# Antimicrobial Activity of *Vitis vinifera* L. Pomace Extract

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**Abstract**
Grape pomace is a winery industry residue of skins, seeds and stems of grapes used in agriculture for animal feed and production of food with biological activity. The aim of this study was to evaluate antimicrobial activity of *Vitis vinifera* L. pomace extract on growth of microscopic fungi and bacteria. The four strains of bacteria (two Gram-positive bacteria *Staphylococcus aureus* CCM 2461, *Bacillus cereus* CCM 2010*;* two Gram-negative bacteria *Escherichia coli* CCM 3988, *Pseudomonas aeruginosa* CCM 1959*)* and four yeasts strains (*Candida albicans* CCM 8186*, Candida glabrata* CCM 8270, *Candida krusei* CCM 8271 and *Candida tropicalis* CCM 8223*)* were studies. For detection of antimicrobial activity, the grape pomace extracts of white variety Pálava and red variety Dornfelder were used. Pálava pomace extracts were less efficient against the microorganisms tested and Dornfelder extracts were more active against Gram-positive bacteria and yeasts.

**Keywords**: antimicrobial activity, Gram negative bacteria, Gram positive bacteria, yeasts, *Vitis vinifera* L.

## 1. Introduction

The composition and properties of grapes have been extensively investigated. It was reported that the grapes contain a large amounts of phenolic compounds. These compounds possess various favourable effects on human health such as lowering of human low-density lipoprotein, reduction of heart disease and cancer [1]. Phenolic compounds, the most numerous and ubiquitous group of plant secondary metabolites, exhibit antibacterial properties against a wide range of pathogenic bacteria, including *Salmonella* spp., *E. coli* O157: H7, *S. aureus*, *L. monocytogenes* and *Bacillus subtilis* [2,3]. It has been proposed that the antibacterial activity could be due to several modes of action, such as damage in the cytoplasmic membrane, inhibition of synthesis of nucleic acids, cell wall components, as well as, cell membrane [4,5]. Also, the phenolic compounds may exhibit a wide range of biological effects including, antiinflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory action, that has been associated with their antioxidant activities. These properties have gained the attention of food industry due to its potential use as natural antibacterial additives [6].

Table grapes are common commercialized in clusters; however, the increased interest in ready to eat products has introduced table grapes in the minimally processed market due to easy to consume and the globalization of food trade [7]. The grape pomace, an important by-product after winemaking and traditional food production [1].

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The aim of this study was to evaluate antimicrobial activity of *Vitis vinifera* L. pomace extract with disc diffusion method and minimal inhibition concentration.

## 2. Materials and methods

### Grape

Ripened grapes from wine cultivars grown in Vrbové (48° 37′ 12″ N, 17° 43′ 25″ E) Slovakia were collected. For antimicrobial activity, the white variety Pálava and red variety Dornfelder grape pomace extracts were used.

### Pomace extract preparation

The pomace extracts were prepared from a single production lot. A portion of the pomace samples (50 g) were immediately freeze-dried after receiving. The samples were extracted with 96 % ethanol at 1:10 ratio (m/v) under overnight shaking. The extracts were filtered through Whatman No. 2 filter paper to remove unwanted residues. After evaporating off the organic solvent, the filtrates were dissolved in dimethyl sulfoxide (DMSO) at 20 $mg.mL^{-1}$ as the stock solution and stored at -20°C for further investigation.

### Microorganisms

Eight strains of microorganisms were tested in this study, including two Gram-positive bacteria *Staphylococcus aureus* CCM 2461, *Bacillus cereus* CCM 2010, two Gram-negative bacteria *Escherichia coli* CCM 3988 and *Pseudomonas aeruginosa CCM 1959* and four yeasts strains: *Candida albicans* CCM 8186, *Candida glabrata* CCM 8270, *Candida krusei* CCM 8271 and *Candida tropicalis* CCM 8223. All tested strains were collected from the Czech Collection of microorganisms (Brno, Czech republic). The bacterial suspensions were cultured in the Muller Hinton broth (MHB, Oxoid, Basingstoke, United Kingdom*)* at 37 °C for 24 h and yeasts were cultured in the Sabouraud dextrose broth (SDB, Oxoid, Basingstoke, United Kingdom*)* at 25 °C for 48 h..

### Disc diffusion method

The agar disc diffusion method was used for the determination of antimicrobial activity of the pomace extracts. Briefly, a suspension of the tested microorganism (0.1 ml of $10^5$ cells $mL^{-1}$) was spread onto Mueller Hinton Agar (MHA, Oxoid, Basingstoke, United Kingdom) and Sabouraud dextrose agar (Oxoid, Basingstoke, United Kingdom) at 25 °C. Filter paper discs (6 mm in diameter) were impregnated with 15 µl of the pomace extract and placed on the inoculated plates. The plates were kept at 4°C for 2 h and after incubated aerobically at 37°C for 24 h and 25 °C for 48 h for bacteria and yeast, respectively. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in triplicate.

### Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) is the lowest concentration of the sample that will inhibit the visible growth of microorganisms. Pomace grape extracts were dissolved in DMSO (conc.20 $mg.mL^{-1}$). MICs were determined by the microbroth dilution method according to the Clinical and Laboratory Standards Institute recommendation [8] in Mueller Hinton broth (Oxoid) for bacteria and Sabouraud dextrose broth (Oxoid) for yeasts. Briefly, the DMSO extracts solutions were prepared as serial two-fold dilutions to obtain a final concentration ranging from 3.9 to 2000 $\mu g.mL^{-1}$. Each well was then inoculated with microbial suspension at the final density of 0.5 McFarland. After 24 h incubation at 37 °C for bacteria and 25 °C for yeasts, the inhibition of microbial growth was evaluated by measuring the well absorbance at 570 nm in an absorbance microplate reader Biotek EL808 with shaker (Biotek Instruments, USA). The 96 microwell plates were measured before and after the experiment. Wells without plant extracts were used as positive controls of growth. Pure DMSO was used as negative control. This experiment was done in eight-replicates for a higher accuracy of the MICs of used pomace grape extracts

## 3. Results and discussion

### 3.1 Disc diffusion method

The results in Table 1 show that the pomace grape extracts were more effective against the Gram-positive bacteria (*S. aureus* and *B. cereus*), compared to the results for the Gram-negative ones (*E. coli* and *P. aeruginosa*). The better antimicrobial activity of grape pomace extract was found in Dornfelder in comparison with Pálava. Most effective the pomace extract of both grape was against *Candida* species in comparison with bacteria. The same results were observed by Boussaada et al. [9] and Michielin et al. [10] studying extracts from other raw materials. The higher resistance of the Gram-negative bacteria is probably related to its two layer cell membrane and to the strong hydrophilicity of the outer membrane acting as a strong barrier, compared to the single membrane of the Gram-positive bacteria which facilitates the penetration of lipophilic compounds [11].

**Table 1.** Antibacterial activity of the extracts from two grape pomaces extract

| | Diameter inhibition zones in mm | |
|---|---|---|
| **Tested microorganisms** | **Pálava** | **Dornfelder** |
| *Staphylococcus aureus CCM 2461* | 4.67±0.58 | 5.67±1.53 |
| *Bacillus cereus CCM 2010* | 5.33±0.58 | 6.67±0.58 |
| *Escherichia coli* CCM 3988 | 3.33±1.33 | 4.67±0.58 |
| *Pseudomonas aeruginosa CCM 1959* | 2.67±1.15 | 4.33±1.15 |
| *Candida albicans CCM 8186* | 3.33±0.58 | 7.33±0.58 |
| *Candida glabrata CCM 8270* | 3.67±1.15 | 6.67±0.58 |
| *Candida krusei CCM 8271* | 4.33±1.15 | 5.67±1.15 |
| *Candida tropicalis CCM 8223* | 3.67±1.15 | 5.33±1.53 |

The antibacterial activity of four grape pomace extracts was evaluated in study of Xu et al. [12]. All extracts exhibited antibacterial activity against *L. monocytogenes* and *S. aureus*, but no antibacterial activity was detected against *E. coli* O157:H7 and *S.* Typhimurium. Our results confirm partially with the previous studies on the antimicrobial activity of whole grape or grape pomace extracts against both Gram-positive and Gram-negative bacteria, with the most pronounced effect effective against Gram-positive bacteria [13].

Grape skin extracts showed no antimicrobial activity against some food spoilage and pathogenic bacteria, including *S. aureus, B. cereus*, *E. coli* and *P. aeruginosa*, while the grape seed extracts rich in fatty acids, provided high antimicrobial activity against the same microorganisms [1].

### 3.2 Minimal inhibition concentration

The minimal inhibition concentration of Gram-negative, Gram-positive bacteria and yeasts are shown in Table 2.

**Table 2.** Minimal inhibition concentration of grape pomace extract

| | Minimal inhibition concentration in μg.mL$^{-1}$ | |
|---|---|---|
| **Tested microorganisms** | **Pálava** | **Dornfelder** |
| *Staphylococcus aureus CCM 2461* | 1000 | 500 |
| *Bacillus cereus CCM 2010* | 500 | 250 |
| *Escherichia coli* CCM 3988 | 2000 | 1000 |
| *Pseudomonas aeruginosa CCM 1959* | 1500 | 1000 |
| *Candida albicans CCM 8186* | 500 | 250 |
| *Candida glabrata CCM 8270* | 1000 | 500 |
| *Candida krusei CCM 8271* | 1000 | 500 |

Supercritical extracts from Merlot grape pomace obtained at 300 bar/50 °C showed MIC values against *S. aureus* of 625±375 μg.mL$^{-1}$, and 1000 μg.mL$^{-1}$ for *E. coli* and *P. aeruginosa* [11]. Antibacterial effect of grape stem extract can be associated with the presence of phenolic compounds. It has been reported that the flavonoid rutin (present in grape stem extract) selectively promoted cleavage of topoisomerase IV, an essential enzyme in *E. coli* survival [14]. Also, the antibacterial activity of quercetin has been attributed to the inhibition of DNA gyrase enzyme [15].

Polyphenols exhibited antibacterial activities with protein-related polyamide polymers [16]. In contrast, polyphenols showed higher antibacterial effect on Gram-positive bacteria than Gram-negative bacteria. It was observed that GPP exhibited dose dependent bactericidal effects [17].

## 4. Conclusions

Dornfelder pomace extracts exhibited better antibacterial activity against Gram-positive *Staphylococcus aureus* CCM 2461, *Bacillus cereus* CCM 2010, Gram-negative *Escherichia coli* CCM 3988 and *Pseudomonas aeruginosa CCM 1959* and four yeasts strains: *Candida albicans* CCM 8186, *Candida glabrata* CCM 8270, *Candida krusei* CCM 8271 and *Candida tropicalis* CCM 8223.

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