

## Phenolic composition and antioxidant activity of mocan seeds (*Visnea mocanera* L.f).

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### Abstract

The fruits from *Visnea mocanera*, named also “mocan”, are consumed fresh and in different drinks by the Canary natives (Spain), due to their medicinal and nutritive properties. The “mocan” fruits are small berries containing small seeds, in which analysis, by HPLC-PAD and HPLC-MS, show a high concentration of flavan-3-ols, mainly catechin and procyanidin dimers and trimers. The evaluation of the free radical scavenging activity of the seed extracts and of the individualised compounds shows high antioxidant activity. The flavan-3-ols identified show differences in that activity, depending on the numbers and structure of units forming the procyanidins.

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**Keywords:** Mocan; Phenolics; Procyanidins; Antioxidant; HPLC-PAD; HPLC-MS

### 1. Introduction

The *Visnea mocanera* L.f., known locally as “mocán” or “mocanera”, is a native wild tree of the Macaronesian region in the Canary Islands (Spain), whose fruits have been used by the Canary natives, from pre-Hispanic times, due to their medicinal and nutritive properties.

The “mocán” fruits, small red-blackish berries, were consumed fresh by the aboriginal population from these islands and were one of the bases of their diet (Navarro & Del Arco, 1987; Viera & Clavijo, 1942). These fruits were also used for preparing drinks, employed as analgesic, anti-inflammatory, stomachic, anti-ulcerogenic, cicatrizing, antihemoptisic, antiemetic, vulnerary and antidiarrhetic agents (Darias, Bravo, Rabanal, Sánchez-Mateo, González-Luis, & Hernández-Pérez, 1989; García Morales, 1987, 1989). Some of these medicinal properties have been proven experimentally with in vivo and in vitro pharmaceutical techniques (Hernández-Pérez, Sánchez-Mateo, Darias, & Rabanal, 1994, 1995a, 1995b). Nowadays interest in

these berries has been aroused by these beneficial properties.

These fruits are rich in soluble carbohydrates (mainly glucose and fructose), dietary fibre (mainly cellulose and lignin), and minerals (Hernández-Peréz, Frias, Rabanal, & Vidal-Valverde, 1994). They are also rich in phenolic compounds (Hernández-Pérez, Hernández, Gómez-Cordovés, Estrella, & Rabanal, 1996) some of which are anthocyanidins, flavonol glycosides and hydroxybenzoic and hydroxycinnamic acids, together with proanthocyanidins. These proanthocyanidins should be located mainly in the seeds of the “mocán” fruits, in the same way as proanthocyanidins of other seeds, such as grape seeds (Castillo et al., 2000; De Freitas, Glories, & Monique, 2000; Fernández de Simón, Hernández, & Estrella, 1993; Peng, Hayasaka, Iland, Sefton, Hoj, & Waters, 2001; Saito, Hosoyama, Ariga, Kataoda, & Yamaji, 1998).

Proanthocyanidins are polymers constituted of a variable number of flavan-3-ols or a catechin nucleus. The most abundant are the procyanidins, formed by the monomers (+)-catechin and (–)-epicatechin, linked by C<sub>4</sub>–C<sub>6</sub> or C<sub>4</sub>–C<sub>8</sub> interflavan bonds (B series), or doubly linked with an additional C<sub>2</sub>–O–C<sub>7</sub> (A series) bond. The dimers, trimers, tetramers and pentamers, constitute the

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proanthocyanidin oligomers and are named condensed tannins (Haslam, 1998).

The majority of the medicinal properties proven for the mocan fruits e.g. antiinflammatory, antiulcerogenic, antihemoptisic) are related to the beneficial properties described for the flavan-3-ols and proanthocyanidins (Bruneton, 1993; Huang, Ho, & Lee, 1992; Masquelier, 1988; Rider, Der Marderosian, & Porter, 1992; Terencio, Sanz, & Paya, 1991).

