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Original Scientific Paper

Grape stalks as a source of antioxidant and antimicrobial substances and their potential application

Ivana D. Radojević[1], Katarina G. Mladenović[2*], Mirjana Ž. Grujović[2] and Sava M. Vasić[1]

1 University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia
2 University of Kragujevac, Institute for Information Technologies, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Republic of Serbia
* Correspondence: katarina.mladenovic@pmf.kg.ac.rs

ABSTRACT:
This research project aimed to analyse the biological potential of aqueous, ethanolic, methanolic, and ethyl acetate extracts of red grape stalks, as well as lyophilised red grape stalks from Krnjevo (Serbia). The concentration of the total phenols and flavonoids, as well as the antioxidant activity of the stalk extracts were measured by means of the spectrophotometric method. *In vitro* antimicrobial activity of 23 selected species of microorganisms (13 species of bacteria and 10 species of fungi) was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC). The results indicated that the highest concentration of total phenols was measured in the ethyl acetate extract (60.08 mg GAE/g of extract), while the highest total flavonoid concentration was observed in the acetone extract (34.24 mg RUE/g of extract). The tested extracts showed poor antioxidant activity compared to chlorogenic acid. The acetone extract probably showed the strongest antimicrobial activity due to the high concentrations of phenols and flavonoids. The tested extracts showed a better effect on Gram-positive bacteria than on Gram-negative bacteria. Although grape stalks are a by-product in the wine industry, they are a potential source of natural compounds which can be used for a variety of purposes in many fields ranging from the food industry to medicine.

Keywords:
bacteria, extracts, flavonoids, fungi, natural preservatives, total phenols

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INTRODUCTION

Nowadays, the use of natural antioxidants and antibacterial compounds in the food industry has become necessary to ensure the high quality of food. Since the composition of winemaking residues depends on the grape variety, climate conditions, and vineyard location, it is relevant to study the antioxidant effects and antibacterial activity of each variety separately and to thus investigate the biotechnological applications of natural polyphenol sources (Sanhueza *et al.* 2014). In order to overcome the side effects of antibiotics, such as the emergence of resistant bacteria, various studies have focused on the antimicrobial activity of natural origin compounds, including grapevines and vine extracts (Darra *et al.* 2012; Čomić *et al.* 2020). It is well known that phenols are the main antimicrobial phytochemicals and grapes are a rich source of these compounds (Nassiri-Asl & Hosseinzadeh 2009).

Winemaking produces a large quantity of waste and by-products with high health and environmental impacts within a short period of time (Bustamante *et al.* 2008). These by-products corresponding to approximately 30% (w/w) of the starting grapes (Rondeau *et al.* 2013; Borddiga *et al.* 2015; Dávila *et al.* 2017), are represented by grape pomace, grape seeds, grape stalks, and wine lees,

as well as wastewater (Bustamante *et al.* 2008; Barba *et al.* 2016; Beres *et al.* 2017; Bordiga *et al.* 2019; Ahmad *et al.* 2020). As has already been reported, winemaking also generates grape stalks, following destemming, and wine lees, the residue which forms at the bottom of the vessels containing the wine after fermentation. The grape stalks [about 7% (w/w) of total grape weight] are usually removed before the fermentation phase to avoid excessive wine astringency (Teixeira *et al.* 2014; Ahmad *et al.* 2020). The research indicated that they are a source of phenolic compounds, such as tannins, flavan-3-ols, hydroxycinnamic acids, monomeric and oligomeric flavonols, and stilbenes (Ping *et al.* 2011; Teixeira *et al.* 2014). As a result, studies have focused on the reuse of grape by-products in the agro-food chain and their possible use in pharmacy. Vine shoots, grape stalks, and wine lees have drawn increasing attention for their applications in the food sector, since they are a good source of functional and bioactive compounds (Troilo *et al.* 2021).

The aim of the present study was to evaluate the concentration of the total phenols and flavonoids in aqueous, ethanolic, methanolic, and ethyl acetate extracts of grape stalks, as well as lyophilised red grape stalks as raw waste from red grapes (Krnjevo, Serbia). The antioxidant and antimicrobial activities of aqueous, ethanolic, methanolic, and ethyl acetate extracts of stalks, as well as lyophilised red grape stalks were also examined in order to determine their potential application as a source of natural antioxidants and antimicrobials. This paper indicates the potential usage of grape stalks despite their being a by-product in winemaking.

