

# Study on the Antioxidant Activity of Grape Stems (*Vitis vinifera*). A Preliminary Assessment of Crude Extracts

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## ABSTRACT

The antioxidant activity of 80% ethanol and 70% acetone extracts of stems from red and white grapes (*Vitis vinifera*) used in wine industry were evaluated to determine their feasibility as natural antioxidants. The results showed that all grape stem extracts were rich in total polyphenolic compounds and flavanols and had clear antioxidant activities. The free radical-scavenging capacity of the extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The extracts obtained from red grape stem present EC<sub>50</sub> values of 0.14 g dm/g DPPH (acetone extract) and 0.20 g dm/g DPPH (ethanol extract) while the extracts obtained from white grape stem present EC<sub>50</sub> values of 0.26 g dm/g DPPH (acetone extract) and 0.37 g dm/g DPPH (ethanol extract). There are significant correlations between the total content of polyphenols and the antioxidant activity ( $R^2 = 0.9352$ ) and between the flavanols content and the antioxidant activity ( $R^2 = 0.9404$ ) of the grape stem extracts obtained.

**Keywords:** Grape Stem; Antioxidant Activity; Stem Extracts; Polyphenols; Radical Scavenging Activity; DPPH

## 1. Introduction

There is a growing interest in the exploitation of the residues generated by the food industry, and this is very attractive to the vegetable/beverage industry, as they will have to sustain increasing costs for treating these solid and liquid wastes. This is particularly true in wine production, which affords a substantial volume of solid organic material. The main by-products are collected during de-stemming (stems) and pressing (pomace). At present, only minimum amounts of these wastes are recycled and this is definitely the case in Europe, where vegetable wastes are generally used for animal feed or compost, without any pre-treatment. Specifically, in grape and wine production zones, a great quantity of residues is generated with little economic interest and is associated with environmental waste issues. Numerous studies indicate that wine, grapes and pomaces contain significant amounts of polyphenolic compounds although less attention has been paid to grape stems, despite that these contain important amounts of polyphenols [1-4]. Furthermore, phenolic constituents present in grapes have aroused great interest due to their antioxidant properties and putative health-promoting effects. Antioxidant compounds are used to prevent or reduce food product deterioration and human degenerative diseases [5]. Free radicals have been claimed to play a key role, in affecting human health by causing life-threatening illnesses by cell

degeneration, such as cancer and cardiovascular diseases. These free radicals can be generated during normal body function, and can be acquired from the environment. Polyphenols are known to be powerful antioxidants as they are hydrogen donating free radical scavengers [6-9].

Therefore, a study of grape by-products such as stems as a possible source of derivatives with antioxidant polyphenolic compounds is of interest, as any useful production from this by-product could represent an economic revaluation of the raw material and an interesting advance in the prevention or reduction of environmental problems caused by residue generation.

The objective of this present study was to evaluate the antioxidant activity of direct stem extracts from red and white grapes (*Vitis vinifera*) used in the wine industry to examine their utilization as natural antioxidants. In addition, correlations between total polyphenolic and flavanol content and antioxidant activity were also evaluated.

## 2. Materials and Methods

### 2.1. Materials and Chemicals

The samples correspond to the stems of the winemaking processes of Manto Negro red grape and Prensal Blanc white grape (*Vitis vinifera*) varieties, these which are natives to and widely grown in Mallorca, Spain, as they are the major red and white grape varieties used in the

local wine industry. The stems used in this study were provided by a Spanish winery (Antonio Nadal S.L., Mallorca). Stems were obtained directly at the beginning of the winemaking process after the grape destemming process. Samples were dried, ground and stored at  $-20^{\circ}\text{C}$  until used.

The following standards and reagents were used: Stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from Aldrich, Germany. Folin-Ciocalteu reagent was purchased from Merck, Germany. (+)-Catechin and DL- $\alpha$ -tocopherol from Sigma, Spain, and gallic acid from Aldrich, Germany.

All reagents used were of analytical grade.

Dry matter was determined by drying the samples to a constant weight at  $105^{\circ}\text{C}$ .

## 2.2. Preparation of Extracts

Extracts from grape stems were prepared using two solvents ethanol: water (80/20, v/v), in some cases, or acetone: water (70/30, v/v), in the others. Grape stems were extracted twice with the selected solvent at room temperature for 1 hour with continuous stirring in each case. The ratio of solvent used was 10mL per gram of sample. After centrifugation, supernatants were combined, concentrated at  $40^{\circ}\text{C}$  and lyophilised. The solid dry extracts were subjected to successive analyses.

## 2.3. Determination of Total Polyphenols and Flavanols

Total polyphenols were spectrophotometrically determined in the solid dry extracts by reading the absorbance at 765 nm (Folin-Ciocalteu method) [10], using gallic acid as a standard and expressing the results as gallic acid equivalents (GAE).

Flavanols were quantified in the solid dry extracts by the vanillin assay [11], using (+)-catechin as standard and expressing the results as (+)-catechin equivalents (CAE).

