–21.538 min); quercetin (compound #14–21.729 min); and naringenin (compound #10–23.482 min).

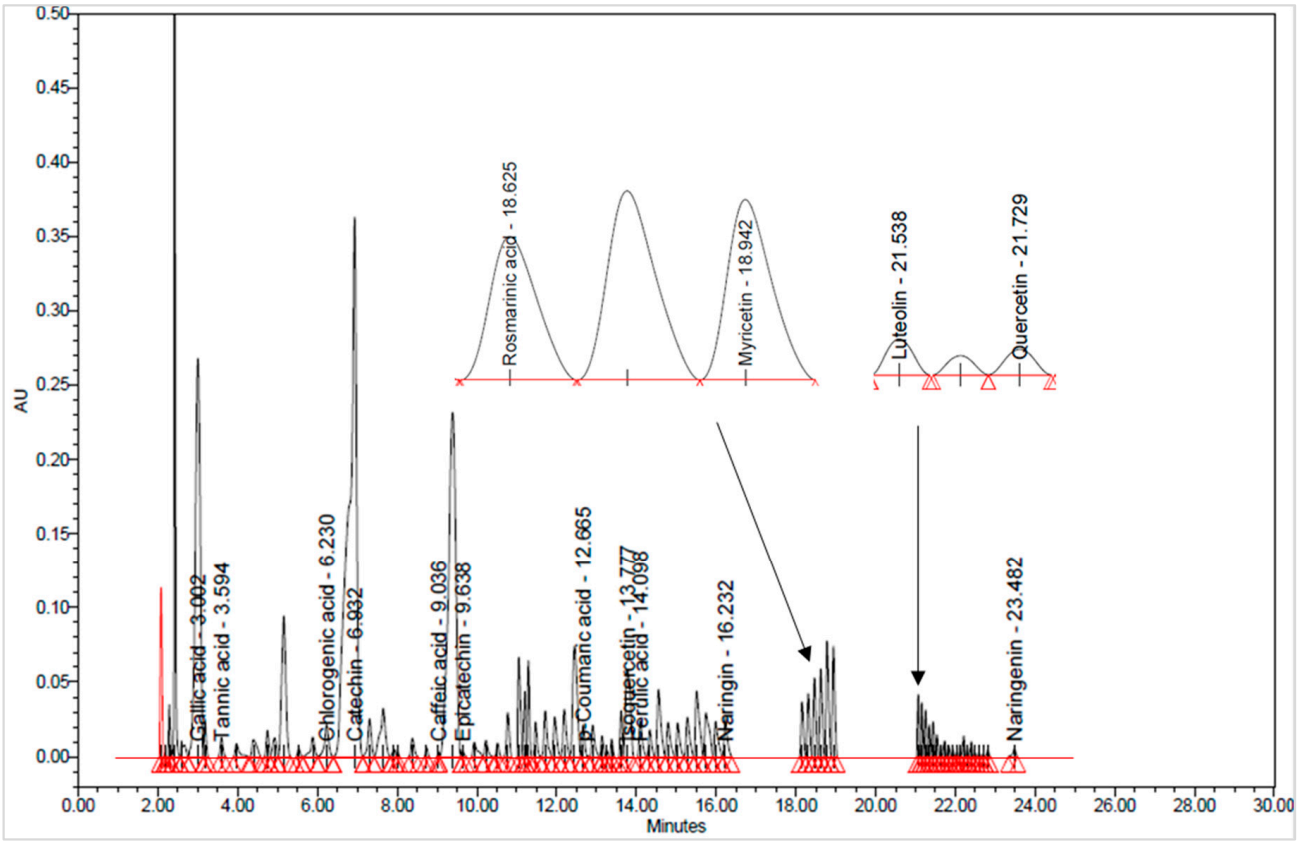

Figure 2. A chromatogram of TRS grape pomace extract at 280 nm in UHPLC.

3.2. Antioxidant Activity

The antioxidant potential of the FN and TR extracts was assessed spectrophotometrically through DPPH assays. The results are shown in Figure 3.

All extracts from the Fetească Neagră cultivar showed the highest antioxidant capacity, especially grape seed extract (90.35%) and whole grape pomace extract (87.19%).

The extracts from the Tămâioasă Românească cultivar showed lower values of antioxidant capacity, except for the seed extract (72.14%). The antioxidant activity registered was less than 50% for the two other extracts.

The structure–activity relationship of bioactive compounds (such as phenolics, flavonoids, and anthocyanins) is a key factor in determining the antioxidant activity of FN and TR extracts. The results demonstrate that FN extracts, which are abundant in total phenols, total flavonoids, and total anthocyanins, display noteworthy antioxidant activity. However, solely the seed TR extract exhibits elevated antioxidant activity, which can be attributed to its elevated content of total phenols and total flavonoids.

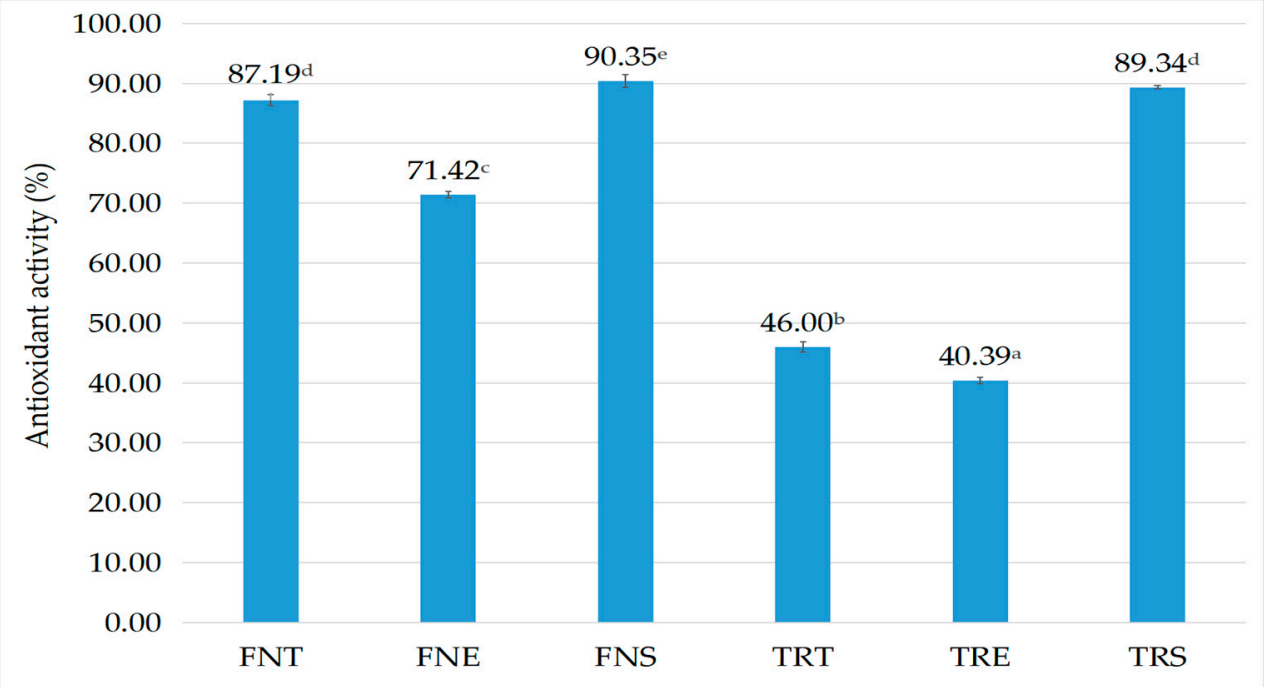

Figure 3. Antioxidant activity of hydroalcoholic extracts from the Fetească Neagră (whole pomace as FNT, skin as FNE, and seed as FNS) and Tămâioasă Românească (whole pomace as TRT, skin as TRE, and seed as TRS) varieties. The values are expressed as the mean ± the standard deviation from three independent experiments. Statistically significant differences ($p < 0.05$) were indicated by different lowercase letters above the error bars.

