AN IMPROVED METHOD TO EXTRACT POLYPHENOLS FROM VITICULTURE WASTE

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Abstract

Wastes generated during vine cultivation (e.g., grapevine stems and leaves) are rich in bioactive molecules, especially polyphenols, therefore their valorization has received considerable attention in the last few years. This study aimed to assess the efficacy of an enhanced technique for extracting polyphenols from vine canes. The approach included a pre-treatment with a mixture of enzymes that degrade cell walls, followed by ethanol:water (1:1) extraction. Substrate concentrations ranging from 1g to 20g of vine cane were used to produce extracts by fluidized bed extraction (P1-P4) and heat extraction (V1, V2). These extracts were then compared regarding their total phenolic content (TPC), hydrolyzable and condensed tannins, antioxidant (DPPH test) and antibacterial activity on *E. coli* ATCC 8739. The extract obtained from 5g of dry vine cane (P2) showed the highest antioxidant activity at 85.64±0.22% and a high value for TCP (83.85±4.62 mg GAE/g dw), compared to extract V2 with TCP 53.1±3.10 mg GAE/g dw. The highest gallotannin content (16.26±0.03 mg tannin acid/g dw) was detected in the extract P4, while the condensed tannins were low in all samples.

This preliminary work suggests the potential of viticulture waste for polyphenol-rich nutraceutical production.

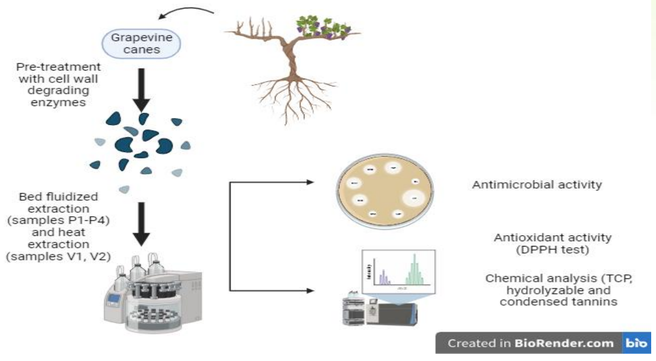

Figure 1. Graphical Abstract

Materials and Methods

1. *Samples collection and preparation*. Vine canes from red (*Cabernet Sauvignon*) and white (*Tămâioasă Românească*) grape varieties were obtained in the spring of 2023, from the Pietroasa area (Romania). The samples were oven-dried at 50^{0}C, 24h, and milled.
2. *Pre-treatment procedure*. Samples with different substrate concentrations (1 to 20g) were mixed with water and 0.5% Viscozyme L (*Sigma-Aldrich*), a cell wall degrading enzymes complex, for 24h, 200rpm.
3. *Extraction methods*. Fluidized bed extraction (for samples P1-P4 containing 1g, 5g, 10g, and 20g of the substrate), room temperature, and heat extraction at 67^{0}C, for 5 min (samples V1 and V2, respectively, both have 4g substrate) were used with a mix of ethanol: water (1:1 v/v). The pH of the extracts was 5.5-6 .
4. *Chemical analysis*. The total phenolic content (TPC) was assessed using ISO 14502-1, expressed as gallic acid (GAE) eq., condensed tannins/ proanthocyanidins (calculated as cyanidin chloride eq.) with a modified butanol-HCl method and hydrolyzable tannins/gallotannins (calculated as tannic acid eq.) with Haslam et al., 1965 method [5-7].
5. *Antioxidant and antibacterial activities*. The DPPH radical scavenging activity (%) was determined based on the protocol previously reported, with ascorbic acid as standard (AcS 1%) [8]. Disk-diffusion method was used to test the antimicrobial effect of non-alcoholic extracts on *Escherichia coli* ATCC 8739. The results were compared to the susceptibility of antibiotic ciprofloxacin CIP1, (inhibition zone 9±1.41 mm) and antimicrobial activity ratio calculated (*Figure 1*).

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Introduction

Cultivating vines for wine production is a longstanding and significant agricultural pursuit on a global scale. However, the wine-making industry produces several waste materials, such as grapevine stems and leaves, and by-products from the vinification process, including grape pomace, bagasse, and wine lees. It is considered that annually 2 to 5 tonnes/hectare of grapevine canes are produced, but only in the last years their valorization has received considerable attention as they are rich in bioactive compounds, especially dietary fibers and polyphenols (stilbenes, gallotannins, and and proanthocyanidins) [1,2].

There is a growing demand to find natural compound mixtures that can be used in the development of innovative products for the nutraceutical industry [3]. In this process, the efficiency of extraction methods to recover bioactive compounds with health-promoting effects is very important. Enzyme-based extraction method has proved to increase the amount of total (free and polysaccharides bound) phenolics and antioxidant activity of extracts [4]. Therefore, our study focused on evaluating the efficacy of an improved method to extract polyphenols from vine canes. This is based on a sample pre-treatment with plant cell wall degradation enzymes that increase the extraction of total phenolics.

