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EVALUATION OF THE POLYPEHNOL EXTRACTS FROM VINE CANES (*Vitis vinifera* L.) OBTAINED BY AN IMPROVED EXTRACTION METHOD

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Abstract

Waste vegetal materials generated during vine pruning are rich in bioactive molecules, especially polyphenols, therefore their valorization received considerable attention in the last years. This work focuses on evaluating the polyphenol composition and antimicrobial activity of vine cane extracts obtained with an improved method using a pre-treatment with cell wall degrading enzymes and fluidized bed extraction. Different quantities of canes (1 to 20 g) were used to obtain several extracts. Total phenolic content (TPC), hydrolysable and condensed tannins, antimicrobial and antioxidant activity (DPPH test) were determined. The extract obtained from 5 g of dry vine cane showed the highest antioxidant activity (85.64±0.22%), a high value of TCP (83.85±4.62 mg GAE/g dw) and a 2.44 times higher antimicrobial activity compared to antibiotic ciprofloxacin on E. coli ATCC 8739. However, the highest gallotannin content (16.26±0.03 mg tannin acid/g dw) was detected in the extract obtained from 20 g of vine canes, while the condensed tannin was low in all samples.

Key words: *improved extraction method, grapevine cane, polyphenol composition, antioxidant activity, antimicrobials.*

INTRODUCTION

Cultivating vines for grape and 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, wine marc and wine lees (Ferrer-Gallego et al., 2022; Taladrid et al., 2023). It is considered that annually 2 to 5 tonnes/hectare of grapevine canes are produced (Arvanitoyannis et al., 2006). Although the vine canes represent 25% of total winery wastes, only in the last years their valorization has received considerable attention, as they are rich in bioactive compounds, especially dietary fibers and polyphenols (Baroi et al., 2022; Rodrigues et al., 2023). It contains a rich array of polyphenols, including stilbenes, gallotannins, proanthocyanidins and phenolic acids (Aliaño-González et al., 2020; Escobar-Avello et al., 2019; Escobar-Avello et al., 2021). The variation in concentration of polyphenolic compounds is correlated with the grape variety, the cultivation conditions, harvesting time and storage conditions (Gorena et al., 2014; Piñeiro et al., 2017). Thus, Escobar-Avello et al., 2019 using high-resolution mass spectrometry identified in grape cane extract a total of 75 phenolic compounds, the most abundant being proanthocyanidins and stilbenoids and their oligomers (Escobar-Avello et al., 2019). In a recent review on the grapevine chemical composition of different vegetative parts, Goufo et al., 2020 found that flavonols (83.43% of total phenolic levels) and flavan-3-ols (61.63%) are the main compounds in stems and leaves (Goufo et al., 2020). The richest in stilbene were Pinot noir and Gewurztraminer cane extracts, containing up to 5800 mg/kg dry weight (Aliaño-González et al., 2020). These phytochemicals, especially stilbenes, exhibit potent antioxidant effects, scavenging free radicals and combating oxidative stress (Aliaño-González et al., 2020). Additionally, grape vine cane extract is replete with vitamins, minerals, and amino acids, further enhancing its nutritional profile. Therefore, the antioxidant, anti-inflammatory, and anti-microbial properties of vine cane extracts have been tested as potential treatments for cardiovascular and neurological diseases, and even cancer, with promising results (Dani, 2010; Selma et al., 2021; Quero et al., 2021; Empl et al., 2018). There is a growing demand to find natural compound mixtures that can be used in the development of innovative products for the nutraceutical industry (Dinu et al., 2023). Different methods for extracting polyphenols from grapevine canes have been studied and recently reviewed (Baroi et al., 2022; Aliaño-González et al., 2020). 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 (Hong et al., 2013).

Therefore, this study aims to evaluate the efficacy of an improved method to extract polyphenols from vine canes. This is based on a cane cuts pre-treatment with plant cell wall degradation enzymes, to help increase the extraction of total phenolics.

MATERIALS AND METHODS

Samples collection and preparation. Vine canes from red (Cabernet Sauvignon) and white (Tămâioasă Românească) grape varieties were obtained in the spring of 2023, from Pietroasa winegrowing center (Romania). The samples were cut into 2-5 cm sections and oven-dried at 50^0C, 24h, and then milled. Samples containing 1 g, 5 g, 10 g, and 20 g of the substrate were coded P1-P4, respectively

Pre-treatment procedure. Samples with different substrate concentrations (P1-P4) were mixed with water and 0.5% Viscozyme L (Sigma-Aldrich), a cell wall degrading enzymes complex, for 24 h, 200 rpm.