3.3. Antimicrobial Activity

The antimicrobial activity of FN and TR extracts was investigated through an agar well diffusion test, whereby the extracts were examined in terms of their capacity to inhibit the proliferation of 18 pathogenic bacteria and 4 *Candida* spp. (Table 3).

All extracts demonstrated antibacterial activity, as evidenced by the presence of a clear zone of inhibition against Gram-positive bacteria. The FN and TR extracts exhibited high antibacterial activity against *P. aeruginosa* and *S. marcescens*, the only Gram-negative bacteria susceptible to them. However, no inhibitory effect was observed on *E. coli*, *S*. Enteritidis, and *S*. Typhimurium. All FN and TR extracts demonstrated high antagonist activity against a range of bacterial strains, including *Staphylococcus* spp., *Enterococcus* spp., *Listeria* spp., *R. equi*, and *P. aeruginosa*, with inhibition diameters exceeding 10 mm. However, all FN and TR extracts demonstrated moderate antibacterial activity against *B. cereus* and *Enterococcus* strains, with inhibition diameters between 6 and 10 mm. All FN extracts exhibited high antibacterial activity against *S. marcescens*.

Furthermore, the antibacterial efficacy of FN and TR extracts was assessed for potential utilization as a biological agent, with the outcomes expressed as the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC). To accomplish this objective, tests were conducted with varying concentrations of the FN and TR extracts against a total of 15 bacterial strains, including 13 Gram-positive and 2 Gram-negative foodborne pathogens (Table 4).

Table 3. In vitro antimicrobial activities of grape pomace extracts.

Reference Microorganisms	Fetească Neagră			Tămâioasă Românească		
	FNT	**FNE**	**FNS**	**TRT**	**TRE**	**TRS**
			Bacteria			
B. cereus ATCC 11778	++	++	++	++	++	++
Ent. faecalis ATCC 29212	++	++	++	++	++	++
Ent. faecium ATCC 6057	++	++	++	++	++	++
Ent. hirae ATCC 10541	++	++	++	++	++	++
L. innocua ATCC 33090	+++	+++	+++	+++	+++	+++
L. ivanovii ATCC 19119	+++	+++	+++	+++	+++	+++
L. monocytogenes ATCC 7644	+++	+++	+++	+++	+++	+++
S. aureus ATCC 33592 MRSA	+++	+++	+++	+++	+++	+++
S. aureus ATCC 6538	+++	+++	++	+++	+++	+++
S. epidermidis ATCC 51625 MRSE	+++	+++	+++	+++	+++	+++
S. epidermidis ATCC 12228	+++	+++	+++	+++	+++	+++
S. pyogenes ATCC 19615	+++	+++	+++	+++	+++	++
R. equi ATCC 6939	+++	+++	+++	+++	+++	+++
E. coli ATCC 8739	-	-	-	-	-	-
P. aeruginosa ATCC 27853	+++	+++	+++	+++	+++	+++
S. enterica Typhimurium ATCC 14028	-	-	-	-	-	-
S. enterica Enteritidis ATCC 13076	-	-	-	-	-	-
S. marcescens ATCC 14756	+++	+++	+++	++	++	++
			Fungi			
Candida albicans ATCC 10231	-	-	-	-	-	-
C. glabrata ATCC 2001	-	-	-	-	-	-
C. parapsilopsis ATCC 20019	-	-	-	-	-	-
C. tropicalis ATCC 44508	-	-	-	-	-	-

Legend: (-) no halo formation indicates the absence of activity; (+) inhibition halo of 1–5 mm diameter indicates low antimicrobial activity; (++) halo of 6–10 mm diameter indicates moderate antimicrobial activity; (+++) halo of >10 mm diameter indicates high antimicrobial activity, as classified by Kouadio et al. [43].

The MIC values for the pathogenic bacteria ranged from 0.56 to 8.92 mg/mL for the FNT extracts, 0.29 to 9.17 mg/mL for the FNE extracts, and from 0.31 to 9.75 mg/mL for the FNS extract, respectively.

The MIC values for the pathogenic bacteria ranged from 0.45 to 7.13 mg/mL for the TRT extracts, from 1.00 to 16.00 mg/mL for the TRE extracts, and from 0.41 to 13.30 mg/mL for the TRS extract.

Of all the tested bacteria, *Streptococcus pyogens* and *Rhodococcus equi* showed the highest sensitivity to the FN and TR extracts. The findings indicate that the FN and TR extracts exhibited low MIC and MBC values, effectively inhibiting bacterial growth and killing the pathogenic bacteria studied.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (expressed as mg/mL) of grape pomace extracts against bacterial pathogens.

Strains	FNT		FNE		FNS		TRT		TRE		TRS	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B. cereus ATCC 11778	8.92 ± 0.00	8.92 ± 0.00	9.17 ± 0.00	9.17 ± 0.00	9.75 ± 0.00	9.75 ± 0.00	7.13 ± 0.00	7.13 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	13.30 ± 0.00	13.30 ± 0.00
Ent. faecalis ATCC 29212	8.92 ± 0.00	8.92 ± 0.00	4.58 ± 0.00	4.58 ± 0.00	2.44 ± 0.00	2.44 ± 0.00	3.56 ± 0.00	3.56 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	1.66 ± 0.00	1.66 ± 0.00
Ent. faecium ATCC 6057	2.23 ± 0.00	2.23 ± 0.00	4.58 ± 0.00	4.58 ± 0.00	2.44 ± 0.00	2.44 ± 0.00	7.13 ± 0.00	7.13 ± 0.00	16.00 ± 0.00	16.00 ± 0.00	13.30 ± 0.00	13.30 ± 0.00
Ent. hirae ATCC 10541	4.46 ± 0.00	4.46 ± 0.00	2.29 ± 0.00	2.29 ± 0.00	2.44 ± 0.00	9.75 ± 0.00	3.56 ± 0.00	3.56 ± 0.00	16.00 ± 0.00	16.00 ± 0.00	13.30 ± 0.00	13.30 ± 0.00
L. ivanovii ATCC 19119	2.23 ± 0.00	2.23 ± 0.00	2.29 ± 0.00	2.29 ± 0.00	2.44 ± 0.00	9.75 ± 0.00	1.78 ± 0.00	3.56 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	1.66 ± 0.00	6.63 ± 0.00
L. monocytogens ATCC 7644	2.23 ± 0.00	2.23 ± 0.00	2.29 ± 0.00	2.29 ± 0.00	2.44 ± 0.00	2.44 ± 0.00	3.56 ± 0.00	3.56 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	6.63 ± 0.00	6.63 ± 0.00
L. innocua ATCC 33090	2.23 ± 0.00	2.23 ± 0.00	2.29 ± 0.00	2.29 ± 0.00	2.44 ± 0.00	2.44 ± 0.00	3.56 ± 0.00	3.56 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	6.63 ± 0.00	6.63 ± 0.00
S. aureus ATCC 33592 MRSA	1.15 ± 0.00	1.15 ± 0.00	2.29 ± 0.00	2.29 ± 0.00	1.22 ± 0.00	1.22 ± 0.00	3.56 ± 0.00	3.56 ± 0.00	8.00 ±0.00	8.00 ± 0.00	1.66 ± 0.00	1.66 ± 0.00
S. aureus ATCC 6538	4.46 ± 0.00	4.46 ± 0.00	2.29 ± 0.00	2.29 ± 0.00	1.22 ± 0.00	9.75 ± 0.00	1.78 ± 0.00	1.78 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	0.83 ± 0.00	6.63 ± 0.00
S. epidermidis ATCC 51625 MRSE	2.23 ± 0.00	4.46 ± 0.00	1.15 ± 0.00	4.58 ± 0.00	2.44 ± 0.00	1.22 ± 0.00	1.78 ± 0.00	1.78 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	1.66 ± 0.00	3.31 ± 0.00
S. epidermidis ATCC 12228	2.23 ± 0.00	4.46 ± 0.00	2.29 ± 0.00	2.29 ± 0.00	2.44 ± 0.00	1.22 ± 0.00	3.56 ± 0.00	3.56 ± 0.00	8.00 ±0.00	8.00 ± 0.00	1.66 ± 0.00	3.31 ± 0.00
Streptococcus pyogens ATCC 19615	0.28 ± 0.00	0.56 ± 0.00	0.29 ± 0.00	0.29 ± 0.00	0.31 ± 0.00	0.31 ± 0.00	0.45 ± 0.00	0.45 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.41 ± 0.00	0.41 ± 0.00
R. equi ATCC 6939	8.92 ± 0.00	8.92 ± 0.00	0.29 ± 0.00	0.29 ± 0.00	1.22 ± 0.00	1.22 ± 0.00	0.89 ± 0.00	0.89 ± 0.00	4.00 ± 0.00	4.00 ±0.00	1.66 ± 0.00	1.66 ± 0.00
P. aeruginosa ATCC 27853	4.46 ± 0.00	4.46 ± 0.00	4.58 ± 0.00	4.58 ± 0.00	2.44 ± 0.00	2.44 ± 0.00	3.56 ± 0.00	3.56 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	3.31 ± 0.00	3.31 ± 0.00
Serratia marcescens ATCC 14756	4.46 ± 0.00	4.46 ± 0.00	2.29 ± 0.00	2.29 ± 0.00	4.88 ± 0.00	4.88 ± 0.00	3.56 ± 0.00	3.56 ± 0.00	8.00 ±0.00	8.00 ± 0.00	6.63 ± 0.00	6.63 ± 0.00

