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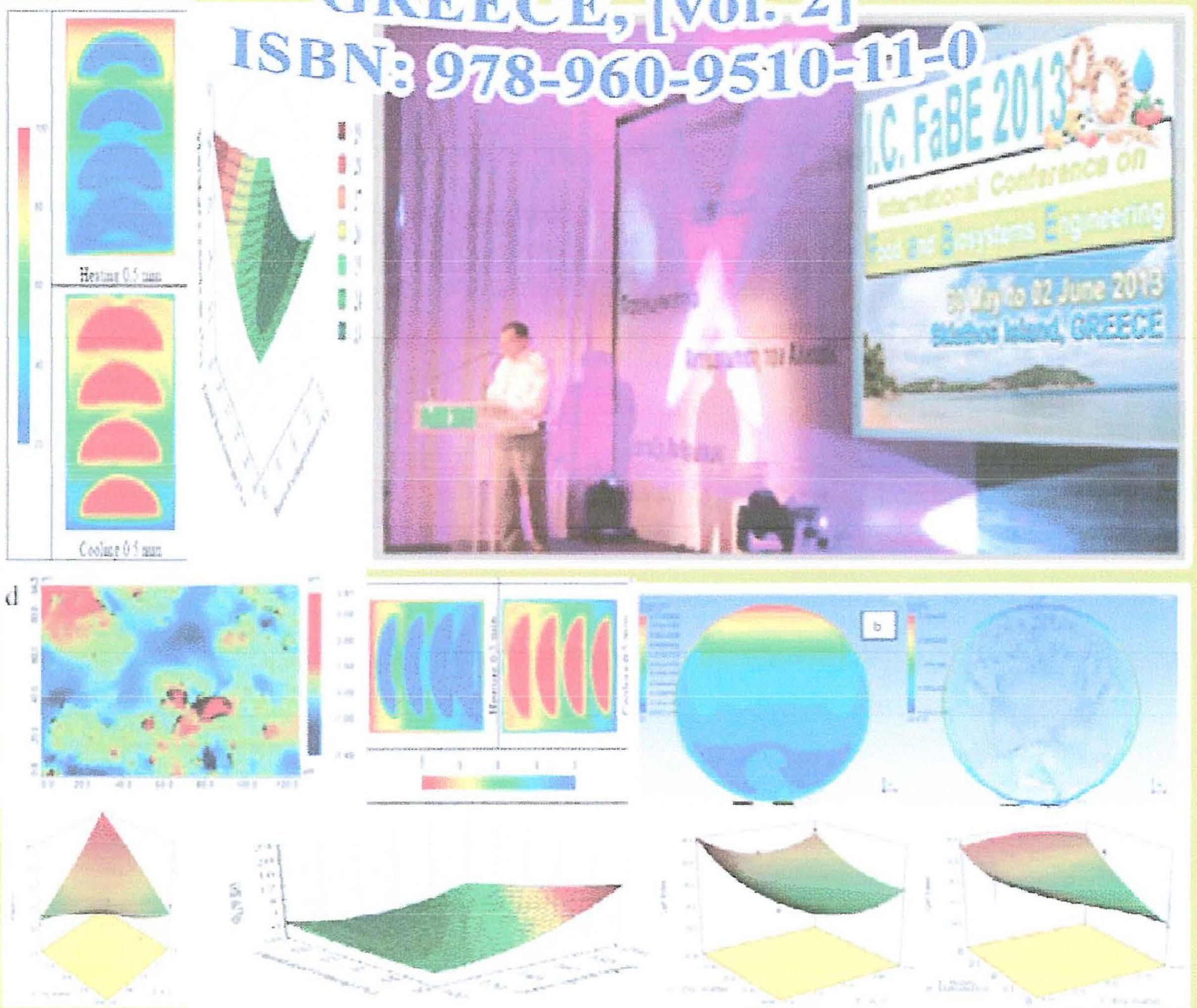

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PHENOLIC COMPOSITION AND ANTIOXIDANT POTENTIAL OF *Vitisvinifera* LEAVES OF SIX VARIETIES HARVESTED IN NORTHEAST OF PORTUGAL

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ABSTRACT:

In the present work it was intended to study the phenolic composition and antioxidant potential of *Vitisvinifera* leaves of six varieties: white – CarregaBranco, Chardonnay and Viosinho, and red – Bastardo, TintaGorda and TourigaFrancesa. All varieties were harvested in the NE of Portugal (Trás-os-Montes region). The phenolic composition of aqueous extracts was determined byHPLC-DADand the antioxidant potential was evaluated by the total reducing capacity (TRC), reducing power, DPPH radical scavenging and hydroxyl radical-scavenging activity assays.