Extraction methods. Fluidized bed extraction with IKA-RET 135.2 was used in the presence of a mix of ethanol: water (1:1 v/v). The pH of the extracts was 5.5-6.

Chemical analysis. The total phenolic content (TPC) was assessed using ISO 14502-1; 2005 and expressed as gallic acid (GAE) eq. Condensed tannins/proanthocyanidins (calculated as cyanidin chloride eq.) were determined with a modified butanol-HCl method (Scalbert et al., 1989; Vermerris and Nicholson, 2006) and hydrolysable tannins/gallotannins (calculated as tannic acid eq.) using KIO_3 method (Haslam *et al.*, 1965; Bate-Smith, 1977; Willis and Allen, 1998).

Antioxidant and antibacterial activities. The DPPH radical scavenging activity (%) was determined based on a protocol previously reported, with ascorbic acid as standard (AcS 1%) (Vamanu and Nita, 2013). 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 the antibiotic ciprofloxacin CIP1 (Oxoid), which presents an inhibition zone of 9±1.41 mm. Antimicrobial activity ratio was calculated as extract inhibition zone/CIP1 inhibition zone.

Statistical analysis. The results presented in figures are average values of at least three replications. Standard deviation was also calculated with the software package Excel.

RESULTS AND DISCUSSIONS

Grapevine cane extract is a treasure trove of bioactive compounds (Souquet et al., 2000). Therefore, in recent years, the valorization of vine grape extract has attracted significant attention for its potential contributions to health and wellness (Niculescu and Ionete, 2023).

This work focused on evaluating the polyphenol composition, antioxidant and antimicrobial activity of vine cane extracts obtained with an improved method. The samples P1-P4 with different substrate concentrations (1 g, 5 g, 10 g, and 20 g, respectively) were pre-treated for 24 h with 0.5% solution of cell wall degrading enzymes, followed by fluidized bed extraction with a mix of ethanol: water (1:1 v/v). The polyphenol composition of different extracts are shown in Figures 1-2.

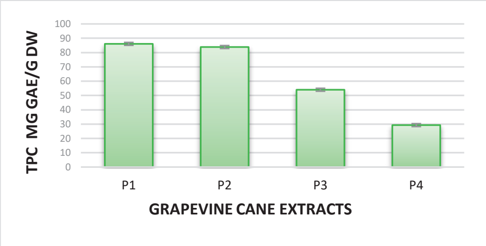

Figure 1. The effect of substrate concentration (samples P1-P4 containing 1 g, 5 g, 10 g, and 20 g of substrate, respectively) on total phenolic content (TPC) of grapevine cane extracts

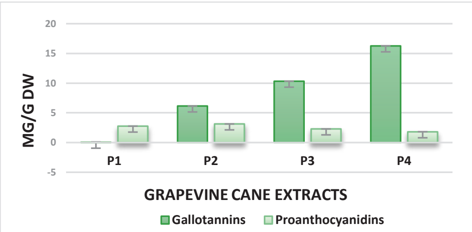

Figure 2. The effect of substrate concentration (samples P1-P4 containing 1g, 5 g, 10 g, and 20 g of substrate, respectively) on hydrolysable and condensed tannins content of grapevine cane extracts

The extract obtained from 5 g of vine cane (P2) showed a high value for TPC (83.85±4.62 mg GAE/g dw), while the gallotannin content was 6.15±0.01 mg/g dw. However, the sample (P4) obtained after bed fluidized extraction of 20 g substrate showed low TCP, but increased gallotannins content (16.26±0.03 mg/g dw). The total phenol and gallotannin contents of grapevine cane extracts were influenced by the amount of substrate used, but not necessarily in a direct dependence with the quantity. Thus, higher TCP was detected in plant extracts obtained from up to 5 g grapevine canes. Hydrolysable tannin content gradually increased with substrate concentration, while the condensed tannins were low in all samples, with a maximum value of 3.13±0.001 mg/g dw for sample P2. Dorosh et al., 2022 using an optimized procedure of subcritical-water extraction obtained cane extracts with TPC 181±12 mg GAE/g dw from one of the Portuguese vine varieties (Dorosh et al., 2020). However, the gallic acid content of up to 15 mg GA/g dw was detected (Dorosh et al., 2020). In another study, methanol extracts obtained by ultrasound-assisted extraction from three Greek vine varieties (Mavrodaphne, Muscat and Rhoditis) were compared. The higher total phenolic content was 374.76 mg GAE/g dw for extract from the Mavrodaphne variety and the predominant phenolics were gallic acid, caffeic acids, quercetin and quercitrin (Veskoukis et al., 2020). It was thus suggested that methods based on plant cell wall degradation increased the polyphenolic content. These works also found that higher levels of total phenols and the presence of gallic acid increased the antioxidant activity of the extracts (Dorosh et al., 2020; Veskoukis et al., 2020).