Proanthocyanidins show beneficial properties such as: potent antioxidant activity (Liu, Zhang, & Lau, 1998; Ricardo da Silva, Darmon, Fernández, & Mitjavila, 1991; Vinson Dabbagh, Serry, & Jang, 1995), and free radical scavenging activity (Saint-Cricq de Gaulejac, Vivas, De Freitas, & Bourgeois, 1999). These compounds inhibit platelet aggregation (Zafirov, Bredy-Dobrev, Litchev, & Papisova, 1990), and the oxidation of low density lipoproteins and they present antiulcerogenic activity against stomach mucosa injury (Saito et al., 1998). They show anti-inflammatory (Gábor, 1986), antihypertensive (Terencio et al., 1991) and antimutagenic (Catteral, Souquet, Cheynier, Clifford, & Iannides, 2000; Gali, Perchellet, Gao, Karchesty, & Perchellet, 1994) properties. It was also observed that proanthocyanidins from grape seeds (*V. vinifera*) present radioprotective effects against chromosomal damage induced by X-rays (Castillo et al., 2000). Most of these properties of the proanthocyanidins, but mainly antioxidant activity, are related to their structural characteristics (Benavente-García, Castillo, Marín, Ortuño, & Del Rio, 1997; Lotito et al., 2000). These compounds are present in complex forms in the natural sources.

To examine the contribution of the phenolic composition of the seed mocan to the whole berry and its influence on the use of the different products obtained from the mocan fruits, as medical source, it is necessary to know their phenolic components and their scavenging activity. In this work we have studied the phenolic composition of the seeds of three different samples of mocan fruits from the Canary Islands. The antioxidant activities of the seed berry extracts and of different isolated compounds from those extracts, were also measured. The relationship between the antioxidant activity and the identified procyanidins of the seeds is discussed, taking into account the structure of the compounds.

## 2. Materials and methods

### 2.1. Samples

The ripped fruits of mocan were collected in three different islands, Tenerife ( $M_1$ ) La Palma ( $M_2$ ) and Hierro ( $M_3$ ), from the Canary Islands (Spain). The seeds of the berries were manually separated, dried at room temperature and ground, before extraction.

### 2.2. Extraction

Ground seeds (2.5 g) were macerated with methanol three times (40 ml $\times$ 3) at room temperature, during 9 h $\times$ 3. The three combined macerates were brought to a fixed volume (120 ml). Part of the resulting liquid was used for the quantification of the phenolic compound families and the evaluation of the antioxidant activity.

An aliquot (100 ml) of the methanol solution was evaporated to dryness, and the residue was dissolved in methanol/water (20:80, v/v). After dissolution, the phenolic compounds were purified and extracted three times by ethyl acetate; the organic fractions were pooled, dried over anhydrous  $Na_2SO_4$ , filtered and the liquid evaporated to dryness under vacuum. The residue was dissolved in methanol/water (1:1, v/v), and analysed by high-performance liquid chromatography with photodiode array detection (HPLC–PAD) and mass spectrometry detector (HPLC–MS):

### 2.3. Quantification of the phenolic compounds families

In the methanol solution, the total polyphenols (PT) were quantified by the Folin–Ciocalteu method (Singleton & Rossi, 1965); catechins (Cat.) by the Swain–Hillis (1959) method and proanthocyanidins (Pro.) by the method of Ribéreau-Gayon and Stonestreet (1966).

### 2.4. Chemical standards

The standards used were of the highest purity commercially available. (+) Catechin was from Aldrich Chimie (Germany); procyanidins  $B_1$  and  $B_2$  were from Extrasynthèse (France), procyanidin  $B_3$  from Sigma (Spain) and three trimers ( $T_2$ ,  $T_3$  and  $C_1$ ) were previously isolated and identified from grape seeds, in our laboratory (Pérez-Illzarbe, Martínez, Hernández, & Estrella, 1992).

### 2.5. HPLC–PAD analysis

The chromatographic system was provided by a 996 photodiode-array detector (Waters, Milford MA, USA). The column was a Nova Pack  $C_{18}$  (300 $\times$ 3.9 mm i.d., 4  $\mu$ m). The solvents used were, solvent A: water/ acetic acid (98:2) and solvent B: water/acetonitrile/ acetic acid (78:20:2). Phenolic compounds were eluted by a gradient as follows: from 0 to 55 min, 100 to 20% A; from 55 to 70 min, 20 to 10% A; from 70 to 80 min, 10 to 5% A and from 80 to 90 min, 5 to 0% A. The flow rates used were, 1.1 ml/min (0–55 min) and 1.2 ml/min (55–90 min). The injection volume was 10  $\mu$ l. Scanning from 210 to 360 was used for detection. A Waters-Millennium (2010) station was used for data collection and manipulation. The samples were analysed in triplicate.

