Phytochemical composition and biological activity of *Vitis Vinifera L.* by-products

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Background

Vitis vinifera L. (Vitaceae) has been used for thousands of years as an important source of active compounds for the pharmaceutical, cosmetic and food industries.

Based on its phytochemical content, modern biological studies reported that the pomace and leaves have antioxidant, anti-inflammatory, cardio-protective, anti-fungal activity, suggesting the winery-derived grape pomace. The information regarding the tendril chemical composition is scarce therefore are needed studies to screen their phytochemical content and their potential biological applications.

Aim

This study aimed to characterize the phytochemical composition of the pomace, leaves and tendrils from V. vinifera and to determine their antioxidant and antimicrobial activity.

Material and methods

***Plant material*:** Plant materials consisted of leaves (L), tendrils (T), red pomace (RP) and white pomace (WP) of several varieties of *Vitis vinifera* , harvested from the experimental fields of SCDVV Murfatlar (Constanta County, Romania), in 2019. The sample RP is a mixture of equal parts of red pomace from varieties of grape vine: Pinot Noir, Feteasca neagra, Cabernet Sauvignon and Mamaia. The WP sample is the mixture of equal parts of white pomace from the varieties Muscat Ottonel and Sauvignon Blanc. The leaves and tendrils were collected of Fetească neagră variety. The plant materials were reduced to a powder of a proper degree of fineness.

***Preparation of plant extracts*.** The vegetal extracts from *Vitis vinifera* (leaves, tendrils and red/white pomace) were obtained by reflux method, with 50% ethanol (v/v). The samples were filtered, the extracts were centrifuged, then supernatants were recovered.

Phytochemical analysis

1. Determination of polyphenolic contents. Quantitative determinations of flavonoids, caffeic acid derivatives and total polyphenolic compounds (TPC) were carried out using spectrophotometric methods. The results were expressed as mg rutin (RE), mg caffeic acid (CAE), and mg gallic acid equivalents (GAE) *per* g dry plant material.

2. LC/MS Analysis of Polyphenols.

The phytochemical analyse of the extracts was performed by LC/MS method ,using an Agilent 1100 HPLC Series system equipped with G1322A degasser, G13311A binary gradient pump, column thermostat, G1313A autosampler, and G1316A UV detector. The HPLC system was coupled with an Agilent Ion Trap 1100 SL mass spectrometer. The identification of the polyphenols was made according to their retention times, as compared with the pure standards and their quantification was based on linear calibration plots of the peak area against concentration.

Antioxidant activity evaluation

The samples were evaluated for the antioxidant capacity using two *in vitro* spectrophotometric methods. The scavenging effect on the DPPH radical of the samples was calculated as the Trolox equivalent antioxidant capacity and the FRAP results were converted to µmoles of Trolox equivalents/mL plant extract.

Antimicrobial activity evaluation

A qualitative difusimetric method adapted from disk/well method was used. All samples were inoculated on round, sterile swabs placed in the wells of the solid culture medium which was previously inoculated with a known bacterial culture.

The microorganisms used in this test were *Streptococcus mutans* ATCC 25175, *Porphyromonas gingivalis* ATCC 33277, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Klebsiella sp*., and *Candida albicans* ATCC 10231 (ATCC—American Type Culture Collection, Manassas, VA, USA).

The culture media were: Nutrient agar, Sabouraud Dextrose agar, and Müeller-Hinton agar were used to grow the bacterial and fungal strains in Petri dishes. All culture media were prepared according to the manufacturer's instructions

Statistical Analysis. The samples were analysed in triplicate; the mean values and standard deviation were calculated using Microsoft Excel Software.

Results and discussion

Total phenolic contents and antioxidant activity

The results obtained by spectrophotometric determinations for the all extracts of *Vitis vinifera* are presented in Table 1.

Table 1. Total phenolic contents and antioxidant activity of *Vitis vinifera* extracts

Samples	TFC (mg RE/g)	Caffeic acid derivatives (mg CAE/g)	TPC (mg GAE/g)	$IC_{50(DPPH)}$ (µg/mL)	FRAP (µmol TE/mL)
Tendrils (62,5-312,5 µg/mL)	14.21±0.20	4.14±0.07	35.65±0.33	155±0.04	10.60±0.39
Leaves (62,5-312,5 µg/mL)	16.75±0.12	6.39±1.11	28.62±0.24	248±0.01	6.29±0.20
White Pomace (37.5–262.50 µg/mL)	1.89±0.10	17.64±1.35	37.80±0.19	76.33±2.67	39.08±1.28
Red Pomace (37.5–262.50 µg/mL)	0.54±0.07	9.11±1.38	32.00±0.76	112.02±3.98	26.52±6.49
Trolox	-	-	-	0.011±0.00	-

Legend: TPC: TFC: total flavonoid content; Total polyphenols content; CAE: caffeic acid equivalents GAE: gallic acid equivalents; RE: rutin equivalents; TE: Trolox equivalents.

