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Research Article

Antimicrobial Activity of Grape Pomace Extracts Against Different Species of Microorganisms

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The food sector has become interested in grape pomace for its numerous health benefits and high concentration of bioactive chemicals. In this study, the antibacterial properties of grape pomace obtained from by-products of white and blue grapes were investigated. The aim of our study was antimicrobial activity of grape pomace extracts from blue varieties (Alibernet, Dornfelder, Cabernet Sauvignon), and white varieties (Blaufränkisch, Sauvignon Blanc, Welschriesling, Weisser Riesling, Irsai Oliver, Pinot Blanc, Palava, Müller-Thurgau, Grűner Veltliner, and Feteasca Regala). The antimicrobial activity of grape pomace extracts was evaluated against nine microorganisms Gram-positive, Gram-negative bacteria and yeasts with disc diffusion method. The best antimicrobial activity of blue grape pomace extract was found against *Bacillus subtilis*. White varieties Sauvignon Blanc, Welschriesling, Weisser Riesling, Irsai Oliver, Pinot Blanc pomace extracts were the most effective ones against *B. subtilis* and Müller-Thurgau grape pomace extract was the most effective one against *C. koseri* and Grűner Veltliner and Feteasca Regala against *B. subtilis*. The most sensitive bacteria were *B. subtilis*.

Keywords: white and blue grape variety, antimicrobial activity, antibiotic resistance, pomace extract

1 Introduction

Grapes are classified as belonging to the genus Vitis, of which there are numerous species, cultivars, and variations. Wine grapes, of which Merlot and Chardonnay are examples of blue and white cultivars/varieties, respectively, are part of the *Vitis vinifera* species. Additionally, table grapes and juice are produced from this type. Popular grape cultivars/varieties used for table (fresh eating), juice, and preserves are concord grapes. Muscadine grapes are members of the *Vitis rotundifolia* species, whereas Concord grapes belong to the *Vitis labrusca* species (Hassan et al., 2019).

The main sources of pomace, which is a by-product of the industrial processing of grapes, are the skins, stems, and seeds from winemaking. Grape marc is also produced in the manufacture of juices and jellies. Approximately 25% of weight of grapes are discarded during the winemaking process (Dwyer et al., 2014), making them a rich, affordable, and underrated source of active phytochemicals. Dried grape pomace or purified grape pomace extract can be proposed to circumvent certain obstacles in the use of grape pomace, such as toxicity, storage stability, and viability of the regeneration process. In recent research, several new extraction techniques have been investigated to obtain polyphenols with biological, antibacterial, and antioxidant properties from different grape pomace extracts (Gerardi et al., 2020; Pintać et al., 2018; Sirohi et al., 2020).

Numerous studies have been conducted on the antimicrobial properties of grape pomace extracts in relation to foodborne pathogens. The varied phenolic content of grape pomace extracts is usually attributed to their antibacterial activity. Numerous studies have shown that the primary phenolic molecules responsible for antibacterial activity are not flavonoids but phenolic acids. In particular, hydroxycinnamic acids have been found to have a higher ability to penetrate cell membranes than hydroxybenzoic acids (Campos et

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al., 2003). Recently, there was a substantial correlation found between total flavan-3-ols and the antibacterial activity of grape pomace extracts (Peixoto et al., 2018). Furthermore, blue grape pomace extract's polyphenols enhanced the action of several antibiotic classes against *Staphylococcus aureus* and *Escherichia coli*, making them especially helpful in limiting the development of clinical isolates that are resistant to several drugs (Sanhueza et al., 2017; Silva et al., 2018).

Similar to other wine by-products, the antibacterial activity of grape pomace extracts against populations of spoilage microorganisms reduced the deleterious effects of microbial metabolism, including gas formation (Ribeiro et al., 2013; Yamakoshi et al., 2001), slime (Furiga et al., 2014), acid (Thimothe et al., 2007), and biogenic amines (Alberto et al., 2007; Wang et al., 2015). As a result, grape pomace can be used in the food industry as a natural preservative to enhance food safety and quality.