## 2.6. HPLC–MS analysis

Mass spectra were obtained using a Hewlett Packard 1100MS (Palo Alto, CA) chromatograph equipped with PAD and MS detectors. A gradient of solvent A (water/acetic acid, 98:2, v/v) and solvent B (water acetonitrile/acetic acid, 78:20:22, v/v/v) was applied to a reversed phase Nova-pack C<sub>18</sub> (3.9×150 mm, 4 μm) as follows: 80% B from 0 to 55 min, 90% B from 57 to 70 min, 95% B from 70 to 80 min, 100% B from 80 to 90 min; the flow rate was 0.7 ml/min. ES conditions were as follows: negative mode, nitrogen was used for the nebulizing pressure, 40 psi, drying gas, 10 l/min at 340 °C; voltage at capillary entrance, 4000 V; variable fragmentator voltage, 80 V ( $m/z < 200$ ), 200 V ( $m/z 200–1000$ ), 200 V ( $m/z 1000–3000$ ). Mass spectra were recorded from  $m/z$  100 to 3000.

## 2.7. Identification and quantification of the compounds

Identification was achieved by comparing retention times, UV spectra and data of UV spectral parameters (Bartolomé, Hernández, Bengoechea, Quesada, Gómez-Cordovés, & Estrella, 1996), with those of standards, or those of previously purified and identified procyanidins (Bartolomé et al., 1993; Pérez-Illarbe et al., 1992), recorded under the same chromatographic conditions. The dimers and trimers of procyanidins have been identified by HPLC–MS.

Quantitative determinations were made using the external standard method with commercial standards. The dimers and trimers of procyanidins, were quantified as (+)-catechin. The calibration curves were obtained by injection of different volumes of standard solutions under the same conditions as for the samples analysed.

## 2.8. Antioxidant activity

In the methanol solution of the three samples, M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub>, the antioxidant activity (IC<sub>50</sub>), as free radical scavenging activity, was determined by the Brand-Williams, Cavelier, and Berset (1995), with 2,2'-diphenyl-1-picrylhydrazyl (DPPH). The percentage of remaining DPPH against the sample concentration was plotted to obtain the amount of antioxidant (μg) necessary to decrease free radicals by 50%. A smaller IC<sub>50</sub> value corresponds to a higher antioxidant activity.

The chromatographic peaks from M<sub>1</sub> were collected individually from the column in several injections of this sample. The individually collected peaks were concentrated in a vacuum and the residue was dissolved with methanol/water (1:1 v/v). In each collected peak, free radical scavenging capacity was determined.

The antioxidant activities of the standard solutions of (+)-catechin and (–)-epicatechin were also measured.

## 2.9. Statistical analysis

All data were expressed as means±standard deviations of the mean of the three determinations. Analysis of variance was applied to the data using Statgraphic Statistical Graphics 4.0 System Software for Windows.

## 3. Results and discussion

Table 1 shows the results obtained in the quantitative study of different groups of phenolic compounds in the methanol solution of M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub>, and their antioxidant activities. The three samples were rich in phenolic compounds, mainly catechins and proanthocyanidins; thus, the percentage of total polyphenols (PT) was similar in the three samples and lower than the corresponding catechins (Cat) and proanthocyanidins (Pro). The sample M<sub>1</sub> was the richest, also presenting the highest antioxidant activity, while the M<sub>2</sub> and M<sub>3</sub> samples showed no significant difference ( $P < 0.05$ ). As had been expected, the seeds of the mocan were very rich in flavan-3-ols, as found in the seeds of grape (Castillo et al., 2000; Fernández de Simón et al., 1993; Peng et al., 2001; Pérez-Illarbe et al., 1992). The differences in the concentrations of phenolic compounds in M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub> could be due to the different geographical locations of the three samples.