Six phenolic compounds were detected, namely: *trans*-caffeoyltartaric acid, *trans*-coumaroyltartaric acid, myricetin-3-*O*-glucoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside and kaempferol-3-*O*-glucoside. The sum of these compounds varied between 23918 and 36859 mg/kg extract (dry basis) for the white varieties and 29706 and 43325 mg/kg extract (dry basis) for the red varieties. Regarding TRC, the white and red varieties displayed the following antioxidant capacities: 203 to 550 and 222 to 360 mg GAE/g extract, respectively. Some differences among the six varieties were observed in what concerns the reducing power and DPPH radical scavenging capacity. The highest values of reducing power were observed for Chardonnay (white) and TourigaFrancesa (red) varieties. On contrary, the highest DPPH radical scavenging capacities were observed for the Viosinho (white) and TintaGorda (red) varieties. All extracts presented hydroxyl radical scavenging activity, being the CarregaBranco (white) variety the one that presented the highest potential.

Keywords: *Vitisvinifera* leaves; Varieties; Phenolic composition; Antioxidant activity.

1. INTRODUCTION

In Portugal grapes (*V. vinifera* fruit) production has great economic importance, being generally the fruits used for fresh consumption and wine making. In 2011, the world production exceeded 69 million tons of grapes, contributing Portugal with 744823 tonnes (FAO, 2012). Several studies have been performed on grapes, wine and grape pomaceon their composition and biological properties. However, during the industrial processing of grapes other products and byproducts are obtained, such as *V. vinifera* leaves. These have been used in medicine and culinary. Nevertheless, few works have been performed until now on *V. vinifera*varieties harvested in Portugal. In spite of this, in the present work it was intended to study the phenolic composition and antioxidant potential of *V. vinifera* leaves of six varieties: three white – CarregaBranco, Chardonnay and Viosinho, and three red – Bastardo, TintaGorda and TourigaFrancesa, harvested in the NE of Portugal (Trás-os-Montes region).

2. MATERIALS AND METHODS

2.1 Plant material

Samples were harvested in July 2006 in an experimental field in Sendim, Bragança, Portugal. Six *V. vinifera* varieties were selected, namely: white – CarregaBranco, Chardonnay and Viosinho, and red – Bastardo, TintaGorda and TourigaFrancesa. After harvest, the leaves were bagged and taken to the laboratory, where they were kept at -20 °C until lyophilization. All samples were freeze-dried, powdered and stored in a desiccator in the dark.

2.2 Aqueous extracts preparation

Five grams of *V. vinifera* leaves were boiled for 45 min in 250 mL of water. The resulting extracts were filtered and then lyophilized. Then the extracts were kept in a dessicator in the dark, until analysis. All extractions were done in triplicate.

2.3 Phenolic composition of the extracts

Each lyophilized extract was redissolved in water, filtered (0.2 □m) and 20 □L were injected in an analytical HPLC unit (Gilson), equipped with a Spherisorb ODS2 (250×4.6 mm; 5□m, particle size) column, coupled to a Diode Array Detector (DAD) (Gilson), according to the procedure described onFernandes et al. (2013).

2.4 Antioxidant activity

2.4.1 Total Reducing Capacity

Total reducing capacity of aqueous extracts was evaluated by the Folin-Ciocalteu's method. The procedure used was adapted from Delgado, Malheiro, Pereira and Ramalhosa(2010) with some modifications. Briefly, 1 mL of sample aqueous extract was mixed with 1 mL of Folin-Ciocalteu's phenol reagent and 7 ml of distilled water. The mixture was kept in the dark for 90 min, followed by absorbance reading at 725 nm (Thermo Electron Corporation Genesys 10UV spectrophotometer). Gallic acid was used as standard, being the results expressed in mg of gallic acid equivalents (GAE)/g of extract.