The antibacterial properties of grape pomace extracts, as well as their ability to suppress various pathogenic microbes, have been studied for a long time. In terms of efficacy and doses used, the studies provided mixed but encouraging results. The aim of this study was to assess the antibacterial activity in order to investigate their potential as a natural source of antimicrobial agents.

2 Material and Methods

2.1 Grape

At Vrbové (48° 37′ 12" N, 17° 43′ 25" E) in Slovakia, ripe grapes from wine cultivars were gathered. Four blue grape varieties Alibernet, Dornfelder, Cabernet Sauvignon, and white varieties Blaufränkisch, Sauvignon Blanc, Welschriesling, Weisser Riesling, Irsai Oliver, Pinot Blanc, Palava, Müller-Thurgau, Grűner Veltliner, and Feteasca Regala vintage of year 2023 were utilized for their antibacterial properties.

2.2 Grape Pomace Extract

Pomace extracts were made from just one batch of product. After being received, a 50 g part of the pomace samples was freeze-dried right away. The samples were shaken overnight while 96% ethanol was extracted at a ratio of 1 : 10 (m.v^{-1}). To get rid of undesirable contaminants, the extracts were filtered through Whatman no. 2 filter paper. Following the organic solvent's evaporation, the filtrates were diluted in 20 mg.mL^{-1} of dimethyl sulfoxide (DMSO) to create the stock solution, which was then kept at -20 °C for future research.

2.3 Microorganisms Tested

In this study, six strains of microorganisms were tested: three strains of Gram-positive bacteria, namely *Bacillus subtilis* subsp. Spizizenii (CCM 1,999), *Listeria ivanovii* (CCM 5884), *Micrococcus luteus* (CCM 732), three strains of Gram-negative bacteria, namely *Citrobacter koseri* (CCM 2535), *Enterobacter aerogenes* (CCM 2531), and *Shigella sonei* (CCM 1373); and three strains of yeast, namely *Candida albicans* (CCM 8186), *Candida glabrata* (CCM 8270), and *Candida tropicalis* (CCM 8223). Every strain that was put to the test came from the Czech Collection of Microorganisms, which is located in Brno, Czech Republic. The yeasts were grown in the Sabouraud Dextrose Broth (SDB, Oxoid, Basingstoke, United Kingdom) at 25 °C for 24 hours, while the bacterial suspensions were cultured in the Muller Hinton Broth (MHB, Oxoid, Basingstoke, United Kingdom) at 37 °C for 24 hours.

2.4 Disc Diffusion Method

The antibacterial activity of grape pomace extracts was evaluated using agar disk diffusion technique. Briefly, Mueller Hinton agar (MHA, Oxoid, Basingstoke, UK) and Sabouraud dextrose agar (Oxoid, Basingstoke, UK) were coated with a suspension of the test microorganism (0.1 mL 10^5 cells mL) at 25 °C. Filter paper discs (6 mm diameter) were placed on the inoculation plates and inoculated with 15 µL of extract from the extracts. The sensitivity of the bacteria studied was assayed using tetracycline as a positive control. The plates were incubated aerobically for 24 h at 37 °C and 48 h at 25 °C for bacteria and yeasts, respectively, then stored at 4 °C for two hours. Gentamicin, cefoxitin and fluconazole were used as positive controls, and each experiment was performed in triplicate.

2.5 Statistical Analyses

One-way ANOVA, followed by the Tukey's HSD test at $p \leq 0.05$ significance, was performed using online Astatsa Anova One Way.

3 Results and Discussion

The best antimicrobial activity of Alibernet variety pomace grape extract was found against *E. aerogenes*, *B. subtilis* and *L. ivanovii* (7.33 mm). Donfelder variety pomace extract showed Table 1 and the best antimicrobial activity against *C. koseri* and *B. subtilis* (8.67 mm), Cabernet Sauvignon variety pomace grape extrakt against *B. subtilis*, and Blaufränkisch variety pomace extract against *B. subtilis* (8.67 mm). The blue varieties of grape pomace extracts were the most effective against *B. subtilis*. The white varieties Sauvignon Blanc, Welschriesling, Weisser Riesling, Irsai Oliver, Pinot

