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

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Introduction

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. The Douro Demarcated region is responsible for the annual production of over 50 thousand tons of winery by-products. 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. 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.

The aim of this work…

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 mg/ml and the initial extract solution was diluted with DMSO to 75,50,25 and 10 mg/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.

Results & Discussion

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 seeds, 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 shoat and skin extracts showed a lower activity, the shoat 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 mg/mL.

Bacteria	MIC(mg/mL) Inhbition zone (mm)			
	Skin	Shoat	Seeds	Stem
E. coli 1	-	25 (9)	10 (10)	10 (9)
E. coli 2	75 (9)	-	-	-
E. coli 3	-	-	-	-
E. coli 4	-	-	-	-
E. coli 5	-	-	-	-
E. coli 6	-	-	-	-
E. coli 7	-	-	-	-
E. coli 8	100 (9)	-	50 (9)	-
E. coli 9	-	-	-	-
L. monocytogenes	-	100 (9)	25(9)	50 (9)
S. aureus	-	-	25(10)	50 (9)
S. epidermidis	-	-	25 (12)	50 (10)
B. cereus	-	-	50 (9)	-
K. pneumoniae	-	-	-	-
E. faecalis	-	-	50 (9)	75 (9)
S. enteritidis	-	-	-	-
E. faecium	-	-	-	-
Methicillin-resistant S. aureus (MRSA 12)	-	50 (9)	10 (8)	75 (8)
Methicillin-resistant S. aureus (MRSA 34)	10 (9)	10 (9)	25(9)	75 (9)
Methicillin-resistant S. aureus (MRSA 25)	-	-	25 (8)	50 (7)
Methicillin-resistant S. aureus (MRSA 2)	-	10 (9)	50 (9)	75 (8)
P. aeruginosa	-	-	-	-

Figure 1. MICs of the studied extracts against bacteria in study

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 CuPRAC 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.

Touriga Franca	DPPH	FRAP	CUPRAC
Skin	1,90 ± 0,09	0,580 ± 0,008	0,550 ± 0,002
Shoat	0,97 ± 0,03	0,548 ± 0,001	0,541 ± 0,003
Seed	0,50 ± 0,01	0,14 ± 0,0005	0,14 ± 0,0005
Stem	0,86 ± 0,04	0,16 ± 0,0001	0,17 ± 0,001

Figure 1. Antioxidant activity results

Conclusions

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 multi-resistance 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.

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