2.4.2 DPPH Radical Scavenging Effect

The ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was monitored according to the method reported by Hatano, Kagawa, Yasuhara and Okuda (1988).DPPH radical scavenging effect was calculated as follows:

$$\%\ \text{DPPH radical scavenging capacity} = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$$

, where A_S was the absorbance of the solution when the sample extract was added and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of scavenging effect percentage against the extract concentration.

2.4.3 Reducing power

Reducing power was determined according to the procedure described by Berker, Güçlü, Tor and Apak(2007).The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance registered at 700 nm against the correspondent extract concentration.

2.4.4 Hydroxyl Radical Assay

Hydroxyl radical scavenging activity was determined according to the procedure described by Valentão, Fernandes, Carvalho, Andrade, Seabra and Bastos(2002).

3. RESULTS AND DISCUSSION

3.1 Phenolic composition of the extracts

All samples revealed the presence of two hydroxycinnamic acid derivatives (*trans*-caffeoyltartaric and *trans*-coumaroyltartaric acids) and four flavonoids glicosides (myricetin-3-*O*-glucoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside and kaempferol-3-*O*-glucoside). The contents of these compounds of the leaves of the six varieties studied in the present work are presented in Table 1.

TABLE 1 – Concentrations of the *trans*-caffeoyltartaric acid (1),*trans*-coumaroyltartaric acid (2), myricetin-3-*O*-glucoside (3), quercetin-3-*O*-glucoside + quercetin-3-*O*-galactoside (4+5) and kaempferol-3-*O*-glucoside (6) (mg/kg dry basis) determined on six varieties of *V. vinifera*leaves.

Variety	Phenolic compound (mg/kg dry basis)					
	1	2	3	4+5	6	Σ
White						
Carrega Branco	6562.8±171.5	566.8±12.2	364.5±13.2	25567.3±891.8	3797.1±132.9	36858.5
Chardonnay	6963.3±62.2	664.1±7.8	335.7±0.8	24822.0±275.6	2698.1±14.2	35483.2
Viosinho	5378.4±33.7	507.3±15.2	153.5±1.0	16235.7±214.3	1642.6±23.5	23917.5
Red						
Bastardo	8394.8±71.7	591.2±3.9	295.6±12.2	26405.2±408.1	2714.3±74.1	38401.1
Tinta Gorda	8515.6±754.5	951.1±64.5	275.8±3.1	28809.5±15.5	4773.5±93.4	43325.4
Touriga Francesa	6232.0±94.8	673.2±12.2	333.0±36.8	19872.7±289.9	2595.5±31.4	29706.5

The sum of these compounds varied between 23918 and 36859 mg/kg extract (dry basis) for the white varieties and 29706 and 43325 mg/kg extract (dry basis) for the red varieties, suggesting different phenolic contents among the six varieties. The presence of *trans*-coumaroyltartaric acid and myricetin-3-*O*-glucoside had already been reported on stems of *V. vinifera* (Souquet, Labarbe, Guerneve, Cheynier and Moutounet, 2000) and myricetin is a compound generally found in *V. vinifera* leaves; however, in the present work it appeared in glycosilated form.

3.2 Antioxidant activity

The results obtained in the TRC, Reducing Power and DPPH radical scavenging capacity methods performed to evaluate the antioxidant activity of the aqueous extracts are presented in Table 2.

TABLE 2 –TRC (mg GAE/g extract) and EC_{50} (mg/mL) values determined in the reducing power and DPPH radical scavenging capacity methods determined on six varieties of *V. vinifera*leaves.

Variety	TRC (mg GAE/g extract)	Reducing power EC_{50} (mg/mL)	DPPH radical scavenging capacity EC_{50} (mg/mL)
White			
Carrega Branco	323±38	0.334	0.136
Chardonnay	203±18	0.232	0.128
Viosinho	550±62	0.825	0.098
Red			
Bastardo	360±47	0.795	0.194
Tinta Gorda	332±57	0.310	0.089
Touriga Francesa	222±42	0.289	0.119

All aqueous extracts showed TRC; however, some differences between white and red varieties were observed.The Viosinho white variety was the one that presented the highest TRC (550±62 mg GAE/g extract), followed by the Bastardo red variety (360±47 mg GAE/g extract).