These results agree with those found for the whole berry of “mocan” (Hernández-Pérez et al., 1996), in which the total polyphenol content was lower than catechin and proanthocyanidin concentrations, and these were at similar concentrations. From these data it seems that the seeds of the mocan furnish the largest part of the flavan-3-ols found in the berry.

From the analysis by HPLC–PAD, the phenolic compounds identified in the mocan seeds are mainly procyanidins and (+)-catechin (Fig. 1), and only traces of (–)-epicatechin have been found. Among procyanidins we identified B<sub>3</sub> as catechin (4α→8)-catechin, B<sub>1</sub> as epicatechin (4β→8)-catechin, B<sub>2</sub> as epicatechin (4β→8)-epicatechin, and among the trimers, T<sub>2</sub> as epicatechin

Table 1  
Quantification of the phenolic compounds and antioxidant activity of “mocan” seed samples

Samples	PT	Cat.	Pro.	IC <sub>50</sub>
M <sub>1</sub>	99.0±5.19b	176±9.31b	131±7.79b	0.03±0.00a
M <sub>2</sub>	36.2±3.24a	49.3±5.50a	58.0±5.81a	0.10±0.03b
M <sub>3</sub>	41.8±2.14a	61.6±8.79a	64.5±6.09a	0.11±0.01b

PT: Total Polyphenols (mg gallic acid/g sample); Cat: Catechin (mg (+)-catechin/g sample); Pro: Procyanidins (mg cyanidin chloride/g sample); IC<sub>50</sub>: Antioxidant activity (μg sample needed to decrease free radicals by 50%). Values are means±S.D. of three independent determinations. Means followed by different letters in each row are significantly ( $P < 0.05$ ) different from one another.

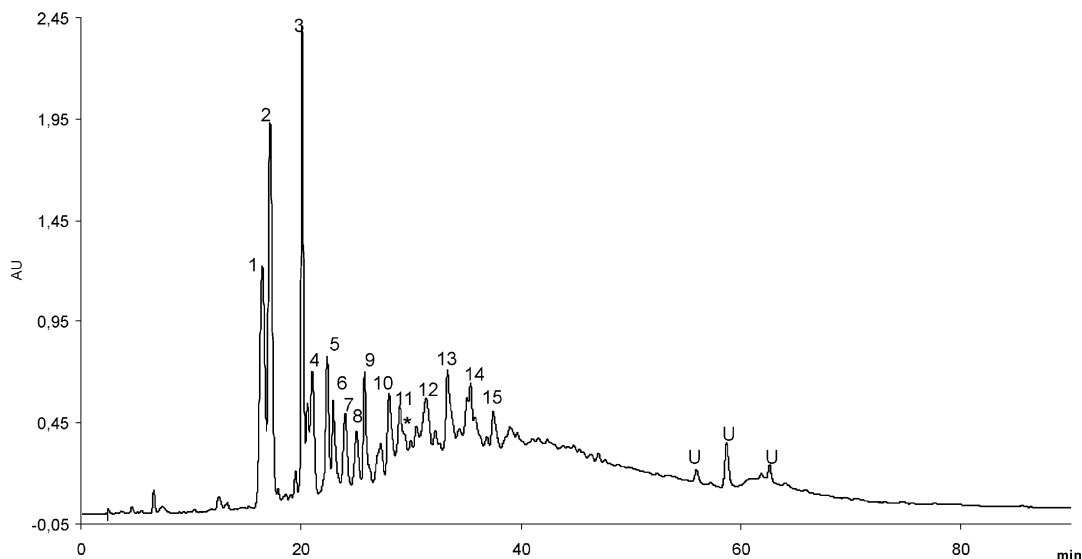


Fig. 1. Chromatogram at 280 nm of the mocan seeds ( $M_1$ ). 1:  $B_3$  [catechin ( $4\alpha\rightarrow 8$ )-catechin]; 2:  $B_1$  [epicatechin ( $4\beta\rightarrow 8$ )-catechin]; 3: (+)-catechin; 4:  $T_2$  [epicatechin ( $4\beta\rightarrow 8$ )-epicatechin ( $4\beta\rightarrow 8$ )-catechin]; 5: procyanidin trimer; 6:  $T_3$  [epicatechin ( $4\beta\rightarrow 6$ )-epicatechin ( $4\beta\rightarrow 8$ )-catechin]; 7: procyanidin dimer; 8:  $C_1$ ; 9:  $B_2$  [epicatechin ( $4\alpha\rightarrow 8$ )-epicatechin]; 10–15: procyanidin trimers; U: unknown; \*: (–)-epicatechin.