The values are expressed as the mean ± the standard deviation from three independent experiments.

4. Discussion

The winemaking industry by-products are rich in nutraceutical compounds and complex carbohydrates [54]. The processes could be rendered more economically efficient if these molecules were extracted. Grape pomace is a by-product of the winemaking process, comprising a rich source of phenolic compounds that have been demonstrated to possess notable antioxidant and antimicrobial properties [11,16,79,93]. Only 30–40% of these compounds is extracted during winemaking; the remainder is present in the pomace. The phenolic compounds present in grape pomace can be classified into two main categories, simple phenols and polyphenols, which, in turn, are divided into flavonoids (anthocyanins, chalcones, flavanones, flavanonols, flavan-3-ols, flavonols, and flavones) and non-flavonoid (hydroxybenzoic and hydroxycinnamic acids, and stilbenes) [56]. This material has been demonstrated to exhibit noteworthy antioxidant and antimicrobial characteristics [11,16,79].

A variety of methodologies have been developed to ensure the efficient and sustainable extraction of biologically active compounds from grape pomace. These include conventional techniques such as Soxhlet extraction, maceration, hydroxide distillation, solid–liquid extraction, and accelerated solvent extraction [21–25]. In this study, a hydroethanolic solution (50% v/v) was selected for use as the solvent for the extraction of phenolic compounds from grape pomace. The use of ethanol is justified by its established suitability for the extraction of phenolic compounds (owing to its relatively high polarity) and its low boiling point, which minimizes energy expenditure and streamlines the removal of the solvent from the samples [23,25,27]. Moreover, ethanol is a Generally Recognized As Safe (or GRAS) chemical substance. Among the conventional and non-conventional extraction methods described in the literature [21–25,30–34], it is revealed that the combination of Soxhlet extraction and maceration selected for this study was proven to be both an efficient and accessible approach for the recovery of the highest quantity of phenolic compounds from the grape pomace of the Fetească Neagră and Tămâioasă Românească varieties.

In our study, the total phenolic content (TPC) and total flavonoid content (TFC) of grape seed extracts were found to be higher than those of grape skins and whole extracts derived from both Romanian grape cultivars, except for the total anthocyanin content (TAC). The findings suggest that FNS extract exhibits the highest phenol content, with a value of 93.52 mg GAE/g, which is approximately 1.15 times higher than the phenolic amount identified in pomace and skin extracts. The results are consistent with those of other studies on the same variety [94] and even exceed the findings of other researchers [14]. Concerning TR seed extract (TRS), the phenol content (90.43 mg GAE/g) was found to be comparable with that evaluated in the red wine variety, but two to three times higher than the content identified in pomace and skin. The results were comparable with those of other studies on grape pomace [95]. The TPC obtained in this study is significantly higher than that reported by Radulescu et al. [79]. In the study conducted by Radulescu et al. [79], the TPC of hydroalcoholic extracts obtained by two methods (maceration and ultrasonication) from the by-products of the Tămâioasă Românească variety (stems, skins and seeds, and pomace, respectively) exhibited a range of 3.32 ± 0.34 to 15.32 ± 1.93 mg GAE/g DM. Moreover, the results of the present study were contrary to those previously reported by Frîncu et al. [9], who had quantified the low phenol content of Tâmâioasă Românească grape pomace at 24.94 mg GAE/g DM and Fetească Neagră grape pomace at 27.41 mg GAE/g DM.

The Fetească Neagră seed extract (FNS) was found to have the highest flavonoid content (86.86 mg RE/g), which is similar to the Cabernet franc content evaluated by Xu et al. [96]. This value was found to be 1.15 times higher than the flavonoid content observed in the pomace and skin. The flavonoid content of TRS extract was found to be the highest, at 61.01 mg RE/g, which is comparable with the flavonoid content of Viognier pomace [96]. In contrast, the TFC of all TR extracts obtained in this study is significantly higher than that reported by Radulescu et al. [79], which was markedly lower for the hydroalcoholic extracts obtained from the by-products of the Tămâioasă Românească variety, with values ranging from 1.15 ± 0.07 to 3.53 ± 0.40 mg QE/g.

The total anthocyanin content was found to be at a high concentration of 36.75 mg cyd-3-gluE/g in FNE (Fetească Neagră skin), with a similar content calculated for the pomace, and the lowest value registered for the seeds. The results were comparable with those of other studies on the same variety [94]. The presence of anthocyanins in seeds is not typically observed; however, this may be attributed to the diffusion of these compounds from the skins during the winemaking process [97]. While anthocyanins are typically absent in white grape varieties, the TR extracts exhibited a relatively low anthocyanin content, with values between 1.06 and 1.69 mg cyd-3-gluE/g, comparable to other studies on white wine varieties [98].

A comprehensive characterization of the grape extracts (FN and TR extracts) was conducted via UHPLC analysis, whereby the individual components present within the extracts were identified and quantified. In our study, the polyphenolic compounds quantified are the hydroxybenzoic acids (gallic acid and tannic acid), hydroxycinnamic acids (p-coumaric acid, ferulic acid, caffeic acid, gallic acid, tannic acid, chlorogenic acid, and rosmarinic acid), flavonols (quercetin, isoquercetin, and myricetin), flavanols (catechin and epicatechin), flavanones (naringenin and naringin), and flavones (luteolin). The polyphenolic profile of the FN and TR extracts aligns with the literature [99,100].

The concentrations and types of phenolic compounds can vary depending on the grape cultivar from which grape pomace is derived, the specific parts of the grape used, or the processing methods employed. It is established that grape seeds contain a higher total polyphenolic content than grape skins. Regarding the phenolic acids, gallic acid presents the highest quantity in grape seeds compared to grape skins. Moreover, Di Stefano et al. [101] reported that grape seeds present the highest quantity of gallic acid (647.88 μg/g) compared to grape skins (181.30 μg/g). In the grape extracts, hydroxybenzoic acids present the highest quantity compared to hydroxycinnamic acids [102].

