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Sustainable Utilization of Grape Waste by Phytochemical and Bioactivity Assessment for Nutraceutical Application of Sundried Seeds and Peel

Pemanfaatan Berkelanjutan Limbah Anggur melalui Uji Fitokimia dan Bioaktivitas untuk Pemakaian Nutraseutik Biji dan Kulit yang Dikeringkan di Bawah Sinar Matahari

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ABSTRACT

Background: Grape (*Vitis vinifera L.*), a widely cultivated fruit, generates substantial waste during wine production. Traditionally discarded, this waste was reviewed for various applications, including chemicals, bio-products, and pigments.
Objectives: Sundried seeds (SDS) and peel (SDP) of grapes (*Vitis vinifera L.*) were investigated for phytochemical composition and potential bioactivity as waste material for potential health benefits.
Methods: Grapevine waste, specifically seeds and peel, was sun-dried and employed for the experimental study with convenient sampling for quantitative analysis. The dried samples were subjected to Fourier-transform infrared (FTIR) spectroscopy to identify primary and secondary metabolites. Ultraviolet-visible (UV-Vis) spectrophotometry was used to quantify specific compounds, including flavonoids, alkaloids, steroids, and phenolic compounds. Antioxidant activity was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay with different solvents (aqueous, ethanolic, and methanolic). Antimicrobial assays were tested against *Staphylococcus aureus* and *Escherichia coli*.
Results: Sundried seeds (SDS) and peel (SDP) exhibited respective concentrations of 128 mg/ml and 141 mg/ml for flavonoids, 95 AE/g and 103 AE/g for alkaloids, 10.2 mg/ml and 9.6 mg/ml for steroids, and 170 GAE/g and 187 GAE/g for phenolic compounds.IC_{50} yield of SDS extracts exhibited in aqueous(5.84 ppm) and methanolic solvents(6.75 ppm), while SDP extracts showed moderate to strong activity in ethanol(47.71ppm) and methanol(84.50 ppm). *Staphylococcus aureus* inhibited the zone of 9 mm and 10mm and *Escherichiacoli* ruptured the membrane in 10 mm and 8 mm on both samples.
Conclusions: Sundried seeds and peel from grapevine waste exhibited promising antioxidant and antimicrobial properties, promising further research for potential value-added applications.

INTRODUCTION

The burgeoning global population, coupled with an escalating demand for sustainable and nutritious food sources, necessitates innovative approaches to food production and waste management[1]. The agricultural sector, a significant contributor to the global economy, generates substantial amounts of byproducts and waste, often underutilized or mismanaged[2]. This also applies to the important sector of the wine and juice business that is grapevine agriculture. Grapes are meticulously processed, and a considerable quantity of waste, including seeds, peel, and stems, is generated[3]. Traditionally, these byproducts have been disposed of through incineration or landfilling, leading to environmental concerns[4]. The concept of circular economy, which emphasizes resource efficiency and waste minimization, has gained traction in recent years[5]. By transforming grapevine waste into value-added products, such as nutraceuticals, it is possible to contribute to a more sustainable and resilient food system[6]. Nutraceuticals, defined as food-derived products with demonstrated health benefits beyond basic nutrition, have witnessed a surge in popularity due to their potential to prevent and manage chronic

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diseases[7]. The integration of bioactive compounds from grapevine waste into functional foods can offer a unique opportunity to address both nutritional and environmental challenges[8]. Grapevine waste is a complex matrix, comprising a diverse array of phytochemicals, including polyphenols, flavonoids, and resveratrol[9]. These compounds have been extensively studied for their antioxidant, anti-inflammatory, and anti-cancer properties[10]. Polyphenols, in particular, have garnered significant attention due to their potential to protect against oxidative stress, a major contributor to chronic diseases[11]. Resveratrol, a stilbenoid found in grape skins and seeds, has been linked to cardiovascular health, neuroprotection, and anti-ageing effects[12].

This study aimed to explore the potential of grapevine waste as a sustainable source of nutraceutical ingredients. By investigating the phytochemical composition of different grapevine waste fractions, we seek to identify the most promising components for nutraceutical development. In addition to offering consumers functional meals that support optimum health and well-being, our research aimed to advance grapevine waste management into the possible use of value-added products focusing on peel and seeds.