MATERIALS AND METHODS

Plant material and the preparation of plant extracts. The grape stalks were collected from the red grapes in the Krnjevo vineyards (Central Serbia), prior to the winemaking process. Before the wine-making process, the stalks are mechanically separated from the grapes. The collected grape stalks were air-dried in darkness at ambient temperature. The homogeneity and traceability of the stalks were ensured by crushing the stalks in a mortar before immersion in the solvents. The dried, crushed grape stalks were extracted by maceration with aqueous, acetone, diethyl ether, methanolic, and ethanol extracts. Briefly, 30 g of the grape stalks was soaked in 150 ml of the solvent. The grape stalks were macerated three times at room temperature using fresh solvent every 24 hours. After every 24 hours, the samples were filtered through filter paper and the filtrates were collected and evaporated to dryness using a rotary evaporator (IKA, Germany) at 40°C. After the evaporation, we collected only secondary metabolites-antioxidants, which are used for future analysis. Therefore, during the evaporation, the effect of the solvents is neutralized, and the secondary metabolites remain in the powder extract. The obtained extracts were kept in sterile sample tubes and stored at -20°C before testing. The lyophilised red grape stalks produced in the Krnjevo winery (Serbia) were also analysed.

Determination of the total phenol content. The total phenol content in the tested extracts was quantified according to the Folin-Ciocalteu method as described by Wootton-Beard *et al.* (2011). Gallic acid (Sigma-Aldrich, St. Louis, MO, USA) was used as the standard and the total phenolic content was expressed as milligram of gallic acid equivalents (GAE) per gram of extract (mg GAE/g of extract). All of the tests were performed in triplicate and the mean values were recorded.

Determination of the total flavonoid content. The total flavonoid content of the extracts was determined using the aluminium chloride method as described by Quettier-Deleu *et al.* (2000). Rutin (Sigma-Aldrich, St. Louis, MO, USA) was used as the standard and the flavonoid concentrations were expressed as milligram of rutin equivalents (RUE) per gram of extract (mg of RUE/g of extract). All of the tests were performed in triplicate and the mean values were recorded.

DPPH radical scavenging capacity assay. The ability of the red grape stalk extracts to scavenge DPPH free radicals was assessed using the method described by Takao *et al.* (1994). The tested extract concentrations ranged from 15.625 μg/ml to 1000 μg/ml. Diluted solutions of the extracts (2 ml each) were mixed with 2 ml of DPPH methanolic solution (40 μg/ml). Ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) was chosen as the standard. All of the tests were performed in triplicate. Radical scavenging activity is expressed as the inhibition percentage calculated using the following formula:

$$\text{Scavenging activity (\%)} = 100 \times [(A_{control} - A_{sample})/A_{control})]$$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the extract.

The effective concentration at which 50% of the DPPH radicals were scavenged (IC_{50}) was obtained from the graph of scavenging activity versus the concentration of the samples. A low IC_{50} value indicates the strong ability of the extract to act as a DPPH scavenger. The antioxidant activity was expressed as the antioxidant activity index (AAI), calculated using the following equation:

$$AAI = FC_{DPPH} / IC_{50}$$

where FC_{DPPH} is the final concentration of 2,2-diphenyl-1-picrylhydrazyl.

The estimation of AAI was done in the following way: AAI <0.5 indicated poor antioxidant activity, AAI from 0.5 to 1.0 indicated moderate antioxidant activity, AAI from 1.0 to 2.0 indicated strong antioxidant activity

and AAI >2.0 indicated very strong antioxidant activity (Vasić *et al*. 2012).