Regarding the reducing power, the highest values were observed for the Chardonnay (white) and the TourigaFrancesa (red) varieties because both presented the lowest EC_{50} values. These results indicated that the aqueous extracts of these two varieties possessed compounds able to reduce the Fe^{3+}ferricyanide complex to its ferrous form.

On contrary, the highest DPPH radical scavenging capacities were observed for the Viosinho (white) and TintaGorda (red) varieties, suggesting the presence of compounds able to reduce the DPPH free radical to its hydrazine form by electron donation.

The hydroxyl radical (•OH) is the most reactive radical that it is known. It can attack and damage almost every molecule found in living cells. This radical is able to interact with the purine and pyrimidine bases of DNA. It can also abstract hydrogen atoms from biological molecules, including thiols, leading to the formation of sulfur radicals able to combine with oxygen to generate oxysulfur radicals, also able to damage biological molecules (Halliwell, 1991).

Hydroxyl radicals are produced *in vivo* by the Fenton reaction**(1)**, in which the reduced form of a transition metal (e.g., iron) react with hydrogen peroxide, leading to the formation of the hydroxyl radical.

$$Fe^{2+}\text{-EDTA} + H_2O_2 \rightarrow \quad Fe^{3+} - \text{EDTA} + OH^- + HO^\bullet \qquad \textbf{(1)}$$

The presence of reducing agents, e.g. ascorbic acid, increases the rate of reaction **(1)**, due to the occurrence of the reduction of the transition metal:

$$Fe^{3+}\text{-EDTA} + \text{ascorbicacid} \rightarrow \quad Fe^{2+}\text{-EDTA} + \text{dehidroascorbicacid} \qquad \textbf{(2)}$$

The pentose sugar 2-deoxyribose is attacked by $HO^\bullet$radicals to yield a mixture of products.On heating with thiobarbituric acid at low pH, some orall ofthese products react to form a pink chromogen that can be measured by its absorbance at 532 nm; thischromogen is indistinguishable from a thiobarbituricacid-malondialdehyde (TBA-MDA) adduct.

All *V. vinifera* leaves showed ability to scavenge hydroxyl radicals as expressed by the percentages of scavenging activity, presented in Tables 3 and 4. Some differences were noticed between white (Table 3) and red (Table 4) varieties.The CarregaBranco (white) variety was the one that presented the highest potential because the highest radical scavenging activities were achieved. Radical scavenging activities higher than 30% were never obtained with the red varieties. On the other hand, the aqueous extracts showed some prooxidantpotential because when performing the deoxyribose assay in the absence of ascorbic acid the absorbancevalues increased with extract concentration. The tubes without ascorbic acid allowed the evaluation of extracts ability to reduce Fe^{3+} to Fe^{2+} that will cause an increase in hydroxyl radical production. Damage to deoxyribose also occurs if the Fe^{3+}-ascorbate/H_2O_2 -induced generation of hydroxyl radicals is performed in the absence of EDTA,because omission of the chelator allows iron ions to bind directlyto the sugar (Valentão, Fernandes, Carvalho, Andrade, Seabra and Bastos, 2002). Compounds that can inhibit deoxyribosedegradation in the absence of EDTA are those with iron ion-binding capacity and which can withdraw the iron ions andrender them inactive or poorly active in Fenton reactions (Valentão, Fernandes, Carvalho, Andrade, Seabra and Bastos, 2002).Thus, when performing the deoxyribose assay in the absence of EDTA it is possible to evaluatethe extracts metal chelation potential, leading to a reduction on the formation of the pink chromogen.

TABLE 3 – Hydroxyl radical scavenging activity determined on three white varieties of *V. vinifera* leaves.