METHODS

This experimental study was performed at the in-house laboratory of the Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women. Sampling has been done in convenient sampling for quantitative analysis of grapevine waste at the Coimbatore region in Tamil Nadu, India, for reducing the waste material that was traditionally utilized for animal feed, fertilizer, and biogas. However, we collected the immediate waste from the vineyards concerning food safety and hazards and separated it into seeds and peels for further use. Additionally, we authenticate the sample from the Tamil Nadu Agriculture University (TNAU) to confirm the species of the grapevine (BSI/SRC/5/23/2022/Tech/491)**.** Sigma Chemical Company supplied the analytical and food grade ethanol, analytical and high-performance liquid chromatography (HPLC) grade methanol, Folin-Ciocalteau reagent, and genuine standards of flavonoids, alkaloids, steroids, and phenolic compounds were analyzed in Ultra-violet visible spectrophotometry by determining the electronic transitions of these compounds within the 200-800 nm wavelength range using an Evolution 300 series spectrophotometer. Standard stock solutions were made using ethanol and methanol, sealed in aluminum foil, and kept at -20°C. Since our study involved neither the use of hazardous chemicals nor animals, we did not seek any kind of ethical clearance.

Sample Collection and Selection

Viticulture waste was purchased at the Madhampatty farm (elevation: 1401ft. N 11°010567619814541) near Coimbatore (South), Tamil Nadu (India). Coimbatore, situated in the rain shadow region of the Western Ghats, enjoys a pleasant climate throughout the year. This favorable climatic condition, characterized by moderate temperatures and adequate rainfall, is conducive to viticulture. The region's ecoclimatic aptitude index, a measure of its suitability for wine production, is rated as excellent, promising high oenological potential. Wine production waste materials were collected during the November–February growing season. These materials were subsequently dried under the sun during the month of May when temperatures ranged from 38 to 42 degrees Celsius between 12 PM and 3 PM for three consecutive days. Waste materials were separated into seeds and peel, cleaned completely, and sundried for the experimental application because of their limited shelf life. Grapes are only in season for a brief period in the summer. Throughout the year, it was improved to be more adaptable and useful for a range of product processing methods[13].

Identification of Metabolites Through Fourier-transform infrared spectroscopy (FT-IR)

Shizmandu FT-IR spectrometer was used to conduct infrared spectroscopy measurements between 2500 nm (4000 cm-1) and 25000 nm (400 cm-1). The annotated spectrum illustrated how the wavelength of light absorbed defines the chemical bond. The chemical bonds of a molecule can be discovered by analyzing its infrared absorption spectra. The MIRacle single-reflection Attenuated Total Reflectance (ATR's) 2 mm spot size and liquid sampling funnel make it easier to evaluate small materials, powders, and liquids. The infrared range of ZnSe is micrometer-regulated and spans from 20,000 to 650 wavenumbers. The data required to determine functional groups were gathered from a single milligram of sun-dried powder sample.

Determination of Alkaloid:

The hydro-ethanolic sample was prepared by dissolving it in Dimethyl Sulfoxide (DMSO), acidifying it with Hydrochloric Acid (HCL), and filtering. Liquid-liquid extraction with chloroform was performed after adding bromocresol green and phosphate buffer. A calibration curve was constructed using atropine standards, and the total alkaloid content of the sample was determined spectrophotometrically at 470 nm and expressed as mg of atropine equivalents (AE) per gram of both extracts.

Estimation of Steroids:

An aliquot of 1 mL hydro-ethanolic extract was combined with varying concentrations of prednisone standard solution in 10 ml volumetric flasks. The mixture was acidified with 2 mL of 4N sulfuric acid, followed by the addition of 2 mL of 0.5% iron (III) chloride and 0.5 mL of 0.5% potassium hexacyanoferrate (III) solution. After incubation in a shaking water bath at 700°C ± 20°C for 30 minutes, the solution was diluted to volume with distilled water. Absorbance was measured at 780 nm against a reagent blank. The total steroid content was expressed as milligrams of prednisone equivalents per gram of extract.