## 2.4. Determination of Antioxidant Activity

The antioxidant activity of the solid extracts was measured in terms of radical scavenging ability, according to the DPPH method reported by Brand-Williams *et al.* [12], modified by Sánchez-Moreno *et al.* [13]. The  $\text{EC}_{50}$  parameter, which reflects 50% depletion of DPPH free-radical, was expressed as grams of dry extract per gram of DPPH. This characteristic parameter is called efficient concentration ( $\text{EC}_{50}$ ) or oxidation index, and the lower the value, the higher the antioxidant activity of the examined product. For rational reasons of clarity, the antiradical activity  $A_{\text{AR}}$  was determined as the inverse value of the efficient concentration  $\text{EC}_{50}$ , representing a comparable term for the effectiveness of antioxidant and

radical scavenging capacity:  $A_{\text{AR}} = 1/\text{EC}_{50}$ . The larger the  $A_{\text{AR}}$ , the more efficient is the antioxidant. For comparison purposes, the efficient concentrations of vitamin C and vitamin E were also evaluated.

## 2.5. Statistical Analysis

The determinations of total polyphenols and flavanol contents, and the antioxidant activity were carried out at least in quadruplicate. Results are presented as the mean value with the standard deviation (SD). Correlation between total polyphenols and flavanol contents and antioxidant activity was verified by the determination coefficient  $R^2$  of the linear regression.

## 3. Results and Discussion

### 3.1. Yield, Total Polyphenolic and Flavanol Content of Solid Extracts

**Table 1** shows the yield obtained for all the grape stem extracts from red and white grape stems. The values were close to 30% of grape stem dry weight (27.3% - 32.1%). Concentration of total polyphenols and flavanols obtained for the grape stems extracts are shown in **Table 1** and are referred to as g to 100 g dry matter (dm).

The solid extracts obtained from an 80% ethanol extraction process are RS-ETOH (from red grape stem) and WS-ETOH (from white grape stem) and those corresponding to 70% acetone extraction process are RS-ACET (from red grape stem) and WS-ACET (from white grape stem).

The high polyphenolic content for all the grape stem extracts can be observed in **Table 1**. The quantities of total polyphenols present in the red grape stem extracts were higher than those of the white stem extracts, independent of the solvent used. Concentrations of total polyphenols in the RS-ETOH and RS-ACET extracts were equal to 29.4 and 38.4 (g GAE/100g dm), respectively; whereas the quantities of polyphenols contained in

**Table 1. Yield, total polyphenols and flavanols of grape stem extracts.**

Solid Extract	Yield (% dm)	Polyphenols (g GAE/100g dm)	Flavanols (g CAE/100g dm)
RS-ETOH	29.5 ± 0.6	29.4 ± 0.4	21.7 ± 0.3
WS-ETOH	27.3 ± 0.5	17.2 ± 0.2	12.6 ± 0.2
RS-ACET	30.1 ± 0.6	38.4 ± 0.5	27.0 ± 0.4
WS-ACET	32.1 ± 0.6	22.9 ± 0.3	16.2 ± 0.2

Results are expressed as the mean ( $n = 4$ ) ± SD; GAE = gallic acid equivalent; CAE = catechin equivalent; RS-ETOH = solid extract from red grape stem with 80% ethanol; WS-ETOH = solid extract from white grape stem with 80% ethanol; RS-ACET = solid extract from red grape stem with 70% acetone; WS-ACET = solid extract from white grape stem with 70% acetone.

the related extracts obtained from white grape stem, WS-ETOH and WS-ACET, were 17.2 and 22.9 (g GAE/100g dm), respectively. This fact is in accordance with the higher polyphenolic content of the red grape stem variety Manto Negro in opposite to the white grape stem variety Prensal Blanc previously described, 11.6 and 8.7 g GAE/100g dm, respectively [2,4]. Püssa *et al.* [14] reported that red varieties stems have higher polyphenol values than those of white varieties.

Moreover, **Table 1** illustrates that the acetone extracts obtained from either grape stem varieties were richer in polyphenols than that the ethanol extracts. In fact, for the extracts corresponding to the red grape stem, RS-ACET was richer than RS-ETOH (38.4 to 29.4 g GAE/100g dm, respectively), and analogously, for extracts corresponding to the white grape stem, WS-ACET was richer than WS-ETOH (22.9 to 17.2 g GAE/100g dm, respectively).

Therefore, the results show the high polyphenolic content for all the solid grape stem extracts obtained in this work that are between 17.2% and 38.4% of dm (**Table 1**).

Additionally, **Table 1** gives the flavanol content of the grape stems extracts. Similar in pattern to the results shown above for the polyphenols, the flavanol content of the red grape stem extracts, RS-ETOH and RS-ACET, were higher than that in the white stem extracts, WS-ETOH and WS-ACET (21.7 and 27 g CAE/100g dm compared to 12.6 and 16.2 g CAE/100g dm, respectively). Again, the acetone extract obtained from either red or white grape stem varieties was richer in flavanols than that the ethanol extract.

