# Antimicrobial and antioxidant activities of grape by-products extracts: sustainable use and valorization of winery by-products

A. Silva[1-4], V. Silva[1-4], A. Aires[7], R. Carvalho[8], J.E. Pereira[5,6], L. Maltez[5,6], G. Igrejas[2,3,4], V. Falco[9], and P. Poeta[1,2,5,6]

[1]Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal.
[2]LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Universidade Nova de Lisboa, Caparica, Portugal.
[3]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal.
[4]Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal.
[5]CECAV – Veterinary and Animal Research Centre, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal.
[6] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal.
[7] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), 5001-801 Vila Real, Portugal;
[8] Department of Agronomy, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), 5001-801 Vila Real, Portugal;
[9]Chemistry Research Centre (CQ-VR), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal.
Keywords: Wine by-products, sustainability, antibiotic resistance, antimicrobial potential
Presenting author email: ppoeta@utad.pt

Wine production is considered one of the most important agricultural activities. However, this industry is the cause of many environmental and economic issues, so it is important the valorization of winery by-products within the scope of sustainability (V. Silva 2018). The Douro Demarcated region is responsible for the annual production of over 50 thousand tons of winery by-products (V. Silva et al. 2020). These by-products contain valuable chemical compounds, such as polyphenols and recent studies suggested a well-recognized health benefits namely antibacterial and antioxidant activities which may become useful therapeutic tools. More attention has been paid to the search for naturally occurring substances able to act as alternative antimicrobials to combat the lack and urgent need for new antimicrobial agents (A. Silva et al. 2021). Phenolic compounds are found in winemaking by-products, including in seeds, skins, stems and shoat of grapes. Circular economy concerns turned the focus to the presence of bioactive phenolic compounds in by-products, namely to those generated in the winemaking process (A. Silva et al. 2021;V. Silva et al. 2020).

Therefore, we aimed to extract phenolic compounds from grape by-products and evaluated the antimicrobial activity of grape by-products: shoat, steam, seeds and skins from a Douro variety, Touriga Franca, against 22 strains: 9 *Escherichia coli (E. coli)*, 1 *Listeria monocytogenes (L. monocytogenes)*, 1 *Staphylococcus aureus (S. aureus)*, 1 *Staphylococcus epidermidis (S. epidermidis)*, 1 *Bacillus cereus (B. cereus)*, 1 *Klebsiella pneumoniae (K. pneumoniae)*, 1 *Enterococcus faecalis (E. faecalis)*, 1 *Enterococcus faecium (E. faecium)*, 1 *Salmonella enteritidis (S. enteritidis)*, 1 *Pseudomonas aeruginosa (P. aeruginosa)* e 4 *Staphylococcus aureus* methicillin resistance (MRSA).

Grape by-products were freeze-dried, mill-powdered and stored in a desiccator. The extraction of phenolic compounds was performed using a mixture of ethanol and water (80/20). The dry residues were redissolved in DMSO to a final concentration of 100 µg/m and the initial extract solution was diluted with DMSO to 75,50,25 and 10 µg/mL. After the extraction, the antimicrobial susceptibility assay was performed using the Kirby-Bauer disk diffusion method. The evaluation of the antioxidant properties was performed using 3 methods: DPPH, FRAP and CuPRAC.

All the extracts had an inhibitory effect on the growth of the strains, but it was found that the extract with a greater antimicrobial activity was the grape shoat, since it showed activity in 50% (11/22) of the strains studied. The grape stem showed activity in 40.9% (9/22) of the strains and the grape leaf and skin extracts showed a lower activity, the leaf extract showed activity against 22.73% (5/22) of the strains and grape skin extract only showed activity in 13.64% (3/22) of the strains. It was possible observe that Gram-positive bacteria MRSA was considered to be most susceptible to extracts of the wine by-products. The diameter of the inhibition zones ranged from 7 to 12 mm at the maximum concentration tested. Overall, the MICs of the extracts ranged from 10 to 100 µg/mL.

Results from all antioxidants assays are expressed in effective concentration (EC50), and the lower the value, the higher is the antioxidant activity. Finally, all extracts showed a high antioxidant activity and the EC50 values of the DPPH, FRAP and ABTS assays. The grape by-product that had the higher antioxidant activity were

the seed extracts activity in all groups tested and in both assays. Nevertheless, seeds presented a higher antioxidant power which may be due to their elevated content in tannins and proanthocyanidins.

Gram-negative bacteria demonstrate low susceptibility to polyphenols when compared to Gram-positive bacteria due to the repulsion between these compounds and the lipopolysaccharide present in the surfaces of gram-negative bacteria. Also, the seed extracts were more effective against multiresistance bacteria which shows that polyphenols may have potential usefulness. These wine by-products may represent a potential formula to use as adjuvants or substitutes to tackle two the biggest problematics, the antibiotic resistance and environmental issue produces in winery industries.

**References**


Silva, Adriana, Vanessa Silva, Gilberto Igrejas, Isabel Gaivão, Alfredo Aires, Naouel Klibi, Maria de Lurdes Enes Dapkevicius, Patrícia Valentão, Virgílio Falco, e Patrícia Poeta. 2021. «Valorization of Winemaking By-Products as a Novel Source of Antibacterial Properties: New Strategies to Fight Antibiotic Resistance». *Molecules* 26 (8): 2331. https://doi.org/10.3390/molecules26082331.

Silva, Vanessa. 2018. «Chemical Composition, Antioxidant and Antimicrobial Activity of Phenolic Compounds Extracted from Wine Industry by-Products». *Food Control*, 7.

Silva, Vanessa, Rupesh Kumar Singh, Nelson Gomes, Bruno Gonçalves Soares, Adriana Silva, Virgílio Falco, Rosa Capita, et al. 2020. «Comparative Insight upon Chitosan Solution and Chitosan Nanoparticles Application on the Phenolic Content, Antioxidant and Antimicrobial Activities of Individual Grape Components of Sousão Variety». *Antioxidants* 9 (2): 178. https://doi.org/10.3390/antiox9020178.



***Acknowledments***

This work was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). This work was supported by the Associate Laboratory for Green Chemistry - LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020). Adriana Silva is grateful to FCT (Fundação para a Ciência e a Tecnologia) for financial support through the PhD grant SFRH/BD/04576/2020.