Determination of Total Phenolic Content:

The Folin-Ciocalteu technique was used to calculate the total phenolic content. A color shift occurred from the reaction of a plant extract with sodium carbonate and Folin-Ciocalteu reagent. This

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solution's absorbance was measured at 550 nm and contrasted with a standard curve for gallic acid. Gallic acid equivalents (GAE), or milligrams per gram of sample, were used to express the total phenolic content.

Determination of Flavonoid Content:

The aluminum chloride colorimetric test was used to evaluate the flavonoid concentration. A colorful complex was produced via the reaction of sodium hydroxide, aluminum chloride, and sodium nitrite with both of the sundried samples. The complex's absorbance was measured at 510 nm and compared to the flavonoid concentration of the sample using standard curves for Isorhamnetin, kaempferol, and quercetin.

Antioxidant Activity

The antioxidant activity of sundried samples with different solvents was detected in scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals. In brief, SDS and SDP were prepared with 10, 50, 150, 250, 350, 500 and 750 µl/ml. DPPH solution with a concentration of 0.2 mmol/l was prepared using vitamin C as standard. Two ml sample solution with different concentration gradients was added into a 10 ml brown reagent tube, and then 2 ml DPPH solution was added. After mixing, the absorbance was measured at 517 nm after reaction for 30 min.

Antimicrobial Potential

Evaluation of the effectiveness of sundried sample extracts against pathogenic bacteria has been aided by *in vitro* research. To find the zones of inhibition and minimum inhibitory concentrations (MICs) for bacterial and fungal strains, respectively, microdilution assays and agar diffusion procedures have been widely used. In addition, research on time-kill kinetics has shed light on the microbial growth inhibition kinetics and the bactericidal or fungicidal properties of extracts obtained from grapes. These investigations have shown the grapevine waste extracts' encouraging antibacterial effectiveness, underscoring their potential as natural substitutes for conventional antimicrobial agents.

Inoculum Preparation (Kirby-Bauer Method) and Test Plates

A pure culture of bacteria is prepared by isolating a single colony from an agar plate and inoculating it into a nutrient broth. The culture is incubated until it reaches a desired turbidity, then it is adjusted to a specific concentration, typically containing approximately 1–2 billion bacterial cells per milliliter for *E. coli* and *Staphylococcus aureus*. A standardized bacterial lawn was prepared for antimicrobial susceptibility testing. A standardized inoculum was created by adjusting a broth culture to a specific turbidity. A sterile swab was dipped into this suspension and used to evenly distribute bacteria across the surface of a nutrient agar plate. After allowing excess moisture to evaporate, antimicrobial disks were applied to the plate. Following incubation, the diameter of inhibition zones surrounding the disks was measured to assess antimicrobial activity.

RESULTS AND DISCUSSIONS

Metabolite Identification Through Fourier-Transform Infrared Spectroscopy (FT-IR)

Significant information about the molecular makeup of extracts can be compiled by Fourier-transform infrared spectroscopy (FT-IR) analysis. Several data points of the grapevine waste extracts under study were produced and the functional groups contained in the composition of sundried waste material extracts were identified through this technique. The FT-IR spectra show the presence of several functional groups that could indicate the presence of polyphenolic chemicals in the extract: C = C aromatic (1640 cm-1), C-H aromatic (650 cm-1), and -OH alcoholic/phenolic (3200 - 3400 cm-1). Infrared (IR) spectra display absorption bands representing the interaction of a molecule with infrared light. The x-axis indicates wavenumber, while the y-axis represents absorbance intensity. IR spectra are typically divided into two regions: the group frequency region (above 1500 cm^{-1}) and the fingerprint region (below 1500 cm^{-1}). The former contains characteristic peaks associated with specific functional groups, aiding in their identification. The latter is unique to each molecule, providing a molecular fingerprint for comparison and identification.Specific bond vibrations, such as O-H and C-H stretching, occur within these ranges. For example, O-H stretching is typically observed between 3700 and 2500 cm^{-1}, while C-H stretching appears around 2900-2800 cm^{-1} (Table 1)[14,15].

The fingerprint region of the IR spectrum, below 1500 cm^{-1}, is highly informative. Due to overlapping peaks, the presence or absence of bands in this region can significantly aid in molecular identification. Certain functional groups contribute to specific absorption patterns in this area. Additionally, this region provides insights into the molecule, including information about proteins, lipids, and carbohydrates[16].