Table 1 Antimicrobial activity of with disc diffusion method in mm

Grape variety	*Citrobacter koseri*	*Enterobacter aerogenes*	*Sigella sonei*	*Bacillus subtilis*	*Listeria ivanovii*	*Micrococcus luteus*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*
Alibernet	5.67 ±0.58^{a}	7.33 ±0.58^{b}	6.33 ±0.58^{b}	7.33 ±0.5 8^{b}	7.33 ±0.58^{b}	6.33 ±0.58$^{a, b}$	5.33 ±0.58^{a}	5.67 ±0.58^{a}	5.33 ±0.58^{a}
Dornfelder	8.67 ±0.58^{a}	8.33 ±0.58^{a}	7.33 ±0.58$^{a, b}$	8.67 ±0.58^{a}	8.33 ±0.58^{a}	7.33 ±0.58$^{a, c}$	4.67 ±0.58^{d}	5.33 ±0.58^{d}	6.33 ±0.58$^{b, c, d}$
Cabernet Sauvignon	7.33 ±0.58^{a}	6.33 ±0.58$^{a, b}$	5.67 ±0.58$^{b, c}$	7.67 ±0.58^{a}	7.67 ±0.58^{a}	6.67 ±0.58$^{a, c, d}$	5.33 ±0.58$^{b, d\ e}$	5.33 ±0.58$^{b, d, e}$	4.33 ±0.58$^{c, e}$
Blaufränkisch	8.33 ±0.58^{a}	6.67 ±0.58^{b}	6.33 ±0.58^{b}	8.67 ±0.58^{a}	8.33 ±0.58^{a}	7.33 ±0.58$^{a, b}$	6.33 ±0.58^{b}	5.33 ±0.58^{b}	5.67 ±0.58^{b}
Sauvignon Blanc	6.67 ±0.58^{a}	6.33 ±0.58^{a}	6.67 ±0.58^{a}	7.67 ±0.58$^{a, b}$	8.33 ±0.58^{b}	6.33 ±0.58^{a}	6.33 ±0.58^{a}	4.33 ±0.58^{c}	4.67 ±0.58^{c}
Welschriesling	6.67 ±0.58^{a}	7.33 ±0.58$^{a, b}$	5.67 ±0.58$^{a, c}$	8.33 ±0.58$^{b, d}$	7.33 ±0.58$^{a, d}$	7.67 ±0.58$^{a, d}$	5.33 ±0.58$^{a, e}$	4.33 ±0.58$^{c, e, f}$	3.67 ±0.58^{f}
Weisser Riesling	5.67 ±0.58^{a}	6.67 ±0.58$^{a, b}$	6.33 ±0.58$^{a, c}$	8.67 ±0.58^{d}	7.67 ±0.58$^{b, c, d}$	7.67 ±0.58$^{b, c, d}$	5.33 ±0.58^{a}	4.67 ±0.58^{a}	4.33 ±0.58^{a}
Irsai Oliver	6.33 ±0.58^{a}	5.67 ±0.58$^{a, b}$	6.33 ±0.58^{a}	8.33 ±0.58^{c}	7.33 ±0.58$^{a, c}$	7.33 ±0.58$^{a, c}$	4.67 ±0.58^{b}	4.33 ±0.58^{b}	4.33 ±0.58^{b}
Pinot Blanc	5.33 ±0.58^{a}	4.33 ±0.58^{a}	5.67 ±0.58^{a}	7.67 ±0.58^{b}	7.33 ±0.58^{b}	7.33 ±0.58^{b}	5.67 ±0.58^{a}	4.67 ±0.58^{a}	4.33 ±0.58^{a}
Pálava	7.67 ±0.58^{a}	8.33 ±0.58^{a}	6.67 ±0.58^{a}	7.67 ±0.58^{a}	7.67 ±0.58^{a}	7.67 ±0.58^{a}	4.67 ±0.58^{b}	4.33 ±0.58^{b}	3.67 ±0.5 8^{b}
Müller-Thurgau	7.67 ±0.58^{a}	7.33 ±0.58^{a}	5.33 ±0.58^{b}	7.33 ±0.58^{a}	6.33 ±0.58$^{a, b}$	6.33 ±0.58$^{a, b}$	4.67 ±0.58^{b}	5.33 ±0.58^{b}	4.33 ±0.58^{b}
Grűner Veltliner	6.67 ±0.58^{a}	6.33 ±0.58^{a}	5.67 ±0.58$^{a, b}$	7.67 ±0.58^{a}	7.33 ±0.58^{a}	7.33 ±0.58^{a}	5.67 ±0.58$^{a, c}$	4.33 ±0.58$^{b, c, d}$	3.67 ±0.58^{d}
Feteasca Regala	6.33 ±0.58^{a}	6.67 ±0.58^{a}	5.33 ±0.58$^{a, b}$	8.67 ±0.58^{c}	8.33 ±0.58^{c}	6.33 ±0.58^{a}	4.67 ±0.58^{b}	4.33 ±0.58^{b}	4.67 ±0.58^{b}