	Extract concentration (mg/mL)					
	0.1	0.2	0.4	1	2	4
			Carrega Branco			
Abs	0.046	0.047	0.049	0.052	0.056	0.059
Scavenging activity %	18.2±6.4	13.9±12.8	19.3±9.3	41.4±0.7	39.6±0.8	36.3±1.2
Abs (without AA)	0.275±0.003	0.350±0.040	0.394±0.047	0.410±0.050	0.417±0.047	0.434±0.034
Abs (without EDTA)	0.104±0.002	0.099±0.002	0.098±0.001	0.097±0.001	0.098±0.001	0.099±0.001
			Chardonnay			
Abs	0.040	0.042	0.041	0.045	0.046	0.053
Scavenging activity %	10.4±2.1	6.6±0.6	10.0±1.9	17.4±0.8	23.6±1.0	21.7±0.4
Abs (s/ AA)	0.300±0.007	0.319±0.004	0.449±0.016	0.563±0.005	0.594±0.003	0.569±0.025
Abs (s/ EDTA)	0.199±0.010	0.184±0.002	0.157±0.002	0.146±0.002	0.121±0.007	0.121±0.005
			Viosinho			
Abs	0.067	0.070	0.074	0.073	0.075	0.073
Scavenging activity %	6.0±2.0	9.1±1.9	13.0±3.7	15.7±2.0	20.7±1.2	18.3±2.13
Abs (without AA)	0.138±0.001	0.145±0.002	0.145±0.005	0.152±0.002	0.169±0.009	0.192±0.004
Abs (without EDTA)	0.143±0.005	0.141±0.003	0.141±0.004	0.139±0.003	0.140±0.002	0.142±0.005

Note: AA – Ascorbic acid

TABLE 4 –Hydroxyl radical scavenging activity determined on three red varieties of *V. vinifera* leaves.

	Extract concentration (mg/mL)					
	0.1	0.2	0.4	1	2	4
			Bastardo			
Abs	0.059	0.061	0.062	0.063	0.064	0.067
Scavenging activity %	10.8±0.9	3.3±1.3	20.5±3.6	25.1±6.9	29.2±6.5	27.2±4.8
Abs (without AA)	0.109±0.003	0.116±0.003	0.123±0.002	0.128±0.003	0.135±0.003	0.138±0.002
Abs (without EDTA)	0.109±0.005	0.100±0.006	0.094±0.003	0.087±0.004	0.089±0.002	0.095±0.002
			Tinta Gorda			
Abs	0.047	0.049	0.051	0.053	0.054	0.055
Scavenging activity %	19.8±4.4	17.9±6.3	20.1±5.7	21.9±4.8	25.8±6.6	25.4±5.8
Abs (s/ AA)	0.246±0.019	0.433±0.026	0.526±0.062	0.565±0.022	0.653±0.015	0.604±0.011
Abs (s/ EDTA)	0.208±0.004	0.186±0.010	0.166±0.010	0.140±0.006	0.123±0.009	0.112±0.006
			Touriga Francesa			
Abs	0.044	0.044	0.044	0.045	0.050	0.052
Scavenging activity %	9.8±2.1	4.6±3.1	9.6±5.4	11.0±5.7	16.3±2.7	11.7±5.3
Abs (without AA)	0.203±0.019	0.423±0.034	0.455±0.049	0.544±0.024	0.619±0.005	0.500±0.016
Abs (without EDTA)	0.199±0.003	0.184±0.004	0.162±0.009	0.156±0.004	0.148±0.005	0.147±0.007

Note: AA – Ascorbic acid

The Chardonnay (white), TintaGorda (red) and TourigaFrancesa (red) varieties were the ones that presented the highest ability to complex with iron because the absorbance values of the tubes without EDTA decreased with extract's concentration increase.

4. CONCLUSIONS

V. vinifera leavesare rich in phenolic compounds, namely: *trans*-caffeoyltartaric acid, *trans*-coumaroyltartaric acid, myricetin-3-*O*-glucoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside and kaempferol-3-*O*-glucoside, varying between 23918 and 43325 mg/kg extract (dry basis). Furthermore, the six varieties showed different antioxidant properties.The Viosinho white variety was the one that presented the highest TRC, followed by the Bastardo red variety. The highest values of reducing power were observed for Chardonnay (white) and

TourigaFrancesa (red) varieties. On contrary, the highest DPPH radical scavenging capacities were observed for the Viosinho (white) and TintaGorda (red) varieties. All extracts presented hydroxyl radical scavenging activity, being the CarregaBranco (white) variety the one that presented the highest potential.

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