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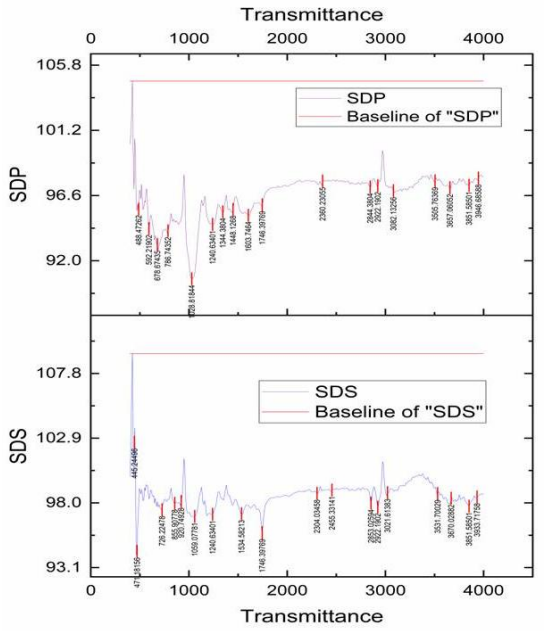
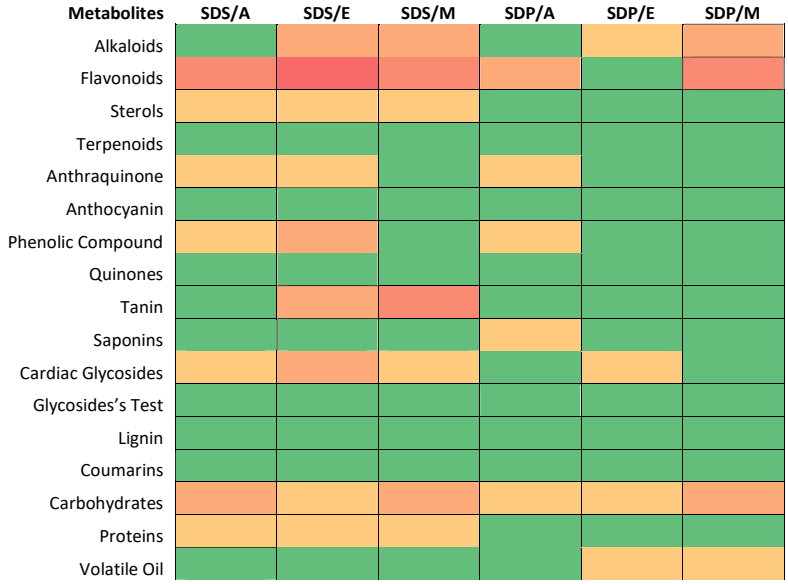


Figure 1. FTIR spectrum of Sundried seeds (SDS) and Sundried peel (SDP) samples

Qualitative and Quantitative Analysis of Phytochemicals

Preliminary phytochemical screening enhanced the proof that the various solvent extracts of sundried seed and peel powder contained alkaloids, flavonoids, phenolic compounds, sterols, anthraquinones, and anthocyanins. Alkaloids and flavonoids were found in higher degrees of precipitation and phenolic compounds, sterols, anthraquinones, and anthocyanins were low in amount in all extracts (Figure 2). The amounts of flavonoids, alkaloids, steroids, and phenolic substances in the test solution were quantified by measuring their absorbance at 424 nm (Table 1). Baroi *et al.*, in 2022[8]stated that the variety of samples, geographical indication, harvesting time, and extraction technique significantly affect the content of active chemicals in grapevine waste. The distinctive features of our experimental sample can be attributed to its traditional drying method and geographic origin.



