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POLYPHENOLIC COMPOSITION AND ANTIMICROBIAL ACTIVITY OF SEED EXTRACTS OF EIGHT GRAPE CULTIVARS

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Abstract: *Polyphenols are compounds with high antioxidant and antimicrobial activity. Grapes have a very high content of polyphenols. The highest percentage of polyphenols is contained in the grape seeds. The content of polyphenols in grape seed extracts of eight types of grapes was studied. The total phenolic content, total flavonoids and procyanidins in grape seed extracts were determined. The highest polyphenolic contents have the seed extracts from Pino Noir, Syrah and Cabernet Franc. The antimicrobial activity of the seed extracts from Pino Noir, Syrah and Cabernet Franc grapes against Staphylococcus aureus, Bacillus cereus and Escherichia coli were studied by agar diffusion method. Structural variations in phenolic compounds alter their antimicrobial activity. The minimum inhibitory concentrations of this grape seed extracts against studied microorganisms were determined. It was concluded that the studied grape seeds have a high potential as source of natural antimicrobial agents and as alternative to reuse of bio-products from winemaking.*

Key words: grape seeds, polyphenolic content, antimicrobial activity, minimum inhibitory concentrations

INTRODUCTION

The wine industry discards about 9 million tons of grape pomace per year worldwide (Constantin, O.E., & Râpeanu, G., 2024). Grape pomace extracts mainly contain phenolic acids, flavonoids, anthocyanins, procyanidins and flavanols (Caponio, G.R., & De Angelis, M., 2023). The pulp consists of seeds, husks and stalks. The content of polyphenols is highest in grape seed extracts, with procyanidins, epicatechin and catechin predominating the most. The indicated polyphenols extracted from grape seeds have a high antioxidant, antimicrobial and antilipid capacity, which makes them very applicable in the food industry, medicine, cosmetics, etc. Foods containing pathogenic bacteria such as Escherichia coli, Staphylococcus aureus and Bacillus cereus are very dangerous to human health (Linscott, A.J., 2011; Tiwari, B.K., & Cullen, P.J., 2009). Currently, synthetic chemical compounds are used as preservatives, but they show cytotoxic effects (Memar, M.Y., & Reza, G., 2019). The application of grape seed extracts as alternative antioxidant and antibacterial agents in food is continuously expanding [Jassy, K.A., & Tiroutchelvame, D., 2020). The aim of this study was to determine the polyphenolic content of seed extracts of eight different types of grapes and their antimicrobial activity against pathogenic microorganisms.

EXPOSITION

The materials used in this study were the wastes (pomaces) from the vinification of eight wines Vitis vinifera L. cv. Pinot Noir, C. Sauvignon, Marselan, Tamyanka, Syrah, Cabernet Franc, Carmenere and Riesling grapes. Skins were separated from the seeds by rubbing the mixture over a plastic sieve. Then the treatment of grape seeds included washing, drying at 40 ºC for 14 h and storing at 4 ºC. For each experiment, a certain amount of dry grape seeds was grinded to powder with a diameter of 2.5–22.5 µm.

Fig. 1. Grape seed powder

The preparation of seed extract were described in detail in our previous manuscript (Krasteva, D., & Godjevargova, T., 2023). The methods for determining the total phenolic content (TPC), total flavonoids (TF), procyanidins (PC), in the obtained extract were described in our previous manuscript (Krasteva, D., & Godjevargova, T., 2023). Absorbance was measured at spectrophotometer 6900 UV-Vis JENWAY, Colmworth, UK.

The antimicrobial and antioxidant potentials of extracts from eight varieties of grape seeds were investigated. First, the extracts were obtained and completely characterized in terms of total polyphenolic parameters. Extracts were obtained from the seeds of eight types of grapes (Pinot Noir, C. Sauvignon, Marselan, Tamyanka, Syrah, Cabernet Franc, Carmenere and Riesling), using the following process: using an extracting agent, which was a mixture of ethanol and water (70:30), the mixture was stirred with a magnetic stirrer for 3 h at room temperature. It is well known that when using extracts in the food industry and in medicine, it is preferable to use ethanol as an extracting agent, since it is safer for health (Tiwari, B. K., & Cullen, P.J., 2009). Then the total polyphenolic content (TPC), total flavonoids (TF), procyanidins (PC) were determined using spectrophotometric methods (Table 1). TPC was found to be the highest in the Pinot Noir and Syrah extracts. According to TPC values, the extracts were ranked in the following order: Pinot Noir > Syrah > Cabernet Franc > Carmenere > Marselan > Cabernet Sauvignon > Riesling > Tamyanka. TF were the highest in the extracts of Syrah, Marselan and Pinot Noir grape seeds. The content of TF in different types of grape seed extracts is closely, but the content of PC varied in very high interval. The PC values in white grape seed extracts are 5-6 times lower than values of seed extract of red grapes. According to the PC values, the extracts were in the following order: Pinot Noir > Syrah > Cabernet Franc > Cabernet Sauvignon > Marselan > Riesling > Tamyanka.