Determination of antimicrobial activity. The antimicrobial activity of the red grape stalk extracts was tested against 13 strains of bacteria (five clinical isolates *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella enterica*); three probiotic strains (*Lactobacillus plantarum*, *Bifidobacterium animalis* subsp. *lactis* and *Bacillus subtilis* IP 5832) and four standard strains (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 12453 and *Escherichia coli* ATCC 25922) and 10 strains of fungi (four isolates *Rhodotorula mucilaginosa*, *Penicillium italicum*, *Penicillium expansum* and *Penicillium chrysogenum*), one probiotic strain (*Saccharomyces boulardii*) and five standard strains (*Candida albicans* ATCC 10231, *Trichoderma viride* ATCC 13233, *Aspergillus flavus* ATCC 9170, *Aspergillus fumigatus* ATTC 204305 and *Aspergillus niger* ATCC 16404). All the clinical isolates were a generous gift from the Institute of Public Health, Kragujevac. The other microorganisms (fungi and ATCC strains) were provided from a collection belonging to by the Microbiology Laboratory, Faculty of Science, University of Kragujevac.

The bacterial and yeast suspensions were prepared by the direct colony method (Andrews 2005). The turbidity of the initial suspensions was adjusted using a 0.5 McFarland densitometer (BioSan, Latvia). The initial bacterial suspensions contained about 10^8 colony forming units CFU/ml and the yeast suspensions contained 10^6 CFU/ml. 1:100 dilutions of the initial suspension were additionally prepared in sterile 0.85% saline. The suspensions of fungal spores were prepared by gentle stripping of the spores from slopes with growing mycelia. The resulting suspensions were 1:1000 diluted in sterile 0.85% saline. The tested extracts were dissolved in 10% dimethyl sulfoxide (DMSO), and then diluted into a liquid nutrient medium. DMSO was obtained from Acros Organics (New Jersey, USA). Resazurin was obtained from Alfa Aesar GmbH & Co. (KG, Karlsruhe, Germany).

The antimicrobial activity was tested by determining the minimum inhibitory concentrations (MIC) and the minimum microbicidal concentration (MMC) using the microdilution plate method with resazurin (Sarker *et al*. 2007). The 96-well plates were prepared by dispensing 100 µl of Mueller–Hinton broth for bacteria and Sabouard broth for fungi into each well. A 100 µl aliquot from the stock solution of the tested extract or component was added into the first row of the plate. The initial concentration was 10 mg/ml. Then, twofold serial dilutions were performed by using a multichannel pipette. The method is described in detail in the reported paper (Muruzović *et al*. 2016). 10% DMSO (as the solvent control test) did not inhibit the growth of microorganisms. Each test included growth control and sterility control. All the tests were performed in duplicate, and the MICs were constant. Minimum microbicidal concentrations were determined by plating 10 µl of samples from those wells where no colour change or mycelia growth was recorded on a nutrient agar medium. At the end of the incubation period the lowest concentration with no growth (no colony) was defined as the minimum microbicidal concentration. Tetracycline (Sigma Aldrich) and fluconazole (Pfizer, New York, New York, USA), dissolved in the nutrient liquid medium, were used as the reference compounds. All of the tests were performed in duplicate and the mean values were recorded.

Data analysis. All the data were presented as means ± standard deviations where appropriate, using Microsoft Excel (Redmond, Washington, DC, USA). Student's T-test was used for a comparison of the antioxidant activity between the extracts and the positive control. The data were analysed using SPSS version 20 (SPSS Inc., Chicago, IL, USA).

RESULTS

Determination of the total phenol and flavonoid content. In this paper, the total phenol and flavonoid content in aqueous, ethanolic, methanolic, acetone, and ethyl ac-

Table 1. Phytochemical characteristics of the grape stalk extracts

Tests	Antioxidant activity		Concentration of chemical compounds	
Type of extract and control	IC_{50}	AAI	Total phenolics	Total flavonoids
Aqueous	407.56±1.01	0.20	26.66±0.13	/
Methanolic	188.49±0.40	0.42	28.12±0.14	7.11±0.03
Acetone	296.16±0.74	0.27	48.45±0.72	34.24±0.05
Ethanol	295.38±0.58	0.27	30.00±0.07	5.71±0.05
Ethyl Acetate	314.92±1.19	0.25	60.08±0.07	17.02±0.07
Chlorogenic acid	11.65±0.52	6.87	/	/