Amongst flavonoids, catechin and epicatechin were the major compounds identified in the grape pomace extracts, results that were also reported by Xu et al. [96] and Abouelenein et al. [103]. Similar to gallic acid, catechin, epicatechin, and naringin are found in higher amount in grape seeds than in grape skins [104]. Flavonoids are proven to be potent antioxidants that play a crucial role in regulating oxidative stress and treating inflammatory and infectious diseases, particularly those caused by resistant and opportunistic bacteria, through various action mechanisms [17,104].

Polyphenols fulfill the criteria for effective antioxidant activity by acting as hydrogen or electron donors, forming stable radical intermediates, and chelating transition metals [17,57,58,80]. The results obtained from our investigation indicate that all of the FN extracts and seed TR extract displayed the highest antioxidant activity, which seems to be linked with the increased phenolic content detected in these extracts, which is in agreement with previously reported results [16,18,57,61,96].

In light of these circumstances, the market volume for novel natural antimicrobials is projected to increase at an estimated annual growth rate of 7.3% over the period spanning from 2019 to 2027 [105,106]. It has been demonstrated that some of the bioactive components of grape by-products can be employed in the prevention and treatment of foodborne bacterial infections. These components have been shown to enhance microbiological food safety, and prevent or treat animal and human illnesses [75–81]. As indicated in previous studies, the agar well diffusion test, the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) are standard methods for the assessment of antimicrobial activities present in grape extracts [91,92]. The results of this study indicated that the FN and TR extracts exhibited a moderate to high antimicrobial activity spectrum against 13 Gram-positive and 2 Gram-negative foodborne pathogens. No antifungal activity was observed against *Candida* strains tested. The present study demonstrated that FN and TR extracts exhibited a pronounced antimicrobial effect against a range of bacterial pathogens, including *L. monocytogenes*, *P. aeruginosa*, and *S. aureus*, which are among the most prevalent agents associated with bacterial foodborne diseases [106]. Many studies suggest that grape extracts or specific bioactive compounds

derived from grapes, pomace, skins, and seeds, can effectively inhibit bacterial growth and kill pathogenic bacteria [14,66–73,80]. Nevertheless, additional research in this area has demonstrated that grape pomace extract or purified phenolic compounds can impede the proliferation of *Escherichia coli*, *Salmonella typhimurium*, and *Candida* strains [107–111]. Corrales et al. [108] demonstrated through agar diffusion testing that 1% (*w*/*v*) grape seed extract did not impede the growth of *E. coli* and *S.* Typhimurium. In contrast to this finding, Baydar et al. [109] employed an identical methodology and observed the inhibition of these foodborne pathogens. Papadopoulou et al. [110] demonstrated that alcohol-free extracts of red wines exhibited antimicrobial activity against *S. aureus*, *E. coli*, and *C. albicans*. In their study, Oliveira et al. [111] observed that Merlot and Syrah grape pomace extracts obtained by supercritical CO_2 exhibited superior inhibitory properties against Gram-positive bacteria, including *B. cereus* and *S. aureus*, compared to Gram-negative bacteria such as *E. coli* and *P. aeruginosa*, as well as the fungal pathogen *C. albicans*.

The extracts of FN and TR were observed to exert an inhibitory effect on bacterial growth and the killing of pathogenic bacteria at relatively low concentrations. Our findings revealed that the MBC values were identical to the MIC values of the pathogenic bacteria tested in almost all cases, although there were a few exceptions. The structure–activity relationship of polyphenolic compounds is a key factor in exerting antibacterial activities via various mechanisms of action [112]. The bioactive compounds display a high affinity for macromolecules, including lipids, hydrocarbons, proteins, and nucleic acids, which have been demonstrated to play an essential role in their biological activity. Previous studies have provided evidence that catechins and epicatechins interact with bacterial cell walls, leading to cell membrane destabilization and alterations to their structural integrity and fluidity [113]. In our study, the elevated concentrations of catechins and epicatechin detected in the FN and TR extracts may be associated with their high antimicrobial efficacy.

The freeze-dried FN and TR extracts will be stored in dark, airtight containers at low temperatures (4 °C or below) to prevent oxidation or degradation due to light, oxygen, or heat. To ensure the efficacy of the storage methods, regular monitoring of TPC, TFC, and TAC will be conducted, providing an overall indication of purity.

Given the finding that grape pomace possesses the ability to inhibit a broad spectrum of pathogens while concurrently stimulating the growth of beneficial microorganisms, promoting health [114,115]; a more exhaustive inquiry is imperative to gain a deeper understanding of its implications. The high antioxidant and antimicrobial activities exhibited by FN and TR extracts render them highly valuable for a number of potential applications. A review of the literature suggests that these compounds may have potential applications in the food industry, particularly as natural preservatives for food products, thereby extending shelf life without the need for synthetic additives [19,20,23,79]. Additionally, their bioactive characteristics could facilitate the development of supplements and anti-inflammatory and antimicrobial therapies within the field of medicine [16,59,60,75–78]. Furthermore, they could be integrated into cosmetic formulations designed to protect the skin from environmental damage and to prevent the signs of ageing, whilst also treating bacterial infections [74,82–84].

Moreover, further research is required to improve the bioavailability of the extracts, investigate potential synergies between the extracts and other substances, such as antibiotics or postbiotics, expand antimicrobial testing, and conduct a comprehensive safety assessment. This will facilitate the identification and optimization of the empirical conditions for the large-scale applications of FN and TR extracts as functional ingredients.

5. Conclusions

The present study sought to assess the polyphenolic profile, and antioxidant and antimicrobial potential of wine by-products derived from Fetească Neagră and Tămâioasă Românească, two distinct Romanian grape varieties. The analysis of the phenolic profile revealed that catechin and epicatechin were the most prevalent compounds identified in the TR and FN seed extracts. Extracts of the Fetească Neagră grape obtained from the

pomace, skins, and seeds, as well as seed TR extracts, have been demonstrated to possess strong antioxidant activity, with values exceeding 70%. Furthermore, all FN and TR extracts are demonstrated to have effective antimicrobial activity against a broad spectrum of the tested pathogenic bacteria, except *E. coli*, *S.* Enteritidis, and *S*. Typhimurium. Moreover, except for a few cases, MBCs are indistinguishable from MICs, which demonstrates the high efficacy of the FN and TR extracts in inhibiting and killing pathogenic bacteria simultaneously. Subsequently, research will be conducted to elucidate the correlation between individual phenolic compounds and the antibacterial and antioxidant properties of the FN and TR extracts. Based on the presented findings, both Fetească Neagră and Tămâioasă Românească extracts offer a promising avenue for innovation across multiple domains. They represent a valuable resource for reducing waste, while simultaneously serving as a valuable source of high-value products with diverse potential applications, including in the pharmaceutical, food, and cosmetic industries.

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

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All obtained datasets are included in this publication.