( $4\beta\rightarrow 8$ )-epicatechin ( $4\beta\rightarrow 8$ )-catechin,  $T_3$  as epicatechin ( $4\beta\rightarrow 6$ )-epicatechin ( $4\beta\rightarrow 8$ ) catechin and  $C_1$  as epicatechin ( $4\beta\rightarrow 8$ )-epicatechin ( $4\beta\rightarrow 8$ )-epicatechin. These compounds were identified by comparison of their retention times, UV spectra and data of UV spectral parameters with those of standards and those of procyanidins previously purified, by HPLC–PAD, and by the spectral mass (HPLC–MS). The trimers are named according to [Cheyner and Ricardo da Silva \(1991\)](#).

Peak 7 was identified as a procyanidin dimer by UV spectra and data of UV spectrum parameters (HPLC–PAD). In the analysis by HPLC–MS, the main ion observed, in the negative ion mode, was  $[M-H]^-$  at  $m/z$  577, corresponding to a procyanidin dimer. This peak could correspond to procyanidin  $B_6$ , catechin ( $4\alpha\rightarrow 6$ )-catechin, according to the results of [Escribano-Bailon, Gutierrez-Fernández, Rivas-Gonzalo, and Santos-Buelga \(1992\)](#).

Peaks 5, 10, 11, 12, 13, 14 and 15 were identified as trimers of procyanidins by their UV spectra and data of UV spectra parameters (HPLC–PAD). In the analysis by HPLC–MS, the main ions observed in the negative ion mode were  $[M-H]^-$  at  $m/z$  865, corresponding to procyanidin trimers. The trimer (peak 5), also agrees with the results of [Escribano-Bailon et al. \(1992\)](#), and may correspond to catechin ( $4\alpha\rightarrow 8$ )-catechin ( $4\alpha\rightarrow 8$ )-epicatechin

The phenolics of mocan seeds are thus mainly flavan-3-ols, as monomers or oligomers, showing a great presence of (+)-catechin in the three samples and high concentrations of dimers and some trimers (Table 2). These results are in concordance with the data for total phenolic compounds (Table 1).  $M_1$  is the richest in phenolic compounds, while  $M_2$  and  $M_3$  present lower

and similar concentrations. The dimers  $B_1$  and  $B_3$  together with the monomer (+)-catechin are the most abundant flavan-3-ols in the three samples. (–)-Epicatechin was not found. Though the samples analysed showed similar percentages of the different compounds (data not shown), there were few differences in their concentration.

The peaks which correspond to identified compounds (from 1 to 15) in the sample  $M_1$  were collected separately during the development of the chromatogram, in order to evaluate their antioxidant activities. Based on the similar compositions of the samples, only sample  $M_1$  was been used in this analysis. In this way it was possible to evaluate the antioxidant activity of each of the most abundant flavan-3-ols found in the mocan seeds and to establish the possible relationship between their structures and their antioxidant activities. Peaks 10–15 (Fig. 1) identified as trimers, were been collected together, due to the difficulty of separating them.