Each value shown is mean ± standard deviation; / - not investigated
IC_{50} – the concentration at which 50 % of the DPPH radicals were scavenged expressed in µg/ml.
AAI – the antioxidant activity index

Table 2. Antibacterial activity of the grape stalk extracts and antibiotics

Species	Extracts												Control	
	Aqueous		Ethanol		Methanol		Acetone		Ethyl -acetate		Lyophilisate		Tetracicline	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
L. plantarum	10	10	2.5	5	2.5	5	0.313	0.313	1.25	2.5	/	/	0.16	1
B. animalis subsp. *lactis*	5	10	2.5	2.5	2.5	2.5	1.25	1.25	1.25	2.5	/	/	4	8
B. subtilis IP 5832	5	10	2.5	2.5	2.5	2.5	0.625	1.25	2.5	2.5	/	/	<0.06	<0.06
B. subtilis	5	10	2.5	2.5	2.5	2.5	0.156	0.313	1.25	1.25	1.25	1.25	<0.06	0.25
B. cereus	5	5	1.25	2.5	1.25	2.5	0.313	0.313	0.625	1.25	2.5	5	0.25	0.5
S. aureus	5	10	2.5	5	2.5	5	0.313	0.625	1.25	5	2.5	5	<0.06	<0.06
S. aureus ATCC 25923	5	5	0.625	1.25	1.25	2.5	0.313	0.313	0.625	0.625	2.5	5	1.5	3
P. aeruginosa	10	>10	10	>10	10	>10	2.5	10	2.5	>10	>10	>10	>128	>128
P. aeruginosa ATCC 27853	>10	>10	5	>10	5	>10	10	>10	10	>10	10	>10	4	32
P. mirabilis ATCC 12453	2.5	5	5	10	2.5	5	5	10	2.5	5	2.5	2.5	>128	>128
E. coli	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	2	6
E. coli ATCC 25922	5	>10	5	>10	5	>10	5	>10	5	>10	>10	>10	4	6
S. enterica	10	10	10	10	10	10	0.625	1.25	5	10	10	>10	2	2

MIC - minimum inhibitory concentration; MBC - minimum bactericidal concentration; the values are given in mg/ml for the extracts and μg/ml for tetracicline

etate extracts of red grape stalks were investigated. The highest content of phenols was measured in the ethyl acetate extract (60.08 mg GAE/g of extracts), and the acetone extract (48.45 mg GAE/g of extracts). A similar content of total phenolic compounds was found in the other three tested extracts (Table 1). The highest flavonoid content was observed in the acetone extract (34.24 mg RUE/g of extracts) and the ethyl acetate extract (17.02 mg RUE/g of extracts), while the lowest content was observed in the ethanolic and methanolic extracts. All the results are shown in Table 1.

Determination of antioxidant activity. The antioxidant activity of the tested extracts of red grape stalks was expressed in the form of IC_{50} values (Table 1). According to the AAI index, the tested extracts showed poor antioxidant activity compared to the positive control (the AAI for chlorogenic acid was 6.87). Among all the tested extracts, the methanolic extract showed the best IC_{50} value (188.49 μg/ml), but also a low AAI index (0.42 poor antioxidant activity). However, there is a significant difference (p= 0.001) in antioxidant activity between chlorogenic acid and the other tested extracts.