Conflicts of Interest: Author Ioan Dopcea was employed by the company "CEBIS International". The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. FAOSTAT. Available online: http://www.fao.org/faostat/en/#data/QCL (accessed on 6 July 2024).
2. OIV. STATE of the World Vine and Wine Sector in 2023. Available online: https://www.oiv.int/sites/default/files/2024-04/OIV_STATE_OF_THE_WORLD_VINE_AND_WINE_SECTOR_IN_2023.pdf (accessed on 6 July 2024).
3. Ministry of Agriculture and Rural Development—Romania. Available online: https://www.madr.ro/horticultura/viticultura-vinificatie.html (accessed on 20 August 2024).
4. Soceanu, A.; Dobrinas, S.; Sirbu, A.; Manea, N.; Popescu, V. Economic aspects of waste recovery in the wine industry. A multidisciplinary approach. *Sci. Total Environ.* **2021**, *759*, 143543. [CrossRef] [PubMed]
5. Van Leeuwen, C.; Friant, P.; Choné, X.; Tregoat, O.; Koundouras, S.; Dubourdieu, D. Influence of climate, soil, and cultivar on terroir. *Am. J. Enol. Vitic.* **2004**, *55*, 207–217. [CrossRef]
6. De Oliveira, J.B.; Egipto, R.; Laureano, O.; de Castro, R.; Pereira, G.E.; Ricardo-da-Silva, J.M. Climate effects on physicochemical composition of syrah grapes at low and high altitude sites from tropical grown regions of Brazil. *Food Res. Int.* **2019**, *121*, 870–879. [CrossRef] [PubMed]
7. Muhlack, R.A.; Potumarthi, R.; Jeffery, D.W. Sustainable wineries through waste valorisation: A review of grape marc utilisation for value-added products. *Waste Manag.* **2018**, *72*, 99–118. [CrossRef]
8. Lyas, T.; Chowdhary, P.; Chaurasia, D.; Gnansounou, E.; Pandey, A.; Chaturvedi, P. Sustainable green processing of grape pomace for the production of value-added products: An overview. *Environ. Technol. Innov.* **2021**, *23*, 101592.
9. Frîncu, M.; Dumitrache, C.; Petre, A.C.; Andrei, M.O.Ț.; Teodorescu, R.I.; Bărbulescu, D.I.; Tudor, V.; Matei, F. Physico-chemical characterization of some sources of grape marc from Pietroasa vineyard. *AgroLife Sci. J.* **2023**, *12*, 81–86. [CrossRef]
10. Niculescu, V.-C.; Ionete, R.-E. An Overview on Management and Valorisation of Winery Wastes. *Appl. Sci.* **2023**, *13*, 5063. [CrossRef]
11. Constantin, O.E.; Stoica, F.; Rațu, R.N.; Stănciuc, N.; Bahrim, 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. [CrossRef]
12. Tomoiagă, L.L.; Iliescu, M.L.; Răcoare, H.S.; Botea, V.; Sîrbu, A.D.; Puşcă, G.; Chedea, V.S. Grape pomance generation from grape cultivars cultivated in Târnave vineyards in the framework of the climate change. *Rom. J. Hortic.* **2020**, *1*, 81–88. [CrossRef]

13. Olejar, K.J.; Fedrizzi, B.; Kilmartin, P.A. Influence of harvesting technique and maceration process on aroma and phenolic attributes of sauvignon blanc wine. *Food Chem.* **2015**, *183*, 181–189. [CrossRef]
14. Geană, E.I.; Ionete, R.E.; Niculescu, V.; Artem, V.; Ranca, A. Changes in polyphenolic content of berry skins from different red grapes cultivars during ripening. *Smart Energy Sustain. Environ.* **2016**, *19*, 95.
15. Tchouakeu Betnga, P.F.; Poggesi, S.; Darnal, A.; Longo, E.; Rudari, E.; Boselli, E. Terroir dynamics: Impact of vineyard and canopy treatment with chitosan on anthocyanins, phenolics, and volatile and sensory profiles of Pinot noir wines from south tyrol. *Molecules* **2024**, *29*, 1916. [CrossRef] [PubMed]
16. Teixeira, A.; Baenas, N.; Dominguez-Perles, R.; Barros, A.; Rosa, E.; Moreno, D.A.; Garcia-Viguera, C. Natural bioactive compounds from winery by-products as health promoters: A review. *Int. J. Mol. Sci.* **2014**, *15*, 15638–15678. [CrossRef]
17. Iacopini, P.; Baldi, M.; Storchi, P.; Sebastiani, L. Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, in vitro antioxidant activity and interactions. *J. Food Compos. Anal.* **2008**, *21*, 589–598. [CrossRef]
18. Guaita, M.; Bosso, A. Polyphenolic characterization of grape skins and seeds of four italian red cultivars at harvest and after fermentative maceration. *Foods* **2019**, *8*, 395. [CrossRef]
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. [CrossRef]
20. Caponio, G.R.; Minervini, F.; Tamma, G.; Gambacorta, G.; De Angelis, M. Promising application of grape pomace and its agri-food valorization: Source of bioactive molecules with beneficial effects. *Sustainability* **2023**, *15*, 9075. [CrossRef]
21. Stalikas, C.D. Extraction, separation, and detection methods for phenolic acids and flavonoids. *J. Sep. Sci.* **2007**, *30*, 3268–3295. [CrossRef]
22. Ćujić, N.; Šavikin, K.; Janković, T.; Pljevljakušić, D.; Zdunić, G.; Ibrić, S. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chem.* **2016**, *194*, 135–142. [CrossRef]
23. Aires, A. Phenolics in foods: Extraction, analysis and measurements. In *Phenolic Compounds—Natural Sources, Importance and Applications*; Soto-Hernandez, M., Palma-Tenango, M., Del Rosario Garcia-Mateos, M., Eds.; IntechOpen: London, UK, 2017; pp. 61–88.
24. Alara, O.R.; Abdurahman, N.H.; Ukaegbu, C.I. Soxhlet extraction of phenolic compounds from *Vernonia cinereal* leaves and its antioxidant activity. *J. Appl. Res. Med. Aromat. Plants* **2018**, *11*, 12–17.
25. 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]
26. Ju, Z.Y.; Howard, L.R. Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. *J. Agric. Food Chem.* **2003**, *51*, 5207–5213. [CrossRef] [PubMed]
27. Zhang, Q.W.; Lin, L.G.; Ye, W.C. Techniques for extraction and isolation of natural products: A comprehensive review. *Chin. Med.* **2018**, *13*, 1–26. [CrossRef] [PubMed]
28. Pintać, D.; Majkić, T.; Torović, L.; Orčić, D.; Beara, I.; Simin, N.; Mimica–Dukić, N.; Lesjak, M. Solvent selection for efficient extraction of bioactive compounds from grape pomace. *Ind. Crops Prod.* **2018**, *111*, 379–390. [CrossRef]
29. Rodrigues, R.P.; Sousa, A.M.; Gando-Ferreira, L.M.; Quina, M.J. Grape pomace as a natural source of phenolic compounds: Solvent screening and extraction optimization. *Molecules* **2023**, *28*, 2715. [CrossRef]
30. Castro, L.E.N.; Sganzerla, W.G.; Silva, A.P.G.; John, O.D.; Barroso, T.L.C.T.; Rostagno, M.A.; Forster-Carneiro, T. Sustainable extraction methods for the recovery of polyphenolic compounds from grape pomace and its biological properties: A comprehensive review. *Phytochem. Rev.* **2024**, 1–28. [CrossRef]
31. Melo, F.d.O.; Ferreira, V.C.; Barbero, G.F.; Carrera, C.; Ferreira, E.d.S.; Umsza-Guez, M.A. Extraction of bioactive compounds from wine lees: A systematic and bibliometric review. *Foods* **2024**, *13*, 2060. [CrossRef]
32. Corrales, M.; Toepfl, S.; Butz, P.; Knorr, D.; Tauscher, B. Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 85–91. [CrossRef]
33. Comuzzo, P.; Marconi, M.; Zanella, G.; Querzè, M. Pulsed electric field processing of white grapes (cv. Garganega): Effects on wine composition and volatile compounds. *Food Chem.* **2018**, *264*, 16–23. [CrossRef]
34. Ferreira-Santos, P.; Nobre, C.; Rodrigues, R.M.; Genisheva, Z.; Botelho, C.; Teixeira, J.A. Extraction of phenolic compounds from grape pomace using ohmic heating: Chemical composition, bioactivity and bioaccessibility. *Food Chem.* **2024**, *436*, 137780. [CrossRef]
35. Bhunia, A.K. General mechanism of pathogenesis. In *Foodborne Microbial Pathogens*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 87–115.
36. Todd, E. Food-borne disease prevention and risk assessment. *Int. J. Environ. Res. Public Health* **2020**, *17*, 5129. [CrossRef] [PubMed]
37. Lencova, S.; Svarcova, V.; Stiborova, H.; Demnerova, K.; Jencova, V.; Hozdova, K.; Zdenkova, K. Bacterial biofilms on polyamide nanofibers: Factors influencing biofilm formation and evaluation. *ACS Appl. Mater. Interfaces* **2021**, *13*, 2277–2288. [CrossRef] [PubMed]
38. Almansour, A.M.; Alhadlaq, M.A.; Alzahrani, K.O.; Mukhtar, L.E.; Alharbi, A.L.; Alajel, S.M. The Silent Threat: Antimicrobial-resistant pathogens in food-producing animals and their impact on public health. *Microorganisms* **2023**, *11*, 2127. [CrossRef] [PubMed]
39. Mulchandani, R.; Wang, Y.; Gilbert, M.; Van Boeckel, T.P. Global trends in antimicrobial use in food-producing animals: 2020 to 2030. *PLoS Glob. Public Health* **2023**, *3*, e0001305. [CrossRef]