*Data are the mean (± SD) of 3 samples; different letters in each row refer to significant differences (Tukey, $p \leq 0.05$)

Blanc pomace extracts were the most effective against *B. subtilis*. Palava pomace extract was the most effective against *E. aerogenes* (8.33 mm). Müller-Thurgau grape pomace extract was most effective against *C. koseri* (7.67 mm) and Grűner Veltliner, Feteasca Regala against *B. subtilis* (7.67 resp. 8.67 mm).

The Table 2 shows antimicrobial resistance against tested bacteria and yeasts. By instantly permeabilizing and clumping bacterial cells, the proanthocyanidin-rich grape seed extracts demonstrated the capacity to quickly inactivate *Listeria monocytogenes* and *Listeria innocua* (Bisha et al., 2010). Furthermore, the checkerboard method was utilized to assess the synergistic effects of grape pomace extracts with numerous commonly used antibiotics against a number of clinical isolates of *Escherichia coli* and *Staphylococcus aureus* (Sanhueza et al., 2017). The same study's findings demonstrated that the extract's constituents – quercetin, gallic acid, protocatechuic acid, and luteolin – when combined with various antibiotic classes – including β-lactam, quinolone, fluoroquinolone, tetracycline, and amphenicol – opposed *S. aureus* and *E. coli* growth in a synergistic manner (Sanhueza et al., 2017).

Using well diffusion assays, extracts of grape skins from conventionally and organically grown Riesling grapes (*V. vinifera* var. *riesling*) were evaluated for their antimicrobial activity against *Salmonella enterica* serovar *Typhimurium*, *E. coli*, *L. monocytogenes*, *S. aureus*, and *Enterococcus faecium*. Because organic grape skins had higher quantities of quercetin, *L. monocytogenes* was marginally more susceptible to the extracts from organic grape skins than the conventional extracts, which shown comparable levels of activity against all Gram-positive bacteria. Nevertheless, neither of the extracts was able to stop the tested Gram-negative bacteria (*Salmonella* and *E. coli*) from growing (Corrales et al., 2010).

In a different investigation, at different concentrations, extracts from the pomace of the Merlot grape (*V. vinifera* var. *merlot*) were able to suppress the growth of *E. coli*, *Morganella morganii*, *P. aeruginosa*, *E. faecalis*, *L. monocytogenes*, methicillin-resistant *S. aureus*, and methicillin-susceptible *S. aureus* (Corrêa et al., 2017). A small number of research looked on the ability of grape pomace extracts to suppress fungi. Recently, a crude phenolic extract was evaluated that was derived from a blend of Chilean grape varietals (Carmènere, Syrah, and Cabernet Sauvignon) and further fractionated using hexane, chloroform, or ethyl acetate (Mendoza et al., 2013). Additionally, it has been documented that pomace can stop the growth of *Candida albicans*, *Candida krusei*, and *Candida parapsilosis* in addition to *Zygosaccharomyces rouxii* and *Zygosaccharomyces bailii* (Han, 2007; Jung et al., 2005; Oliveira et al., 2013; Sagdic, 2011a; Sagdic, 2011b).