Results and Discussion

1. The TCP and gallotannin contents of grapevine cane extracts obtained after enzyme pre-treatment and bed fluidized extraction were influenced by the amount of substrate, while the condensed tannins were low in all samples (*Figures 2-4*).
2. The extract obtained from 5g of vine cane (P2) showed a high value for TCP (83.85±4.62 mg GAE/g dw), the highest antioxidant activity (85.64±0.22%) and strong antibacterial activity (*Table 1*).
3. The sample (P4) with excessive substrate showed low TCP and antioxidant activity, but increased gallotannin content (16.26±0.03 mg/g dw).
4. Vinegrape extract obtained by heat extraction (V2) had an increased TCP (53.10±3.13 mg GAE/g dw) and gallotannins content (12.15±0.03 mg/g dw), compared to sample V1.
5. Five of six extracts showed a high capacity for scavenging the DPPH radical and the results are better than previously reported [9,10].

Table 1. Antimicrobial activity of grapevine cane extracts.

Samples	Inhibition zone (mm)	Antimicrobial activity ratio
P1	14±5.65	1.55
P2	22±0.11	2.44
P3	25±1.41	2.77
P4	22±2.82	2.44
V1	17±1.41	1.88
V2	20±0.10	2.22

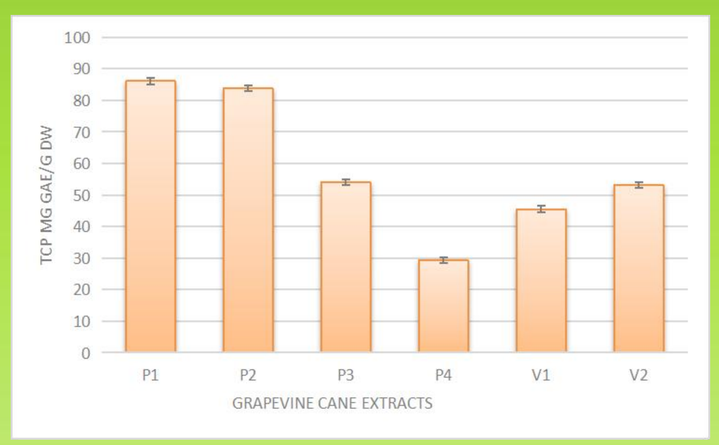

Figure 2. Total phenolic content (TCP) of grapevine cane extracts.

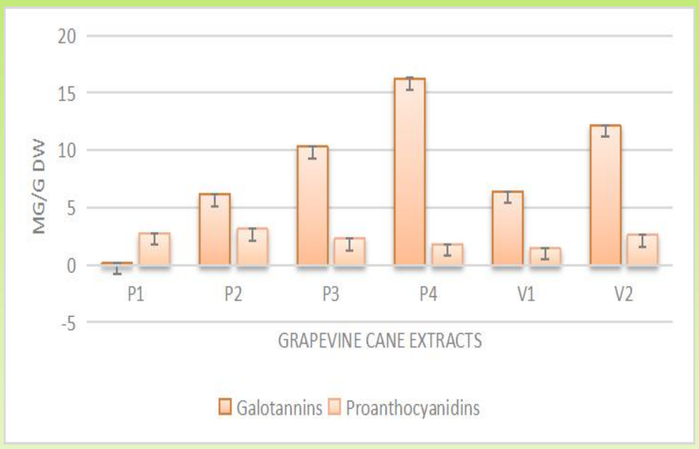

Figure 3. Hydrolyzable and condensed tannins content of grapevine cane extracts.

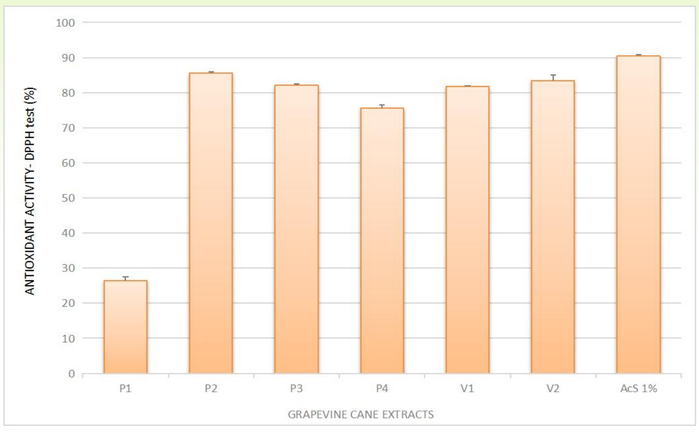

Figure 4. Antioxidant activity of grapevine cane extracts.

Conclusions

The grapevine stems are the least valorized subproduct from the wine industry, despite being produced in huge amounts (25% of total winery wastes). The work proposes a new approach to extract polyphenols from vine canes, based on enzyme-assisted pre-treatment and fluidized bed extraction, which is a relatively simple and cost-effective method. A high value for total phenolic content (83.85±4.62 mg GAE/g dw), and increased gallotannins composition were obtained after extraction from 5g of substrate (P2). In terms of antioxidant activity, the DPPH value for this extract was the highest (85.64±0.22%), while it showed strong antimicrobian activity on the pathogen *E. coli*.

This approach is environmentally friendly, while the polyphenolic profile of obtained vine cane extracts proves their potential to be exploited by the nutraceutical industry. Further works will investigate the polyphenol-rich cane extract modulation effects on dysbiotic gut microbiota and their health-promoting effects.

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