Table 3 shows the  $IC_{50}$  values for the different peaks. Among procyanidin dimers,  $B_2$  [epicatechin ( $4\beta\rightarrow 8$ )-epicatechin], showed the highest antioxidant activity, followed by  $B_1$  [epicatechin ( $4\beta\rightarrow 8$ )-catechin],  $B_6$  [catechin ( $4\alpha\rightarrow 6$ )-catechin], and  $B_3$  [catechin ( $4\alpha\rightarrow 8$ )-catechin]. In the case of trimers,  $C_1$  [epicatechin ( $4\beta\rightarrow 6$ )-epicatechin ( $4\beta\rightarrow 8$ )-epicatechin] had the greatest antioxidant activity, followed by peak 5 [catechin ( $4\alpha\rightarrow 8$ )-catechin ( $4\alpha\rightarrow 8$ )-epicatechin],  $T_3$  [epicatechin ( $4\beta\rightarrow 6$ )-epicatechin ( $4\beta\rightarrow 8$ )-atechin] and  $T_2$  [epicatechin ( $4\beta\rightarrow 8$ )-epicatechin ( $4\beta\rightarrow 8$ )-catechin]. The trimers collected together also had a high antioxidant activity.

The evaluation of the antioxidant activity of the standards solution of (+)-catechin and (–)-epicatechin (Table 3), showed behaviour corresponding to the

Table 2  
Concentration (mg/g) and percentage (%) of procyanidins in *Visnea mocanera* fruit seeds

Peaks <sup>a</sup>	Compounds	M1	% M1	M2	% M2	M3	% M3
1	B <sub>3</sub>	0.337±0.017c	13.6	0.127±0.021a	17.2	0.193±0.014b	14.9
2	B <sub>1</sub>	0.471±0.031b	19.1	0.156±0.040a	21.0	0.257±0.038a	19.8
3	(+) Catechin	0.346±0.048b	14.0	0.082±0.006a	11.1	0.239±0.033b	18.4
4	T <sub>2</sub>	0.099±0.016b	4.0	0.036±0.006a	4.9	0.047±0.007a	3.6
5	Trimer	0.211±0.017c	8.5	0.041±0.008a	5.6	0.128±0.008b	9.9
6	T <sub>3</sub>	0.084±0.010b	3.4	0.018±0.004a	2.5	0.039±0.006a	3.0
7	Dimer	0.101±0.003b	4.1	0.033±0.006a	4.5	0.038±0.014a	2.9
8	C <sub>1</sub>	0.114±0.016b	4.6	0.041±0.003a	5.6	0.050±0.007a	3.8
9	B <sub>2</sub>	0.201±0.006b	8.1	0.060±0.004a	8.1	0.070±0.011a	5.4
10	Trimer	0.115±0.017b	4.7	0.036±0.014a	4.8	0.044±0.006a	3.4
11	Trimer	0.089±0.014b	3.6	0.018±0.004a	2.4	0.042±0.004a	3.2
12	Trimer	0.092±0.010b	3.7	0.019±0.006a	2.6	0.034±0.007a	2.6
13	Trimer	0.133±0.010b	5.4	0.044±0.013a	5.9	0.066±0.010a	5.1
14	Trimer	0.020±0.007a	0.8	0.010±0.003a	1.3	0.013±0.004a	1.0
15	Trimer	0.058±0.013b	2.4	0.019±0.007a	2.5	0.040±0.007ab	3.0

Values are means ± S.D. of three independent determinations. Means followed by different letters in each column are significantly ( $P < 0.05$ ) different from one another.

<sup>a</sup> The number of the peaks corresponds to that of the chromatogram.

Table 3  
Antioxidant activity (IC<sub>50</sub> as µg of compounds) of procyanidins from *Visnea mocanera* fruit seeds

Peaks <sup>a</sup>	Compounds	IC <sub>50</sub>
1	B <sub>3</sub> [catechin (4 $\alpha$ →8)-catechin],	4.47
2	B <sub>1</sub> [epicatechin (4 $\beta$ →8)-catechin]	3.70
3	(+) Catechin	7.15
4	T <sub>2</sub> [epicatechin (4 $\beta$ →8)-epicatechin (4 $\beta$ →8)-catechin]	1.93
5	Trimer [catechin (4 $\alpha$ →8)-catechin (4 $\alpha$ →8)-epicatechin]	1.03
6	T <sub>3</sub> [epicatechin (4 $\beta$ →6)-epicatechin (4 $\beta$ →8) catechin]	1.32
7	Dimer (B <sub>6</sub> ) [catechin (4 $\alpha$ →6)-catechin]	4.30
8	C <sub>1</sub> [epicatechin (4 $\beta$ →6)-epicatechin (4 $\beta$ →8)-epicatechin].	0.35
9	B <sub>2</sub> [epicatechin (4 $\beta$ →8)-epicatechin]	1.74
10–15	Trimers	0.83
	Standard (+)-Catechin	6.29
	Standard (-) Epicatechin	5.63