Notable is the high flavanol content for all the stem extracts obtained. **Table 1** clearly shows that flavanols accounted for approximately 70% of the polyphenolic content of white and red grape stem extracts.

Summarizing, the best grape stem extract for either total polyphenol or flavanols contents was that obtained from a red grape stem, using 70% acetone as solvent.

### 3.2. Antioxidant Activity

**Table 2** shows the antioxidant activity of red and white

grape stem extracts expressed as effective concentration,  $EC_{50}$ , antiradical activity,  $A_{AR}$  and antioxidant activity equivalents of vitamin C and vitamin E. All solid extracts obtained presented a high antioxidant activity, especially the red grape stem extracts, RS-ACET and RS-ETOH, which have an  $EC_{50} = 0.14$  and  $0.20$  g dm/g DPPH, respectively, representing an antiradical activity  $A_{AR}$  of 7.14 and 5 g DPPH/g dm, respectively. These high antioxidant activities correspond to 615 and 430 vitamin C mg-equivalent/g dm, respectively, and 1630 and 1140 vitamin E mg-equivalent/g dm, respectively. Note that both extracts had an antioxidant activity higher than that vitamin E. On the other hand, the extracts obtained from the white grape stem, WS-ACET and WS-ETOH, had an  $EC_{50} = 0.26$  and  $0.37$  g dm/g DPPH, respectively, representing an antiradical activity  $A_{AR}$  of 3.85 and 2.70 g DPPH/g dm, respectively. They corresponded to 331 and 233 vitamin C mg-equivalent/g dm, respectively, and 878 and 616 vitamin E mg-equivalent/g dm, respectively.

The grape stem extract with a higher antioxidant activity was that obtained from red grape stem using 70% acetone as solvent. It should be noted that this follows the same trend as in the cases of total polyphenols and flavanols previously mentioned.

In fact, antioxidant activity was closely correlated with the total content of polyphenols and flavanols. So, by linear regression analysis, there is a significant correlation between the total content of polyphenols and the antioxidant activity of the grape stem extracts studied. The linear equation resulting was: Polyphenols content =  $48.801 - 89.962 EC_{50}$  (determination coefficient,  $R^2 = 0.9352$ ) (negative slope due to the lower the  $EC_{50}$  value, the higher the antioxidant activity). This fact was described by several authors in selected fruits, vegetables and grain products, tea infusion, *Rubus* species and vine grapes and leaves [15-18]. These authors found a significant correlation between the total content of polyphenols and the antioxidant power.

Moreover, there is a significant correlation between the flavanols content and the antioxidant activity of the grape stem extracts studied and the linear equation re-

**Table 2. Effective concentration  $EC_{50}$ , antiradical activity  $A_{AR}$  and antioxidant activity equivalents of vitamins C and E of grape stem extracts.**

Solid Extract	$EC_{50}$ (g dm/g DPPH)	$A_{AR}$ (1/ $EC_{50}$ )	AA-Vit C eq (ascorbic acid) (vit. C mg-eq/g dm)	AA-Vit E eq (DL- $\alpha$ -tocopherol) (vit. E mg-eq/g dm)
RS-ETOH	$0.20 \pm 0.02$	$5.00 \pm 0.50$	430	1140
WS-ETOH	$0.37 \pm 0.04$	$2.70 \pm 0.29$	233	616
RS-ACET	$0.14 \pm 0.01$	$7.14 \pm 0.71$	615	1630
WS-ACET	$0.26 \pm 0.03$	$3.85 \pm 0.44$	331	878

Results are expressed as the mean ( $n = 4$ )  $\pm$  SD; AA-Vit C eq = Antioxidant activity-vitamin C equivalent; AA-Vit E eq = Antioxidant activity-vitamin E equivalent; RS-ETOH = solid extract from red grape stem with 80% ethanol; WS-ETOH = solid extract from white grape stem with 80% ethanol; RS-ACET = solid extract from red grape stem with 70% acetone; WS-ACET = solid extract from white grape stem with 70% acetone.

sulting was: Flavanols content = 34.550 - 62.588 EC<sub>50</sub> (determination coefficient, R<sup>2</sup> = 0.9404).

The use of natural polyphenols from grape stem as food additives can be advantageous. It is well known that the maximum lawful levels for synthetic food antioxidants are established from different toxicological parameters that need not be applicable to naturally occurring compounds. Therefore, the polyphenols from natural extracts could be used at higher levels than the synthetic phenols, thereby increasing their antioxidant effectiveness. Also, the anti-oxidative characteristics of these natural compounds can represent an interesting feature for their application to prevent deterioration of pharmaceuticals and cosmetics.

#### 4. Conclusions

The high contents in total polyphenols as well as in flavanols together with the excellent antioxidant properties of all the grape stem extracts obtained confer a wide range of applications of these products in food, cosmetic and pharmacological industries.

Taking into account that grapes are used on the order of millions of tons in wine producing countries, the results for these four solid extracts obtained from wine grape stems indicate an abundant and valuable source of natural antioxidant sources in which their utilization could also provide at least a partial solution for the wine-making waste.

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