Resveratrol and Blue Frankish pomace extract were tested for their antibacterial efficacy against both Gram-positive and Gram-negative bacteria as well as *Candida* yeasts in a separate investigation. The antimicrobial assay was used to assess three yeast strains (*Candida albicans*, *Candida krusei*, and *Candida tropicalis*) and six bacterial strains (*Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enteritidis* subsp. *enteritidis*). We employed grape pomace extracts of the blue variety Blue Frankish and pure resveratrol. When compared to Gram-negative bacteria and yeasts, the results demonstrate the excellent antibacterial action of resveratrol and blue grape pomace extract against Gram-positive bacteria (Kunová et al., 2019).

Using four strains of bacteria (two Gram-positive, *Staphylococcus aureus*, *Bacillus* cereus; two Gram-negative, *Escherichia coli*, *Pseudomonas aeruginosa*) and four strains of yeast (*Candida albicans*, *Candida glabrata*, *Candida krusei*, and *Candida tropicalis*, Kačániová et al. (2018) the samples. Grape pomace extracts from the blue variety Dornfelder and the white variation Palava were used to find the antibacterial activity. In our investigation, Dornfelder extracts were more effective against Gram-positive bacteria and yeasts, while Palava pomace extracts showed lower efficacy against the microorganisms examined.

According to Xu et al. (2016), four grape pomace extracts were tested for their antibacterial properties. While no antibacterial action was found against *E. coli* O157:H7 and *S. typhimurium*, all extracts showed antibacterial activity against *L. monocytogenes* and *S. aureus*. Our findings, which have the strongest impact against Gram-positive bacteria, are in partial agreement with earlier research on the antibacterial activity of whole grapes or grape pomace extracts against both Gram-positive and Gram-negative bacteria (Darra et al., 2012).

It's important to remember that there are varietal variations in the amount and make-up of phenolic compounds in grape pomaces, as well as in the antibacterial qualities that follow (Teixeira et al., 2014). Furthermore, agronomic techniques and environmental elements (such as climate, soil type, and geographic location) have a significant impact on grape composition and related characteristics. Although few recent studies (De La Cerda-Carrasco et al., 2015; González-Centeno et al., 2013) have explored white grape pomaces, the majority of investigations have focused on pomaces generated from blue grape types.