The antibacterial activity of the GSEs was determined using the agar diffusion method (Krasteva, D., & Godjevargova, T., 2023). Minimum inhibitory concentrations (MICs) were determined by the following way. The microbial inoculum was diluted to obtain a suspension with OD650 nm = 0.3–0.5, corresponding to a cell concentration of 5 x 10^5 CFU/mL. Various concentrations of the extracts (6.00, 4.00, 3.00, 2.00, 1.50, 1.00, 0.50, and 0.25 mg dry weight per mL) were prepared. Then, 2 mL of each extract was added to 6 mL of diluted inoculum in tubes. The cultures were incubated at 37 ^{o}C for 48 h. The final concentrations of extracts in the tubes were 1.50, 1.00, 0.75, 0.50, 0.37, 0.25, 0.12, and 0.06 mg dry weight per mL. The growth of cells in the tubes was monitored by measuring the absorbance at 650 nm at 0, 5, and 24 h on the Jenway 6900 spectrophotometer. MIC was defined as the lowest concentration of GSE that inhibited visible bacterial growth after incubation for 24 h at 37 ^{o}C. Ciprofloxacin was used as the positive control, and the extract was in concentrations from 0 to 150 mg/mL.

It was found that the highest polyphenolic contents have the seed extracts from Pino Noir, Syrah and Cabernet Franc. That way, the antimicrobial activity of the seed extracts from Pino Noir, Syrah and Cabernet Franc grapes against two Gram-positive bacterial species *Staphylococcus aureus, Bacillus cereus* and one Gram-negative bacterial species *Escherichia coli* were studied by agar diffusion method and by a test for determining the minimum inhibitory concentration (MIC).

Table 1. Spectrophotometric determination of phenolic composition of grape seed extracts

№	Grape varieties	Color	TPC, mg GAE/g	TF, mg QE/g	PC, mg CE/g

1.	Cabernet Sauvignon	red	88.22 ± 0,72	45.95 ± 0.14	157.22 ± 2.10
2.	Marselane	red	103.24 ± 1.11	52.01 ± 0.34	152.18 ± 2.05
3.	Pinot Noir	red	111.22 ± 1.28	51.50 ± 0.30	170.45 ± 2.52
4.	Tamyanka	white	79.06 ± 0.65	40.05 ± 0.18	31.44 ± 0.23
5.	Carmenere	red	104.35 ± 1.21	47.12 ± 0.27	149.55 ± 1.26
6.	Syrah	red	110,21 ± 1.10	52.55 ± 0.35	162.38 ± 2.05
7.	Cabernet Franc	red	109.41 ± 1.15	50.15 ± 0.28	160.58 ± 1.95
8.	Riesling	white	81.53 ± 0.67	42.08 ± 0.15	35.08 ± 0.25

Structural variations in phenolic compounds alter their antimicrobial activity. The influence of the GSEs in different concentrations on the growth of *S. aureus, B. cereus*, and *E. coli* was studied. The diameters of the inhibition zones (in mm) corresponding to the tested GSEs (0.50 mg/mL) and the antibiotic (25 mg/L) are listed in Table 2. All assays were carried out in triplicate. The results are expressed as mean ± SD. The most sensitive strain to the extracts was S. aureus, and the least sensitive strain was E. coli. Higher concentrations of the extracts produced stronger inhibition. The results show that the Pinot Noir and Syrah extracts had greater antimicrobial potential compared to Cabernet Franc exstract (Table 2).

It is obvious that depending on the type and concentration of polyphenols in grape seed extracts, the impact on microorganisms is different. In addition, different types of microorganisms have different sensitivities to grape extracts. The mechanism of antimicrobial action of polyphenols can be linked to their following interactions with microbial cells (Fig. 2): reactions with proteins; inhibition of nucleic acid synthesis by bacterial cells or DNA damage; interaction with the bacterial cell wall or inhibition of cell wall formation; alteration of the function of the cytoplasmic membrane, such as modifications of membrane permeability or fluidity, damage to the cytoplasmic membrane and, as a result, disruption of the membrane; inhibition of energy metabolism; changes in cell attachment and inhibition of biofilm formation and others.