<sup>a</sup> The number of the peaks corresponds to that of the chromatogram.

results of Lagune, Vivas, and Glories (1996) and Saint-Cricq de Gaulejac et al. (1999), the antioxidant activity of (-)-epicatechin being stronger than (+)-catechin. The same authors explain that the stereochemical position of the C-3 in the (+)-catechin (2R: 3S) and (-)-epicatechin (2R:3R) affects the antioxidant activity, concluding that the hydroxyl group in C-3 is involved in the scavenging of free radicals.

The antioxidant activity of procyanidin dimers B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>, increases in relation to the function of the number of (-)-epicatechin units and also depends on the position of the monomers in the procyanidin molecule. B<sub>2</sub> has a higher antioxidant activity because the structure presents two units of (-)-epicatechin; however B<sub>3</sub>, that shows a smaller value, contains two units of (+)-catechins. Procyanidin B<sub>1</sub>, with one (+)-catechin and one (-)-epicatechin, shows an intermediary value of antioxidant activity. The structure assigned to peak 7

(B<sub>6</sub>) [catechin (4 $\alpha$ →6)-catechin], tallies with the antioxidant activity value, which is similar to that of B<sub>3</sub> [catechin (4 $\alpha$ →8)-catechin] (Table 3).

The scavenging action is related to this different structural conformation, according to De Freitas, Glories, and Laguerre (1996). Thus, not only the structures of the units of proanthocyanidin oligomers, (+) catechin or (-) epicatechin, influence their antioxidant activity, but also the position of the units in the molecule, and the nature of linkage and the concentration (Saint-Cricq de Gaulejac et al., 1999). The interflavan linkage C4–C6 seems to be more efficient in the radical scavenging activity than the linkage C4–C8.

Among the different identified trimers, C<sub>1</sub> [epicatechin (4 $\beta$ →6)-epicatechin (4 $\beta$ →8)-epicatechin] has the highest antioxidant activity, followed by the fraction containing several trimers, and the lowest are T<sub>3</sub> [epicatechin (4 $\beta$ →6)-epicatechin (4 $\beta$ →8)-catechin] and T<sub>2</sub> [epicatechin (4 $\beta$ →8)-epicatechin (4 $\beta$ →8)-catechin]. The values obtained for these trimers correspond to the assigned structures. In this case it is also observed that the (-)-epicatechin units influence the antioxidant activity as also occurs in the dimers. The C<sub>1</sub> presents three units of (-)-epicatechin whereas the rest of the identified trimers have one or two units of (-)-epicatechin. The highest antioxidant activity, of peaks 10–15, could be explained by a synergism effect between these compounds

The degree of polymerisation of the procyanidins is also related to their scavenging potential for free radicals. Table 3 shows that the procyanidin trimers present a higher antioxidant activity value than the dimers. The protective effect of catechin and procyanidins against the free radical action shows some dependence on the degree of polymerization, being more efficient in the

trimers than the monomers. These results agree with those of Saint-Cricq de Gaulejac et al. (1999), studying the scavenging activity of procyanidins in different fractions of grape seed extract, and also with that of Plumb, De Pascual Teresa, Santos-Buelga, Cheynier, and Williamson (1998), about the effect of the degree of polymerisation on the antioxidant activity, which increases from monomer to trimer.

On the other hand, the relationship between the total antioxidant activity of the extract and the compositional data shows that matrix effects and other synergistic interactions among components are important.

The seeds of *Visnea mocanera* fruits are an important source of catechin and oligomers of flavan-3-ols, compounds responsible for the biological properties of these fruits and to the different preparations from them.

The high antioxidant activity of the mocan seeds, and their apparent lack of toxicity increases the potential interest in these fruits for improving the efficacy of different products as nutraceutical and pharmacological agents. The consumption of *Visnea mocanera* fruits may have a role in prevention of human diseases in which free radicals are involved.

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