40. Naghavi, M.; Mestrovic, T.; Gray, A.; Hayoon, A.G.; Swetschinski, L.R.; Aguilar, G.R.; Weaver, N.D.; Ikuta, K.S.; Chung, E.; Wool, E.E.; et al. Global burden associated with 85 pathogens in 2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet Infect. Dis.* **2024**, *24*, 868–895. [CrossRef]
41. Badea, F.; Diguta, C.F.; Matei, F. The use of lactic acid bacteria and their metabolites to improve the shelf life of perishable fruits and vegetables. *Sci. Bull. Ser. F Biotechnol.* **2022**, *XXVI*, 117–125.
42. Utoiu, E.; Oancea, A.; Stanciuc, A.M.; Stefan, M.L.; Toma, A.; Moraru, A.; Diguta, C.F.; Matei, F.; Cornea, C.P.; Oancea, F. Prebiotic content and probiotic effect of kombucha fermented pollen. *AgroLife Sci. J.* **2018**, *7*, 149–156.
43. Kouadio, N.J.; Zady, A.L.O.; Kra, K.A.S.; Diguță, F.C.; Niamke, S.; Matei, F. In Vitro Probiotic Characterization of *Lactiplantibacillus plantarum* Strains Isolated from Traditional Fermented Dockounou Paste. *Fermentation* **2024**, *10*, 264. [CrossRef]
44. Pristavu, M.C.; Diguță, C.; Coulibaly, W.H.; Youte Fanche, S.A.; Dopcea, G.; Matei, F. Review of postbiotics as new health promoters. *AgroLife Sci. J.* **2022**, *11*, 142–152. [CrossRef]
45. Ma, L.; Tu, H.; Chen, T. Postbiotics in Human Health: A Narrative Review. *Nutrients* **2023**, *15*, 291. [CrossRef]
46. Yan, R.; Zeng, X.; Shen, J.; Wu, Z.; Guo, Y.; Du, Q.; Tu, M.; Pan, D. New clues for postbiotics to improve host health: A review from the perspective of function and mechanisms. *J. Sci. Food Agric.* **2024**, *104*, 6376–6387. [CrossRef] [PubMed]
47. Răducu, A.L.; Popa, A.; Boiu-Sicuia, O.A.; Israel-Roming, F.; Cornea, C.P.; Jurcoane, S. Antimicrobial activity of camelina oil and hydroalcoholic seed extracts. *Rom. Biotecnol. Lett.* **2021**, *26*, 2355–2360. [CrossRef]
48. Roleira, F.M.F.; Tavares-Da-Silva, E.J.; Varela, C.L.; Costa, S.C.; Silva, T.; Garrido, J.; Borges, F. Plant Derived and Dietary Phenolic Antioxidants: Anticancer Properties. *Food Chem.* **2015**, *183*, 235–258. [CrossRef]
49. 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]
50. Caponio, G.R.; Lippolis, T.; Tutino, V.; Gigante, I.; De Nunzio, V.; Milella, R.A.; Gasparro, M.; Notarnicola, M. Nutraceuticals: Focus on anti-inflammatory, anti-cancer, antioxidant properties in gastrointestinal tract. *Antioxidants* **2022**, *11*, 1274. [CrossRef]
51. Lobiuc, A.; Pavăl, N.E.; Mangalagiu, I.I.; Gheorghiță, R.; Teliban, G.C.; Amăriucăi-Mantu, D.; Stoleru, V. Future Antimicrobials: Natural and Functionalized Phenolics. *Molecules* **2023**, *28*, 1114. [CrossRef]
52. Li, S.; Jiang, S.X.; Jia, W.T.; Guo, T.M.; Wang, F.; Li, J.; Yao, Z.L. Natural antimicrobials from plants: Recent advances and future prospects. *Food Chem.* **2023**, *432*, 137231. [CrossRef] [PubMed]
53. Arlet, A.C.I.; Tociu, M.; Balanuca, B.; Israel-Roming, F. Extraction and evaluation of total phenolics content from red corn bran. *Sci. Bull. Ser. F Biotechnol.* **2023**, *27*, 60–67.
54. Baroi, A.M.; Toma, D.I.; Alin, D.I.N.; Vizitiu, D.E.; Fierascu, I.; Fierascu, R.C. Grapevine plant waste utilization in nanotechnology. *AgroLife Sci. J.* **2024**, *13*, 203–216.
55. Cerchezan, G.; Israel-Roming, F. Correlation of the chemical parameters with the sensorial properties of wine. *Sci. Bull. Ser. F Biotechnol.* **2024**, *XXVIII*, 95–104.
56. Hornedo-Ortega, R.; Reyes González-Centeno, M.; Chira, K.; Jourdes, M.; Teissedre, P.-L. Phenolic compounds of grapes and wines: Key compounds and implications in sensory perception. In *Winemaking—Stabilization, Aging Chemistry and Biochemistry*, 1st ed.; Câmara, J., Ed.; Nova Science Publisher: Hauppauge, NY, USA; IntechOpen; London, UK, 2020.
57. Karami, S.; Rahimi, M.; Babaei, A. An Overview on the antioxidant, anti-inflammatory, antimicrobial and anti-cancer activity of grape extract. *Biomed. Res. Clin. Prac.* **2018**, *3*, 1–4. [CrossRef]
58. Chedea, V.S.; Tomoiaga, L.L.; Macovei, S.O.; Magureanu, D.C.; Iliescu, M.L.; Bocsan, I.C.; Buzoianu, A.D.; Vosloban, C.M.; Pop, R.M. Antioxidant/pro-oxidant actions of polyphenols from grapevine and wine by-products-base for complementary therapy in ischemic heart diseases. *Front. Cardiovasc. Med.* **2021**, *8*, 750508. [CrossRef]
59. Chedea, V.S.; Macovei, Ș.O.; Bocsan, I.C.; Măgureanu, D.C.; Levai, A.M.; Buzoianu, A.D.; Pop, R.M. Grape pomace polyphenols as a source of compounds for management of oxidative stress and inflammation—A possible alternative for non-steroidal anti-inflammatory drugs? *Molecules* **2022**, *27*, 6826. [CrossRef] [PubMed]
60. Bocsan, I.C.; Măgureanu, D.C.; Pop, R.M.; Levai, A.M.; Macovei, Ș.O.; Pătrașca, I.M.; Chedea, V.S.; Buzoianu, A.D. Antioxidant and anti-inflammatory actions of polyphenols from red and white grape pomace in ischemic heart diseases. *Biomedicines* **2022**, *10*, 2337. [CrossRef]
61. Radulescu, C.; Buruleanu, L.C.; Nicolescu, C.M.; Olteanu, R.L.; Bumbac, M.; Holban, G.C.; Simal-Gandara, J. Phytochemical profiles, antioxidant and antibacterial activities of grape (*Vitis vinifera* L.) seeds and skin from organic and conventional vineyards. *Plants* **2020**, *9*, 1470. [CrossRef] [PubMed]
62. Luchian, C.E.; Cotea, V.V.; Vlase, L.; Toiu, A.M.; Colibaba, L.C.; Răschip, I.E.; Nadăş, G.; Gheldiu, A.M.; Tuchiluş, C.; Rotaru, L. Antioxidant and antimicrobial effects of grape pomace extracts. *BIO Web Conf.* **2019**, *15*, 04006. [CrossRef]
63. Cotoras, M.; Vivanco, H.; Melo, R.; Aguirre, M.; Silva, E.; Mendoza, L. In vitro and in vivo evaluation of the antioxidant and prooxidant activity of phenolic compounds obtained from grape (*Vitis vinifera*) pomace. *Molecules* **2014**, *19*, 21154–21167. [CrossRef] [PubMed]
64. Louli, V.; Ragoussis, N.; Magoulas, K. Recovery of phenolic antioxidants from wine industry by-products. *Bioresour. Technol.* **2004**, *92*, 201–208. [CrossRef]
65. 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. [CrossRef]