Antibacterial activity. The *in vitro* antibacterial and antifungal activity of the aqueous, ethanolic, methanolic, acetone and ethyl acetate extracts as well as of the lyophilised grape stalks were also investigated. The antimicrobial activity of the examined extracts was determined against 23 species of microorganisms. In this experiment, the values of MIC and MMC were in the range of 0.313 mg/ml to >10 mg/ml (Table 2). The intensity of antibacterial activity depended on both the type of extract and the type of bacteria. The extracts were active in the following ascending order: aqueous methanolic, ethanol, ethyl acetate, acetone. Based on the results, it may be concluded that the acetone and ethyl acetate extracts have better antibacterial activity compared to the other extracts. Gram-positive bacteria showed greater susceptibility to the tested extracts. The lyophilised stalks did not show antibacterial activity against the species *L. plantarum, B. animalis* subsp. *lactis,* and *B. subtilis* IP 5832, while the activity against other Gram-positive bacteria was detected. However, significantly lower antibacterial activity was shown against Gram-negative bacteria (MIC and MBC in the range from 10 mg/ml to >10 mg/ml), except for the species *P. mirabilis* ATCC 12453 where the activity was detected at 2.5 mg/ml. The aqueous extract showed antibacterial activity against Gram-positive bacteria at a concentration of 5 mg/ml. The ethyl acetate, ethanol and methanolic extracts also exhibited better effects against Gram-positive bacteria, but in contrast to the aqueous extract, the activity was better against Gram-negative bacteria (MIC and MMC in the range 2.5 mg/ml to 10 mg/ml), with the exception of *E. coli* (MIC and MMC at >10 mg/ml). The acetone extract showed the best antibacterial effect, with a concentration range between 0.313 and 1.25 mg/ml for Gram-positive bacteria and between 0.625 mg/ml and 10 mg/ml for Gram-negative bacteria, except for *E. coli* (MIC and MMC >10 mg/ml).

The aqueous extract exhibited no activity against the tested fungal species (MIC and MFC at >10 mg/ml), with the exception of *R. mucilaginosa* and *T. viride* ATCC 13233 (MIC and MFC at 10 mg/ml). The ethanol, methanolic and ethyl acetate extracts showed selective antifungal activity, whit concentrations ranging from 1.25 mg/

Table 3. Antifungal activity of grape stalk extracts and antimycotics

Species	Extracts												Control	
	Aqueous		Ethanol		Methanol		Acetone		Ethyl -acetate		Lyophilisate		Fluconazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
S. boulardii	>10	>10	>10	>10	>10	>10	10	>10	10	>10	5	>10	7.81	31.25
R. mucilaginosa	10	>10	2.5	10	5	10	5	10	5	10	5	10	31.25	500
C. albicans ATCC 10231	>10	>10	5	10	10	>10	5	10	5	10	5	>10	31.25	62.5
P. italicum	>10	>10	1.25	5	1.25	5	0.625	1.25	1.25	2.5	2.5	10	250	500
P. expansum	>10	>10	5	5	5	5	2.5	2.5	2.5	5	5	>10	/	/
P. chrysogenum	>10	>10	10	10	5	10	0.315	1.25	1.25	2.5	5	10	1000	1000
T. viride ATCC 13233	10	10	5	10	10	10	2.5	10	5	10	5	10	500	1000
A. flavus ATCC 9170	>10	>10	1.25	10	2.5	10	0.315	5	1.25	>10	5	>10	500	500
A. fumigatus ATTC 204305	>10	>10	1.25	>10	2.5	5	0.315	10	1.25	>10	5	>10	1000	1000
A. niger ATCC 16404	>10	>10	1.25	>10	5	10	0.315	10	2.5	>10	10	>10	1000	1000

MIC - minimum inhibitory concentration; MBC - minimum bactericidal concentration; values are given in mg/ml for the extracts and µg/ml for fluconazole

ml to 10 mg/ml (Table 3). The ethanol and methanolic extracts showed no activity against *S. boulardii*, while the ethyl acetate extract showed activity (MIC at 10 mg/ml). The acetone extract showed the best antifungal activity with concentrations ranging from 0.315 mg/ml to 10 mg/ml. The lyophilised stalks showed good antifungal activity against the tested fungi.