Green: Present in low concentration; A color shift from yellow to red indicates a moderate to high concentration of phytochemicals. SDS: sundried seed; SDP: sundried peel; A: aqueous; E: ethanol; M:methanol; SDS/A: sundried seed sample in aqueous solution; SDS/E: sundried seed sample in ethanolic solution; SDS/M: sundried seed sample in methanolic solution; similarly, SDP/A: sundried peel sample in aqueous solution; SDP/E: sundried peel sample in ethanolic solution; SDP/M: sundried peel sample in methanolic solution

Figure 2. Data visualization in a heatmap-based qualitative phytochemical analysis

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Phenolic compounds are verified as the highest through qualitative screening of the sample with seventeen metabolites and Fourier-transform infrared spectroscopy. We have selected four metabolites to quantify based on this data. Upon analysis, sundried peel from grapevine wasted a significantly greater number of quantified metabolites whereas steroids in the seed sample were comparatively higher than in the peel sample (Table 1). A 2020 study reported a lower concentration of quantified phytochemicals in hydroalcoholic extracts compared to the concentrations observed in our analysis[17].

Table 1. Quantification of identified phytochemicals

Sample Name	Test Name	Result (mg/ml)
Sun-dried Seed (SDS)	Flavonoids	128
	Alkaloids (AE/g)	95
	Steroids	10.2
	Phenolic Compound (GAE/g)	170
Sun-dried Peel (SDP)	Flavonoids	141
	Alkaloids (AE/g)	103
	Steroids	9.6
	Phenolic Compound (GAE/g)	187

g: grams; AE: atropine equivalents; GAE: gallic acid equivalent; mg/ml: milligrams per milliliter

Antioxidant Potential

Vitis vinifera L. peel and seed samples were analyzed rapidly with DPPH (2,2-diphenyl-1-picrylhydrazyl) solution based on the scavenging power of a hydrogen shift to a radical and aqueous, ethanol, and methanol extract. There was a rapid color shift from pale to colorless, indicating that the extract had scavenging properties. Table 2 shows that the largest amounts of scavenged samples appeared in aqueous and methanolic solutions of the seed sample. The IC_{50} values represented the effectiveness of reaching the 50% scavenging capacity, with a reciprocal relationship between the IC_{50} value and the antioxidant value: the lower the IC_{50} value, the greater the scavenging activity[18].

Table 2. IC_{50} scavenging activity of Sundried Seed (SDS) and Sundried Peel (SDP) samples in different solvent

Sample		10 µl/ml	50 µl/ml	150 µl/ml	250 µl/ml	350 µl/ml	500 µl/ml	750 mg/ml	Linear eq.(y)	R^2	IC_{50}	Allusion
SDS	SDS/A	90.98	96.72	96.72	96.72	95.9	95.9	94.26	0.263x + 94.26	0.073	5.8499	P
	SDS/E	88.52	95.08	88.52	77.87	67.21	64.75	43.44	-7.757x + 106.0	0.869	792.3252	Na
	SDS/M	85.25	95.9	95.08	94.26	94.26	92.62	87.7	0.001x + 92.15	3.00E-07	6.7569	P
SDP	SDP/A	54.1	63.11	78.69	84.43	90.98	95.9	95.9	7.259x + 51.40	0.921	142.5712	W
	SDP/E	57.38	76.23	86.89	95.9	95.9	94.26	93.44	5.473x + 63.82	0.679	47.4158	S
	SDP/M	56.56	69.67	80.33	94.26	95.08	93.44	93.44	6.176x + 58.55	0.777	84.5049	M

IC_{50} µl/ml and its allusion:<10 Powerful (P); 10–50: Strong (S); 500–100: Mild (M); 100–250: Weak (W); >250: Not active (Na). A: aqueous; E: ethanol; M: methanol

Flavonoid and phenolic compounds are the strongest antioxidants that can be extracted using polar solvents. In this study, we attempted to optimize by focusing on the potency of the solvents, namely water, ethanol, and methanol. To maximize the yield of the extracted antioxidants solvent mixtures, i.e. hydroethanol and hydromethanol, can be used in future research.

Determination of Antimicrobial Potential

Grape seeds, peels, and leaves contain a variety of bioactive chemicals that have been linked to *Vitis vinifera L's* effectiveness against pathogenic microorganisms, such as bacteria, fungi, and viruses. One such compound, which is still present in grape skins and has shown strong antibacterial activity against fungi as well as both gram-positive and gram-negative bacteria, is resveratrol. Flavonoids found in large amounts in grape seeds and peel, such as quercetin and catechin, have been demonstrated to have antibacterial properties through the disruption of microbial cell membranes and the inhibition of vital enzymes that are involved in microbial development and pathogenicity. These microbes have a well-known method of action that involves breaking down microbial cell membranes, which allows intracellular components to seep out and eventually causes cell death. Various bacterial and fungal species treated with grape seed and peel extracts have shown this membrane-disrupting action. Research has revealed that certain polyphenols derived from grapes disrupt the processes involved in microbial cell-signaling, hence preventing the production of biofilms

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and the expression of virulence factors—both of which are essential for the pathogenicity and survival of microorganisms in their host settings[19].