66. Baydar, N.G.; Özkan, G.; Sağdiç, O. Total Phenolic Contents and Antibacterial Activities of Grape (*Vitis vinifera* L.) Extracts. *Food Control* **2004**, *15*, 335–339. [CrossRef]
67. Chedea, V.S.; Braicu, C.; Chirila, F.; Ober, C.; Socaciu, C. Antibacterial action of an aqueous grape seed polyphenolic extract. *Afr. J. Biotechnol.* **2011**, *10*, 6276–6280.
68. Tseng, A.; Zhao, Y. Effect of different drying methods and storage time on the retention of bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot). *J. Food Sci.* **2012**, *77*, H192–H201. [CrossRef] [PubMed]
69. Sanhueza, L.; Tello, M.; Vivanco, M.; Mendoza, L.; Wilkens, M. Relation between antibacterial activity against food transmitted pathogens and total phenolic compounds in grape pomace extracts from Cabernet Sauvignon and Syrah Varieties. *Adv. Microbiol.* **2014**, *04*, 225–232. [CrossRef]
70. Friedman, M. Antibacterial, antiviral, and antifungal properties of wines and winery byproducts in relation to their flavonoid content. *J. Agric. Food Chem.* **2014**, *62*, 6025–6042. [CrossRef]
71. Silva, A.; Silva, V.; Igrejas, G.; Gaivão, I.; Aires, A.; Klibi, N.; Dapkevicius, M.d.L.; Valentão, P.; Falco, V.; Poeta, P. Valorization of Winemaking By-Products as a novel source of antibacterial properties: New strategies to fight antibiotic resistance. *Molecules* **2021**, *26*, 2331. [CrossRef]
72. da Silva, W.P.; Lopes, G.V.; Ramires, T.; Kleinubing, N.R. May phenolics mitigate the antimicrobial resistance in foodborne pathogens? *Curr. Opin. Food Sci.* **2023**, *25*, 101107.
73. Sateriale, D.; Forgione, G.; Di Rosario, M.; Pagliuca, C.; Colicchio, R.; Salvatore, P.; Paolucci, M.; Pagliarulo, C. Vine-Winery Byproducts as Precious Resource of Natural Antimicrobials: In Vitro Antibacterial and Antibiofilm Activity of Grape Pomace Extracts against Foodborne Pathogens. *Microorganisms* **2024**, *12*, 437. [CrossRef]
74. Draghici-Popa, A.-M.; Buliga, D.-I.; Popa, I.; Tomas, S.T.; Stan, R.; Boscornea, A.C. Cosmetic products with potential photoprotective effects based on natural compounds extracted from waste of the winemaking industry. *Molecules* **2024**, *29*, 2775. [CrossRef]
75. Koutelidakis, A.; Dimou, C. Grape pomace: A challenging renewable resource of bioactive phenolic compounds with diversified health benefits. *MOJ Food Process. Technol.* **2016**, *2*, 262–265. [CrossRef]
76. Lo, S.; Pilkington, L.I.; Barker, D.; Fedrizzi, B. Attempts to create products with increased health-promoting potential starting with pinot noir pomace: Investigations on the process and its methods. *Foods* **2022**, *11*, 1999. [CrossRef]
77. Caponio, G.R.; Noviello, M.; Calabrese, F.M.; Gambacorta, G.; Giannelli, G.; De Angelis, M. Effects of grape pomace polyphenols and in vitro gastrointestinal digestion on antimicrobial activity: Recovery of bioactive compounds. *Antioxidants* **2022**, *11*, 567. [CrossRef] [PubMed]
78. Sinrod, A.J.G.; Shah, I.M.; Surek, E.; Barile, D. Uncovering the promising role of grape pomace as a modulator of the gut microbiome: An in-depth review. *Heliyon* **2023**, *9*, e20499. [CrossRef] [PubMed]
79. Radulescu, C.; Buruleanu, L.C.; Olteanu, R.L.; Nicolescu, C.M.; Bumbac, M.; Gorghiu, L.M.; Nechifor, M.D. *Grape by-Products: Potential Sources of Phenolic Compounds for Novel Functional Foods*; Intech Open: London, UK, 2023; Available online: https://www.intechopen.com/online-first/88679 (accessed on 4 April 2024).
80. Nistor, E.; Dobrei, A.; Dorbei, A.; Bampidis, V.; Ciolac, V. Grape pomace in sheep and dairy cows feeding. *J. Hortic. For.* **2014**, *18*, 146–150.
81. Hassan, Y.I.; Kosir, V.; Yin, X.; Ross, K.; Diarra, M.S. Grape pomace as a promising antimicrobial alternative in feed: A critical review. *J. Agric. Food. Chem.* **2019**, *67*, 9705–9718. [CrossRef] [PubMed]
82. Wittenauer, J.; Mäckle, S.; Sußmann, D.; Schweiggert-Weisz, U.; Carle, R. Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity. *Fitoterapia* **2015**, *101*, 179–187. [CrossRef] [PubMed]
83. Hoss, I.; Rajha, H.N.; El Khoury, R.; Youssef, S.; Manca, M.L.; Manconi, M.; Louka, N.; Maroun, R.G. Valorization of wine-making by-products' extracts in cosmetics. *Cosmetics* **2021**, *8*, 109. [CrossRef]
84. Ferreira, S.M.; Santos, L. A potential valorization strategy of wine industry by-products and their application in cosmetics—Case study: Grape pomace and grapeseed. *Molecules* **2022**, *27*, 969. [CrossRef]
85. Fernández-Bayo, J.D.; Nogales, R.; Romero, E. Winery vermicomposts to control the leaching of diuron, imidacloprid and their metabolites: Role of dissolved organic carbon content. *J. Environ. Sci. Health B* **2015**, *50*, 190–200. [CrossRef]
86. Domínguez, J.; Martínez-Cordeiro, H.; Álvarez-Casas, M.; Lores, M. Vermicomposting grape marc yields high quality organic biofertiliser and bioactive polyphenols. *Waste Manag. Res.* **2014**, *32*, 1235–1240. [CrossRef]
87. Pękal, A.; Pyrzynska, K. Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Anal. Methods* **2014**, *7*, 1776–1782. [CrossRef]
88. Giurescu, I.; Șesan, T.E.; Badju, S.; Lupu, C.; Oancea, F. Preparation of compost from sea buckthorn branches by using a multipurpose *Trichoderma* strain. *Sci. Bull. Ser. F Biotechnol.* **2023**, *27*, 97–104.
89. Teng, Z.; Jiang, X.; He, F.; Bai, W. Qualitative and Quantitative Methods to Evaluate Anthocyanins. *eFood* **2020**, *1*, 339–346. [CrossRef]
90. Brand-Williams, W.; Cuvelier, M.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT* **1995**, *28*, 25–30. [CrossRef]
91. Balouiri, M.; Sadiki, M.; Ibnsouda, S.K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* **2016**, *6*, 71–79. [CrossRef] [PubMed]