Table 2 Antimicrobial resistance with disc diffusion method in mm

	Citrobacter koseri	***Enterobacter aerogenes***	***Sigella sonei***	***Bacillus subtilis***	***Listeria ivanovii***	***Micrococcus luteus***	***Candida albicans***	***Candida glabrata***	***Candida tropicalis***
ATB	27.67 ±0.58^{a}	29.33 ±0.58^{b}	28.33 ±0.58$^{a, b}$	27.33 ±0.58$^{a, c}$	28.67 ±0.58 $^{b, c}$	29.67 ±0.58 ^{b}	30.33 ±0.58 ^{b}	28.67 ±0.58$^{a, b}$	28.33 ±0.58$^{a, b}$
ATB	28.33 ±0.58^{a}	29.67 ±0.58^{a}	28.67 ±0.58$^{a, b}$	27.67 ±0.58 $^{b, c}$	28.33 ±0.58$^{a, c}$	29.33 ±0.58^{a}	29.67 ±0.58^{a}	28.33 ±0.58$^{a, c}$	27.67 ±0.58 $^{b, c}$
ATB	28.33 ±0.58^{a}	28.67 ±0.58^{a}	29.33 ±0.58$^{a, b}$	27.67 ±0.58^{a}	29.33 ±0.58$^{a, c}$	30.33 ±0.58 $^{b, c}$	29.67 ±0.58$^{a, c}$	27.67 ±0.58^{a}	27.67 ±0.58^{a}
ATB	28.67 ±0.58$^{a, b}$	27.67 ±0.58^{a}	28.33 ±0.58$^{a, b}$	29.33 ±0.58^{a}	29.67 ±0.58^{a}	28.33 ±0.58$^{a.b}$	27.67 ±0.58$^{a, b}$	28.67 ±0.58$^{a, b}$	28.33 ±0.58$^{a, b}$
ATB	27.67 ±0.58^{a}	29.33 ±0.58 ^{b}	28.67 ±0.58$^{a, b}$	27.67 ±0.58^{a}	28.33 ±0.58$^{a, b}$	29.33 ±0.58 ^{b}	29.67 ±0.58 ^{b}	28.33 ±0.58$^{a, b}$	27.67 ±0.58^{a}
ATB	28.67 ±0.58^{a}	27.67 ±0.58^{a}	28.33 ±0.58$^{a, b}$	29.33 ±0.58 $^{b, c}$	29.67 ±0.58 $^{b, d}$	28.33 ±0.58$^{a, c, d}$	27.67 ±0.58^{a}	28.67 ±0.58$^{a, c, d}$	28.33 ±0.58$^{a, c}$
ATB	27.67 ±0.58^{a}	29.33 ±0.58 ^{b}	28.33 ±0.58$^{a, b}$	27.33 ±0.58^{a}	28.67 ±0.58$^{a, b}$	29.67 ±0.58 ^{b}	30.33 ±0.58 ^{b}	28.67 ±0.58$^{a, b}$	28.33 ±0.58$^{a, b}$
ATB	28.67 ±0.58$^{a, b}$	27.67 ±0.58$^{a, b}$	28.33 ±0.58$^{a, b}$	29.33 ±0.58^{a}	29.67 ±0.,58^{a}	28.33 ±0.58$^{a, b}$	27.67 ±0.58$^{a, b}$	28.67 ±0.58$^{a, b}$	28.33 ±0.58$^{a, b}$
ATB	27.67 ±0.58^{a}	29.33 ±0.58 ^{b}	28.33 ±0.58$^{a, b}$	28.67 ±0.58$^{a, b}$	27.67 ±0.58^{a}	28.33 ±0.58$^{a, b}$	29.33 ±0.58 ^{b}	29.67 ±0.58 ^{b}	28.33 ±0.58$^{a, b}$
ATB	27.67 ±0.58^{a}	29.33 ±0.58 ^{b}	28.67 ±0.58$^{a, c}$	27.67 ±0.58^{a}	28.33 ±0.58$^{a, b}$	29.33 ±0.58 ^{b}	29.67 ±0.58 ^{b}	28.33 ±0.58$^{a, b}$	27.67 ±0.58^{a}
ATB	27.67 ±0.58^{a}	28.67 ±0.58$^{a, b}$	27.67 ±0.58^{a}	28.33 ±0.58^{a}	29.33 ±0.58 ^{b}	29.67 ±0.58 ^{b}	28.33 ±0.58^{a}	27.67 ±0.58^{a}	28.33 ±0.58^{a}
ATB	27.67 ±0.58^{a}	28.67 ±0.58$^{a, b}$	27.67 ±0.58^{a}	28.33 ±0.58^{a}	29.33 ±0.58 ^{b}	29.67 ±0.58 ^{b}	28.33 ±0.58^{a}	27.67 ±0.58^{a}	28.33 ±0.58^{a}
ATB	27.67 ±0,58^{a}	29.33 ±0.58 ^{b}	28.33 ±0.58$^{a, b}$	28.67 ±0.58$^{a, b}$	27.67 ±0.58^{a}	28.33 ±0.58$^{a, b}$	29.33 ±0.58 ^{b}	29.67 ±0.58 ^{b}	28.33 ±0.58$^{a, b}$

*Data are the mean (± SD) of 3 samples; different letters in each row refer to significant differences (Tukey, $p \leq 0.05$); the antibiotics gentamicin for Gram-positive bacteria, cefoxitin for Gram-negative bacteriaand fluconazole for yeast were used to testantibiotic resistance

4 Conclusion

Comparison of the antimicrobial activity of lyophilized extracts of whole pomace from white and blue grape varieties against Gram-positive and Gram-negative bacteria and yeasts revealed differences in specific antimicrobial activity against pathogenic and spoilage bacteria. Alibernet, Dornfelder, Cabernet Sauvignon and white varieties – Perla, Palava, Müller-Thurgau, Grűner Veltliner, Feteasca Regala, Blaufränkisch, Sauvignon Blanc, Welschriesling, Welser Riesling, Weisser Riesling, Irsai Oliver and Pinot Blanc – appear to have great potential as natural antimicrobials. Subsequent research has focused on the discovery of phenolic chemicals and their correlation with antibacterial properties in grape marc.

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