Table 3. Zone of inhibition in diameter of SDS and SDP

Sample	Zone of Inhibition (mm)	
	Staphylococcus aureus	***Escherichia coli***
Control (Teicoplanin)	9 mm	16 mm
SDP	10 mm	8 mm
SDS	9 mm	10 mm

Teicoplanin-TEI[30] susceptibility test disc 30 mcg

A study on the bacteriostatic and bactericidal properties of the grape seed revealed structure-activity correlation assays against *E. coli* and *S. enteritidis* and was most effective on *Staphylococcus aureus*. The phenolic compounds in grape seeds are partially hydrophobic, which allows them to interact with the bacterial cell wall and lipopolysaccharide layer. This interaction disrupts membrane stability, leading to cell death. A study demonstrated the strong antimicrobial activity of grape seed extract against both strains of Staphylococcus aureus, with a minimum inhibitory concentration (MIC) of 0.625 mg/mL and a minimum bactericidal concentration (MBC) of 1.250 mg/mL[20]. The hydroalcoholic skin extract derived from Muscat grapes exhibited antimicrobial activity against *Staphylococcus aureus* and *Enterococcus faecalis*, as evidenced by zones of inhibition measuring 7 mm and 5.9 mm, respectively[17]. Hence, the findings of this study suggest that sundried grape seed and peel, derived from pomace, possess the potential to inhibit enterotoxin production. Although the observed zones of inhibition were relatively small, the natural origin of the sample makes it a promising candidate for further investigation as a natural antimicrobial agent. Hence, the study result revealed that sundried seeds and peel from pomace can inhibit enterotoxin production. Even though the zone of inhibition for both bacteria is relatively small, based on the source of the sample, it can be said that it can act as a natural ingredient for antimicrobial.

The study focuses on waste material that is frequently thrown away, tackles a pertinent waste management and valuation issue, and offers a comprehensive understanding of the possible advantages of grapevine waste by combining phytochemical analysis and bioactivity assessment. A rigorous scientific approach is demonstrated by the use of UV-Vis spectrophotometry for the measurement of certain molecules and FTIR spectroscopy for the identification of metabolites. The results point to possible uses for grapevine waste in several sectors, including food, cosmetics, and pharmaceuticals. The study helps to promote sustainable practices and lessen the impact on the environment by investigating the potential of grapevine waste material. Grapevine waste materials (seeds and peel) and two bioactivities (antioxidant and antibacterial) are the main subjects of the investigation. Their potential may be better understood by examining a wider variety of waste products and bioactivities. The study shows the extracts' bioactivity, but it skips over the underlying mechanisms that cause these effects. Clarification of the precise chemicals and processes involved requires more investigation. The qualitative study does not go into great depth about the extraction techniques that were employed, which could have an impact on the extracts' yield and composition. By standardizing extraction processes, the results would be more reproducible. The safety and effectiveness of these extracts in live things must be evaluated through in vivo research. The economic viability of removing bioactive chemicals from grapevine recyclables, the scalability and life cycle assessment of the sample need to be analyzed further.

CONCLUSIONS

Flavonoids found in large amounts in grape seeds and peel- quercetin and catechin, have been demonstrated to have antibacterial properties through the disruption of microbial cell membranes and the inhibition of vital enzymes that are involved in microbial development and pathogenicity. Various bacterial and fungal species treated with grape seed and peel extracts have shown this membrane-disrupting action. Research has revealed that certain polyphenols derived from grapes disrupt the processes involved in microbial cell-signaling, hence preventing the production of biofilms and the expression of virulence factors—both of which are essential for the pathogenicity and survival of microorganisms.

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AUTHOR CONTRIBUTIONS

SG: Conceptualization, performed analysis, drafting and editing the original manuscript, and data interpretation. CAK: Supervised, edited, and reviewed the manuscript.

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