92. CLSI. *CLSI Supplement M100S. Performance Standards for Antimicrobial Susceptibility Testing*, 26th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2016.
93. Negro, C.; Aprile, A.; Luvisi, A.; De Bellis, L.; Miceli, A. Antioxidant activity and polyphenols characterization of four monovarietal grape pomaces from Salento (Apulia, Italy). *Antioxidants* **2021**, *10*, 1406. [CrossRef]
94. Muncaciu, M.L.; Zamora Marín, F.; Pop, N.; Babeş, A.C. Comparative polyphenolic content of grape pomace flours from "Feteasc㠠Neagră" and "Italian Riesling" cultivars. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2017**, *45*, 532–539. [CrossRef]
95. Marinelli, V.; Padalino, L.; Nardiello, D.; Del Nobile, M.A.; Conte, A. New approach to enrich pasta with polyphenols from grape marc. *J. Chem.* **2015**, *2015*, 34578. [CrossRef]
96. Xu, Y.; Burton, S.; Kim, C.; Sismour, E. Phenolic compounds, antioxidant, and antibacterial properties of pomace extracts from four Virginia-grown grape varieties. *Food Sci. Nutr.* **2016**, *4*, 125–133. [CrossRef]
97. Gül, H.; Acun, S.; Sen, H.; Nayir, N.; Turk, S. Antioxidant activity, total phenolics and some chemical properties of Öküzgözü and Narince grape pomace and grape seed flour. *J. Food Agric. Environ.* **2013**, *11*, 28–34.
98. Arapitsas, P.; Oliveira, J.; Mattivi, F. Do white grapes really exist? *Food Res. Int.* **2015**, *69*, 21–25. [CrossRef]
99. Chiavaroli, A.; Balaha, M.; Acquaviva, A.; Ferrante, C.; Cataldi, A.; Menghini, L.; Rapino, M.; Orlando, G.; Brunetti, L.; Leone, S.; et al. Phenolic Characterization and Neuroprotective Properties of Grape Pomace Extracts. *Molecules* **2021**, *26*, 6216. [CrossRef] [PubMed]
100. Ramirez-Lopez, L.M.; DeWitt, C.A.M. Analysis of phenolic compounds in commercial dried grape pomace by high-performance liquid chromatography electrospray ionization mass spectrometry. *Food Sci. Nutr.* **2014**, *2*, 470–477. [CrossRef] [PubMed]
101. Di Stefano, V.; Buzzanca, C.; Melilli, M.G.; Indelicato, S.; Mauro, M.; Vazzana, M.; Arizza, V.; Lucarini, M.; Durazzo, A.; Bongiorno, D. Polyphenol Characterization and Antioxidant Activity of Grape Seeds and Skins from Sicily: A Preliminary Study. *Sustainability* **2022**, *14*, 6702. [CrossRef]
102. Sochorova, L.; Prusova, B.; Jurikova, T.; Mlcek, J.; Adamkova, A.; Baron, M.; Sochor, J. The Study of Antioxidant Components in Grape Seeds. *Molecules* **2020**, *25*, 3736. [CrossRef]
103. Abouelenein, D.; Mustafa, A.M.; Caprioli, G.; Ricciutelli, M.; Sagratini, G.; Vittori, S. Phenolic and nutritional profiles, and antioxidant activity of grape pomaces and seeds from Lacrima di Morro d'Alba and Verdicchio varieties. *Food Biosci.* **2023**, *53*, 102808. [CrossRef]
104. Sabra, A.; Netticadan, T.; Wijekoon, C. Grape bioactive molecules, and the potential health benefits in reducing the risk of heart diseases. *Food Chem. X* **2021**, *12*, 100149. [CrossRef]
105. Chagas, M.D.S.S.; Behrens, M.D.; Moragas-Tellis, C.J.; Penedo, G.X.M.; Silva, A.R.; Gonçalves-de-Albuquerque, C.F. Flavonols and Flavones as Potential anti-Inflammatory, Antioxidant, and Antibacterial Compounds. *Oxid. Med. Cell Longev.* **2022**, *6*, 9966750. [CrossRef]
106. WHO. *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics*; World Health Organization: Geneva, Switzerland, 2017.
107. World Health Organization. 2020 Antibacterial Agents in Clinical and Preclinical Development: An Overview and Analysis. Available online: https://www.who.int/publications/i/item/9789240021303 (accessed on 1 July 2024).
108. Corrales, M.; Han, J.H.; Tauscher, B. Antimicrobial properties of grape seed extracts and their effectiveness after incorporation into pea starch films. *Int. J. Food Sci. Technol.* **2009**, *44*, 425–433. [CrossRef]
109. Baydar, N.G.; Sagdic, O.O.E.E.; Ozkan, G.; Cetin, S. Determination of antibacterial effects and total phenolic contents of grape (*Vitis vinifera* L.) seed extracts. *Int. J. Food Sci. Technol.* **2006**, *41*, 799–804. [CrossRef]
110. Papadopoulou, C.; Soulti, K.; Roussis, I.G. Potential antimicrobial activity of red and white wine phenolic extracts against strains of *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. *Food Technol. Biotechnol.* **2005**, *43*, 41–46.
111. Oliveira, D.A.; Salvador, A.A.A.S.; Smânia, E.F.A.; Maraschin, M.; Ferreira, S.R.S. Antimicrobial activity and composition profile of grape (*Vitis vinifera*) pomace extracts obtained by supercritical fluids. *J. Biotechnol.* **2013**, *164*, 423–432. [CrossRef] [PubMed]
112. Bouarab-Chibane, L.; Forquet, V.; Lantéri, P.; Clément, Y.; Léonard-Akkari, L.; Oulahal, N.; Degraeve, P.; Bordes, C. Antibacterial Properties of Polyphenols: Characterization and QSAR (Quantitative Structure–Activity Relationship) Models. *Front. Microbiol.* **2019**, *10*, 829. [CrossRef] [PubMed]
113. Liu, S.; Zhang, Q.; Li, H.; Qiu, Z.; Yu, Y. Comparative assessment of the antibacterial efficacies and mechanisms of different tea extracts. *Foods* **2022**, *11*, 620. [CrossRef] [PubMed]
114. Liu, Z.; de Souza, T.S.P.; Wu, H.; Holland, B.; Dunshea, F.R.; Barrow, C.J.; Suleria, H.A.R. Development of phenolic-rich functional foods by lactic fermentation of grape marc: A review. *Food Rev. Int.* **2023**, *40*, 1756–1775. [CrossRef]
115. Barakat, N.; Bouajila, J.; Beaufort, S.; Rizk, Z.; Taillandier, P.; El Rayess, Y. Development of a new kombucha from grape pomace: The impact of fermentation conditions on composition and biological activities. *Beverages* **2024**, *10*, 29. [CrossRef]