DISCUSSION

In recent years, there has been a growing tendency to use wine by-products, such as grape stalks since they are a rich source of functional compounds. Numerous studies have confirmed this and indicate the presence of phenolic acids (caffeic acid, gallic and p-coumaric acid), flavonoids (catechin, epicatechin, and luteolin), and stilbenes (trans-resveratrol and its trans-ε-viniferin dimer) in red grape stalks (Chaher *et al.* 2014; Gorena *et al.* 2014; Ruiz-Moreno *et al.* 2015; Chagas *et al.* 2016; Sánchez-Gómez *et al.* 2017; Moreira *et al.* 2018; Zwingelstein *et al.* 2020). The present research also confirmed the presence of phenols and flavonoids in grape stalks. The differences between grape stalk extracts directly depend on the type of solvent. Solvents are responsible for extracting and concentrating secondary metabolites. Different amounts of secondary metabolites in the extracts are measured, thus identifying the extracts with the highest concentration of phenols and flavonoids. Certain procedures serve to neutralise the solvents and pure secondary metabolites remain in the extracts. The effect of grape stalks on the colour and phenolic content of red wine was also evaluated by Pascual *et al.* (2016). The grape stalks increased the concentration of catechins, gallocatechins, and proanthocyanidins, but reduced the intensity of colour and increased the astringency and bitterness of the wine. The possibility of replacing bentonite with dried grape stalks was considered by Kosínska-Cagnazzo *et al.* (2020), to remove the unstable proteins which cause turbidity in wines. The addition of 10 mg/ml in a wine model precipitated all the proteins, preserved the total polyphenol content, but caused a change in the colour of the wine at high doses. The number of proteins remaining in the wine was inversely proportional to the addition of grape stalks, due to the interactions between proteins and polyphenols which promote the formation of precipitates. This could be exploited, for example, in white wine rich in insoluble proteins which precipitate slowly and poor in tannins useful for the initial protein precipitation. The quality of the wine was also improved by Miljić *et al.* (2014) by adding grape seeds and stalks during the vinification in red wine. The presence of stalks increased the phenolic content and antioxidant activity compared to the control wines. An innovative liqueur obtained from red fruit and fortified with grape stalks was produced by Barros *et al.* (2016), who used this by-product as a source of polyphenols. The product obtained exhibited higher antioxidant activity due to the presence of bioactive compounds (ortho-diphenols, flavanols, and anthocyanins) compared to a liqueur obtained without the addition of grape stalks. The highest value of antioxidant activity was achieved after 90 days of maceration, also showing a change in colour parameters. Grape stalks (from Barbera red grapes) were kindly provided by a wine-making factory in Piacenza (northern Italy) from the 2003 vintage (Spigno & de Faveri 2007). This study also showed that grape stalks could be a good low-cost source of antioxidants. Due to the health benefits, antioxidant and radical scavenging properties displayed by polyphenols, research into the application of these natural compounds has been growing over the years. It is well-known that the antioxidant activity directly correlates with the phenolic compounds. Howev-

er, Teixeira *et al.* (2018) indicated that the correlation between total phenolic content and antioxidant capacity is not always in correlation, which was confirmed in our study. They also indicated the need for more efficient and precise extraction methods in order to draw more precise conclusions on which experimental conditions serve to extract higher concentrations of polyphenols more efficiently. According to our results, the stalk extracts showed poor antioxidant activity.

On the other hand, the stalk extract showed good antimicrobial activity in our research. All the extracts of the grape stalks and lyophilised stalks showed antibacterial activity against *S. aureus*. Natural antimicrobial substances are a new source of natural preservatives which can be used in food technology. Research is therefore being carried out into the chemical composition and antimicrobial potential of agro-industrial waste against pathogenic bacteria. According to Martin *et al.* (2012) beat stalk, peanut peel, Pinot Noir grape marc, Petit Verdot grape seed and marc, red grape fermentation lees and guava bagasse waste showed antimicrobial activity against *S. aureus*. According to these results the by-products and waste from the wine and food processing industries could be used as natural preservatives in food technology (Martin *et al.* 2012). In a subsequent study, Vázquez-Armenta *et al.* (2018) evaluated the effect of grape stalk extracts, compared to synthetic disinfectants, on the motility, surface energy, and adhesion of *Listeria monocytogenes* on food contact surfaces, such as stainless steel and polypropylene. The results showed a greater reduction of pathogen adhesion. The presence of grape stalk extracts probably induced the synthesis of exopolysaccharides, which is responsible for the inhibition of motility, adhesion, and biofilm formation. This could be one of the mechanisms of grape stalks activity. The *in vitro* antimicrobial activity of grape stalks after 64 days of storage against Gram-positive and Gram-negative bacteria was studied by Gouvinhas *et al.* (2018). Antimicrobial activity was tested against *S. aureus, Enterococcus faecalis, P. aeruginosa, E. coli,* and *Klebsiella pneumoniae*, which are known as foodborne pathogens. All the polyphenolic extracts were competent in inhibiting the bacterial growth of selected Gram-positive and Gram-negative bacteria strains. In our research, the intensity of antibacterial activity depended on both the type of extract and the type of bacteria. Based on the results, it can be concluded that acetone and ethyl acetate extracts have better antibacterial activity than the other tested extracts. The antimicrobial activity of the acetone extracts is probably related to the higher total concentrations of phenols and flavonoids. It was concluded that this concentration of secondary metabolites led to the antibacterial and antifungal activity of the acetone extract. Although the ethyl acetate extract had a higher concentration of phenols, its flavonoid concentration was moderate, so this extract did not show the best antimicrobial effect. Gram-positive bacteria showed higher susceptibility to the tested extracts. None of the tested extracts showed antibacterial activity against *E. coli*. Ruiz-Moreno *et al.* (2015) indicated the lower antifungal effect of grape stem extract against *Saccharomyces cerevisiae*. In our research, the ethanol, methanolic and ethyl acetate extracts showed moderate antifungal activity against the tested fungi (MIC and MFC between 1.25 mg/ml and 10 mg/ml). The ethanolic and methanolic extracts showed no activity against *S. boulardii*, while the ethyl acetate extract was active at a concentration of 10 mg/ml. The acetone extract exhibited the best antifungal activity as well as the highest concentration of flavonoids, a well-known promoter of antimicrobial activity.