Three of four grapevine cane extracts had a high antioxidant activity detected using DPPH test (Figure 3). Sample P2 showed the highest antioxidant activity (85.64±0.22% inhibition), probably correlated with the TCP content. The antioxidant activity for samples obtained from 10 g (P3) and 20 g (P4) grapevine canes was slowly lower than that for P2 sample. However, using the lowest amount of canes (1 g) significantly decreased the antioxidant activity to 26.46±1.10% inhibition.

Grape canes of black and white *Vitis vinifera* grapes varieties were collected from Czech vineyards and phenols extracted were by 40% ethanol (Gharwalova et al., 2020). The antioxidant activity of the samples (DPPH test) ranged between 29.46-71.46% inhibition and the TPC varied between 6.30-20.44 mg GAE/g dw (Gharwalova et al., 2020). Testing the antioxidant activities in polyphenol-rich grape cane extracts from 44 European varieties, Ferrier et al., 2022 noted that extracts from Savagnin blanc, Villard noir and Magdeleine noire des Charentes had higher antioxidant capacities based on ORAC, ABTS, DPPH, FRAP, CUPRAC and chelation assays (Ferrier et al., 2022). Moreover, the higher level of some molecules, especially E-resveratrol (3), E-piceatannol (4), E-ε-viniferin (13), hopeaphenol (36), isohopeaphenol (37) and Z/E-vitisin B (41) were found to be the main drivers of ABTS and DPPH capacities.

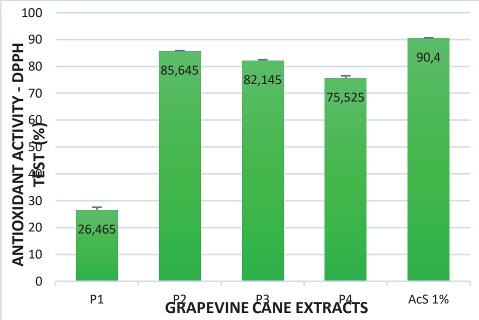

Figure 3. Antioxidant activity of grapevine cane extracts

All extracts obtained after enzyme pre-treatment and bed fluidized extraction proved to have a strong antibacterial activity on strain *E. coli* ATCC 8739 (Figure 4). In the case of P2 and P4 samples, the antimicrobial activity ratio was 2.44 higher compared to the antibiotic ciprofloxacin (CIP1). However, the larger inhibition zone (25±1.42 mm) was noted for extract obtained from 10 g of vine canes (P3).
In another work *Vitis vinifera* var. Red Globe cane extracts were used as a sanitizer and were effective in reducing the populations of pathogens *Listeria monocytogenes*, *Staphylococcus aureus, Salmonella enterica* subsp. *enterica* serovar *typhimurium*, and *Escherichia coli* O157: H7 (Vázquez-Armenta et al., 2017). Some promising results were obtained by exploiting the antioxidant and/or antimicrobial activity of vine canes or their extracts. They were able to replace the most common oenological additives and inhibit the activity of major food pathogens (Troilo et al., 2021; Blackford et al., 2021).

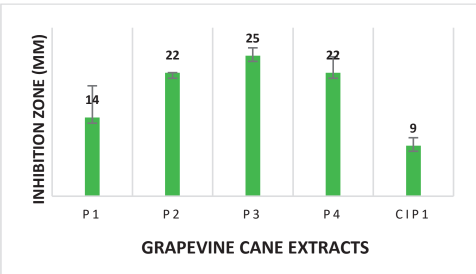

Figure 4. Antimicrobial activity of grapevine cane extracts and antibiotic ciprofloxacin (CIP1)

CONCLUSIONS

The grapevine stems are the least valorized subproduct from the wine industry despite being produced in huge amounts. 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 5 g of substrate (P2). As regards the antioxidant activity, the DPPH value for this extract was the highest (85.64±0.22%), while it showed strong antimicrobial 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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