Table 2. Effect of grape seed extracts on the growth of pathogenic microorganisms

	Zone of inhibition in mm

Bacterial species	Pinot Noir	Syrah	Cabernet Franc
Staphylococcus aureus	14 ±0.25	13 ±0.11	10 ±0.33
Bacillus cereus	10 ±0.15	9 ±0.55	8 ±0.15
Escherichia coli	8±0.15	7±0.22	6 ±0.11

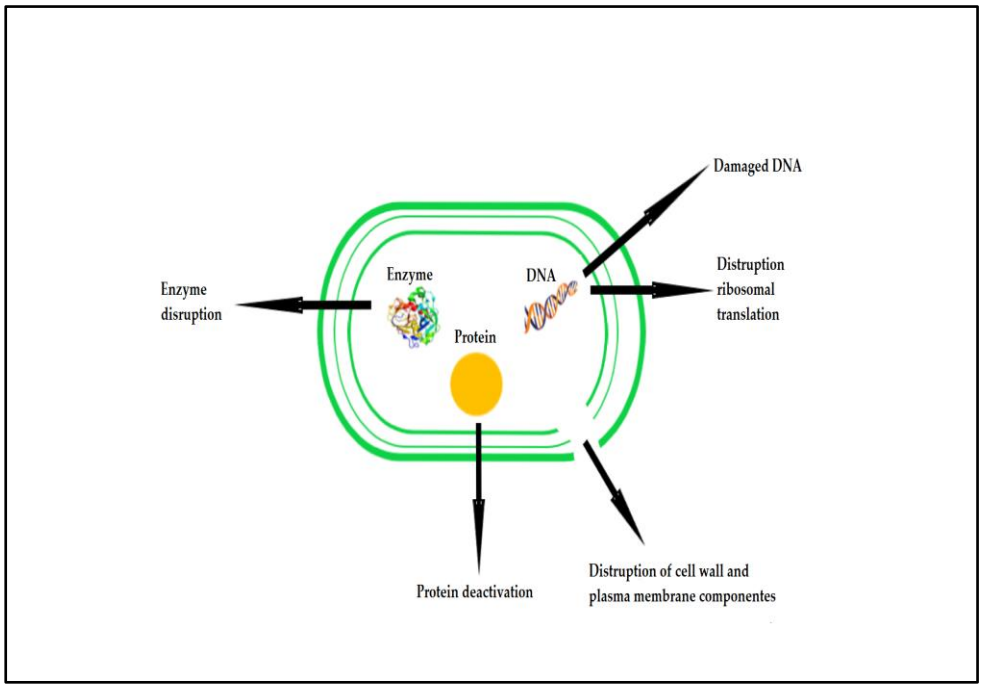

Fig. 2. Mechanisms of antimicrobial effect of grape seed extract

The MICs of the Pinot Noir, Syrah and Cabernet Franc extracts were studied. The MIC values of all the GSEs are presented in Table 3. All tested GSEs had significant antimicrobial activity against the investigated bacteria. According to the MIC values, the extracts were ranked in the following order: Pinot Noir > Syrah > Cabernet Franc. It was found that the extracts were more effective against Gram-positive bacteria than against Gram-negative bacteria. According to the sensitivity of the strains to the studied extracts, they were ranked in the following order: *S. aureus* > *B. cereus* > *E. coli*.

Table 3. Minimum inhibitory concentrations (mg/mL) of grape seed extracts (GSE) against pathogenic microorganisms

№	Grape varieties	Color	Microorganisms			References
			S. aureus	*B. cereus*	*E. Coli*	
1	Pinot Noir	red	0.12	0.25	0.50	In this work
2	Syrah	red	0.13	0.27	0.55	

3	Cabernet Franc	red	0.15	0.31	0.58	
4	Touriga Nacional	red	0.05	0.01	--	Silva, V., &Poeta, P., 2018.
5	Preto Martinho	red	0.010	0.050	--	
6	Red grape	red	35	20	225	Jalali, M., & Ali, S., 2017.
7	Pinot Noir	red	0.78	-	25	Trust, M. P., & Cletos, M., 2019.

The results we obtained for the antimicrobial activity of the investigated GSEs against the tested bacteria and the results obtained by other authors are compared in Table 3. It is obvious that MIC values vary widely in different studies, even for the same microorganism species and the same grape variety. This is due to the different grape varieties, climate, extraction solvents, bacterial species, etc. The comparison shows that the obtained MIC values indicate very good sensitivities of the tested bacteria to the investigated grape seed extracts.

CONCLUSION

The content of polyphenols in grape seed extracts of eight types of grapes was studied. TPC was found to be the highest in the Pinot Noir and Syrah extracts. TF were the highest in the extracts of Syrah, Marselan and Pinot Noir grape seeds. The PC values in white grape seed extracts are 5-6 times lower than the values of seed extract of red grapes. According to the PC values, the extracts were in the following order: Pinot Noir > Syrah > Cabernet Franc > Cabernet Sauvignon > Marselan > Riesling > Tamyanka. Structural variations in phenolic compounds alter their antimicrobial activity. The studied seed extracts showed a low minimum inhibitory concentration against *S. Aureus, B. Cereus and E. Coli.* It was concluded that the studied grape seeds have a high potential as source of natural antimicrobial agents and as alternative to reuse of bio-products from winemaking.

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