CONCLUSION

There are numerous studies about antioxidants from natural sources. This one investigated the potential application of waste in the wine industry – red grape stalks. This by-product in the wine industry originating from grapes is an additional source of secondary metabolites, which can be used as antioxidants or antimicrobial substances. Our results indicated that lyophilised and extracts of grape stalks can be used to control potential pathogens, such as *S. aureus*. In addition to medical purposes, these secondary metabolites can also be used in food technology, i.e. for making low-cost foods rich in antioxidants. Nowadays, the need for natural antioxidants has increased and their use is widespread. Grape stalks certainly present a cheap source of natural antioxidants. Nevertheless, in the future, there is a need for the development of more efficient and precise extraction methods, and for investigating which experimental conditions can extract higher concentrations of polyphenols more efficiently.

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REZIME

Botanica SERBICA

Peteljke grožđa kao izvor antioksidativnih i antimikrobnih komponenti i njihova potencijalna primena

Ivana D. Radojević, Katarina G. Mladenović, Mirjana Ž. Grujović i Sava M. Vasić

Ovaj rad je imao za cilj analizu biološkog potencijala vodenog, etanolnog, metanolnog i etil acetatnog ekstrakta stabljika crvenog grožđa, kao i liofilizata stabljika crvenog grožđa iz Krnjeva (Srbija). Spektrofotometrijskom metodom merena je koncentracija ukupnih fenola i flavonoida, kao i antioksidativna aktivnost ekstrakta stabljike. *In vitro* antimikrobna aktivnost na izabrane 23 vrste mikroorganizama (13 vrsta bakterija i 10 vrsta gljiva) je procenjena određivanjem minimalne inhibitorne koncentracije (MIC) i minimalne mikrobicidne koncentracije (MMC). Rezultati su pokazali da je najveća koncentracija ukupnih fenola izmerena u ekstraktu etil acetata (60.08 mg GAE/g ekstrakta), dok je najveća koncentracija ukupnih flavonoida zabeležena u ekstraktu acetona (34.24 mg RUE/g ekstrakta). Ispitani ekstrakti su pokazali slabu antioksidativnu aktivnost u poređenju sa hlorogenskom kiselinom. Ekstrakt acetona pokazao je najjaču antimikrobnu aktivnost verovatno zbog visokih koncentracija fenola i flavonoida. Testirani ekstrakti su pokazali bolji efekat na Gram-pozitivne bakterije nego na Gram-negativne bakterije. Iako stabljike grožđa predstavljaju otpadnu sirovinu u industriji vina, one su potencijalan izvor prirodnih jedinjenja koja se mogu koristiti u različite svrhe, od prehrambene industrije do medicine.

Ključne reči: bakterije, ekstrakti, flavonoidi, gljive, prirodni konzervansi, ukupni fenoli