The highest amount of the total flavonoids (TFC) was determined in the leaves extract (16.75 mg/g) followed by the tendrils extract (14.21 mg/g), and the lowest amount was determined in red pomace (0.54 mg/g). The WP extract contained the highest amount of total polyphenols, and phenylpropane derivates (37.80, and 17.64mg/g, respectively), followed by the RP extract (32.00, and 9.11mg/g, respectively). These results are in agreement with those obtained by other authors.

The evaluation of the antioxidant activity (Table 1) showed that the WP extract showed the highest activity, compared to the rest of the extracts, by both methods. The results are in good agreement with the TPC values listed in Table 1, so that WP with a higher content of TPC (37.80 mg/g) exhibited greater antioxidant activity (76.33 µg/mL, and 39.08 µmol TE/mL, respectively) than the other samples (> 100 µg/mL).

Chromatographic analysis of Vitis vinifera extracts

Both qualitative and quantitative differences were observed regarding the phytochemical content of *Vitis vinifera* by-products extracts (Table 2). Leaves and tendrils can be major sources of flavonoids (hyperoside, isoquercitrin, rutin, quercitrin), and grape pomaces are raw materials of catechin and epicatechin, but also of phenolic acids (gallic and protocatechuic acids).

Table 2. Polyphenols determined in *Vitis vinifera* extracts by LC-MS/MS (µg/g plant material)

Polyphenolic compounds	tR ± SD (min)	Leaves	Tendrils	WP	RP
Gallic acid	1.50±0.01	5.50±0.05	6.90±0.11	128.19±0.93	146.87±1.13
Protocatechuic acid	2.80±0.01	1.71±0.02	7.92±0.08	57.17±0.24	34.88±0.12
Caftaric acid	3.54±0.05	< 0.02	< 0.02	< 0.02	< 0.02
Gentisic acid	3.52±0.04	-	-	< 0.02	< 0.02
Catechin	6.00±0.03	23.31±0.36	51.72±1.06	539.14±1.86	561.93±4.07
Vanillic acid	6.70±0.01	-	-	-	45.00 ±0.88
Syringic acid	8.40±0.01	-	-	4.38± 0.12	72.22±0.62
Epicatechin	9.00±0.01	7.10±0.09	2.60±0.09	513.52±2.48	425.78±4.22
Hyperoside	18.60±0.12	147.09±1.79	127.39±2.61	< 0.02	-
Isoquercitrin	19.60±0.10	903.49 ± 6.51	541.34±6.44	29.70±0.30	6.59±0.09
Rutin	20.20±0.15	385.63±3.36	617.21±6.79	2.63±0.07	-
Quercitrin	23.64±0.13	188.74±2.26	100.87±2.13	26.08±0.37	-
Quercetin	26.80±0.15	10.54±0.24	8.89±0.11	6.69±0.02	9.99±0.01
Luteolin	29.10±0.19	-	-	3.50±0.04	-

Antimicrobial activity evaluation

Table 3. The diameter of inhibition zone (mm).

Bacterial/fungal strain	Inhibition diameter for the tested samples (mm) ± SD				
	C	1	2	3	4
Porphyromonas gingivalis ATCC 33277	0 ± 0.00	10± 0.00	13± 1.41	12± 1.41	11± 1.41
Enterococcus faecalis ATCC 29212	0± 0.00	10± 0.00	12± 1.41	11± 1.41	11± 1.41
Streptococcus mutans ATCC 25175	0± 0.00	10± 0.00	11.5± 0.71	11± 1.41	10± 1.41
Staphylococcus aureus ATCC 25923	0± 0.00	14.5± 0.71	12± 1.41	10± 1.41	11± 1.41
Escherichia coli ATCC 25922	0± 0.00	10± 1.41	11.5± 0.71	10± 0.00	11± 1.41
Klebsiella sp.	0± 0.00	0± 0.00	0± 0.00	10± 0.00	11± 1.41
Candida albicans ATCC 10231	0± 0.00	0± 0.00	0± 0.00	10± 0.00	9± 1.41

Legend: 1= TE; 2= LE; 3=WP; 4=RP; C = EtOH 50°

The studied extracts showed similar biologic activities with slight differences between extracts, which could be attributed to main components, but also to the synergic or antagonistic effects characteristic of the phytocomplexes.

Conclusions

By-products of *Vitis vinifera* are important sources of antioxidant bioactive compounds, so that the leaves and tendrils are rich in flavonoids, and grape pomace contains large amounts of phenolic acids and catechin isomers. As a result of the mentioned composition, both extracts displayed an antioxidant potential in the DPPH and FRAP assays.

Regarding the antioxidant action, the extracts showed an antiradical potential, the white pomace being distinguished by a high antioxidant power, in relation to a high content of total polyphenols.

The extracts had notable antimicrobial effects on several pathogenic bacteria from oral cavity, such as *Porphyromonas gingivalis, Enterococcus faecalis, Streptococcus mutans*, and *Staphylococcus aureus,* which may be associated with oral pathology. These results encourage the use of Vitis vinifera by-product extracts for the formulation of oral care products.

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