Phenolic Composition of the "Mocán" (*Visnea mocanera* L.f.)

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The leaves and fruits of the "mocán" (*Visnea mocanera* L.f.) have been analyzed by TLC and HPLC to establish their phenolic composition. The fruits are richer than the leaves in phenolics with 4% procyanidins, 4% catechins, 3% total polyphenols, 0.6% low-polymerization polyphenols, and 0.1% anthocyanins. Benzoic acids (*p*-hydroxybenzoic, protocatechuic, and gallic), benzoic aldehydes (*p*-hydroxybenzoic, vanillic, and syringic), cinnamic acids (*p*-coumaric and ferulic), 3-flavanols [(+)-catechin, (−)-epicatechin, and procyanidins], flavonols (quercetin, myricetin, and kaempferol) and their glycosides (isorhamnetin 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, quercetin 3-*O*-rhamnoside, and quercetin 3-*O*-galactoside), and anthocyanins (glycosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin) have been identified. The presence of these families of compounds in mocán could account for the antimicrobial, antiinflammatory, analgesic, antiulcerogenic, hemostatic, astringent, cicatrizant, vulnerary, and psychostimulant activities found in previous studies.

Keywords: *Visnea mocanera; fruits; phenolic compounds*

INTRODUCTION

Visnea mocanera L.f. (Theaceae), known locally as "mocán" or "mocanera", is a native tree of the Macaronesian region used by the canary natives (named guanches) since pre-Hispanic times due to its medicinal and nutritive properties.

The "yoyas" (mocan fruits), small red-blackish berries when fully ripe and the size of a cherry, were consumed fresh by the aboriginal population and were the basis of their diet (Viera y Clavijo, 1942; Navarros Mederos and Del Arco Aguilar, 1987). In the same way, these fruits were used for preparing a psychostimulant drink (mocan wine) and a syrup named "chacerquén" or "chacherquen", which was employed as an analgesic, antiinflammatory, stomachic, antiemetic, antihemoptisic, cicatrizant, antiulcerogenic, vulnerary, and antidiarrhetic agent (Berthelot, 1846; Darias *et al*., 1986, 1989; García Morales, 1987, 1989). The leaves were also used to heal scars and as an antiulcerogenic agent (Darias *et al*., 1989).

Some of these medicinal properties such as scar healing, vulnerary, analgesic, antiinflammatory, intestinal astringent, hemostatic, and psychostimulant (anxiogenic nature) properties have been proven experimentally with *in vivo* and *in vitro* pharmacological techniques (Hernández-Pérez *et al*., 1994a,b, 1995a,b).

At the same time and as a consequence of the frequent use of these fruits by the old island colonists, a proximate nutritive composition study was carried out showing that they are rich in soluble carbohydrates (mainly glucose and fructose), dietary fiber (mainly cellulose and lignine), and minerals (Hernández-Pérez *et al*., 1994c).

Whereas the compounds of low and medium polarity are well-characterized (Hernández-Pérez *et al*., 1994a), the fractions corresponding to compounds of high polarity (including phenolic substances) have not been well-defined chemically due to their complex structures and difficulties in their separation and identification by means of customary chromatographic and spectroscopic techniques.

These facts, together with the interesting pharmacological activities found in these fractions (intestinal astringent, hemostatic, analgesic, antiinflammatory, and psychostimulant) and the extensive use of the mocan fruits as food and medicine, have led us to quantify and characterize, mainly in fruits, the major phenolic compounds through chromatographic (TLC and HPLC) and spectrophotometric techniques.

MATERIALS AND METHODS

(1) Collection. The leaves and fruits (fully ripe) of *V. mocanera* L.f. were collected in July and September 1992 in Tenerife (Canary Islands, Spain). The plant material was dried in a stove at 40 °C with free circulation of air, pulverized, and stored in the absence of light and humidity and at a temperature below 25 °C, in the herbarium of the Facultad de Farmacia, Universidad de La Laguna, Tenerife.

(2) Extraction. All samples were processed in the same way; 100 mg of each sample was macerated and homogenized in 100 mL of methanol/hydrochloric acid (1000:1 v/v) with an M50 hand mill (Taurus) and during 1 min. Then, the sample was filtered through sintered glass no. 1 under vacuum. Part of the resulting liquid was used for the total quantitation, by spectrophotometric methods, of phenolic compounds as total polyphenols, low-polymerization polyphenols, catechins, procyanidins, or condensed tannins and anthocyanins, as well as for the identification of anthocyanins by TLC and HPLC (previously filtered through a Millipore membrane, 0.5 μm).

The other part was concentrated to dryness with a rotatory evaporator (35 °C) and the resulting residue dissolved in 25 mL of water/ethanol (80:20 v/v).

After dissolution, an extraction with ethyl acetate (3 × 20 mL) was made and the organic extracts were dried with anhydrous sodium sulfate and concentrated to dryness. The resulting residue was redissolved in 2 mL of methanol/water (1:1 v/v). Analysis of the methanol solution was by TLC and HPLC for procyanidins, flavonols, and low-molecular-weight phenols.

(3) Total Quantitation of the Phenolic Compound Family. Total polyphenols were quantitated by the Folin−

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Ciocalteu method (Singleton and Rosi, 1965), low-polymerization polyphenols by the method of Masquelier *et al*. (1965), catechins by the Swain−Hillis method (1959), procyanidins by the Ribéreau-Gayen−Stonestreet method (1966), and total anthocyanins by Paronetto's method (1977).

(4) TLC Analysis. *Analysis of Procyanidins (Lea et al., 1979).* Monodimensional chromatography silica gel plates were run in the solvent toluene/acetone/formic acid (60:20:20 v/v/v); two-dimensional cellulose TLC was carried out on purchased MN300 sheets in the solvent systems isoamyl alcohol/acetic acid/water (50:25:25 v/v/v) and acetic acid/water (2:88 v/v). Spots were visualized by spraying with chlorhydric vanillin (1% vanillin in 70% hydrochloric acid) reagent.

Analysis of Flavonols (Marckham, 1982). The adsorbent was cellulose MN300. Method 1 solvents were BAW [upper phase of *n*-butyl alcohol/acetic acid/water (4:1:5 v/v/v)] and 15% acetic acid. The method 2 (monodimensional) solvent was Forestal [acetic acid/water/hydrochloric acid (30:10:3 v/v/v)] in plates previously washed with 15% acetic acid. Detection of the spots was made by UV light, before and after saturation with ammonia vapor, and spraying with Neu's reagent (Neu, 1957) and UV light.

Analysis of Low-Molecular-Weight Phenolics and 3-Flavanols (Gómez-Cordovéz et al., 1978; Diez de Bethencourt et al., 1980). The adsorbent was cellulose MN300. Method 1 solvents were 2% formic acid and isopropyl alcohol/ammonium hydroxide/water (8:1:1 v/v/v). Method 2 solvents were 2% acetic acid and 20% potassium chloride. Detection of the spots was made by UV light at 254 and 260 nm, before and after saturation with ammonia vapor, and spraying with 25% lead(II) acetate. Other spraying reagents were diazotized *p*-nitroaniline (mixture in 2:8 v/v proportions of 0.5% *p*-nitroaniline in 2 N HCl and 20% sodium acetate, plus three drops of 0.5% sodium nitrite) and the 15% sodium carbonate, chlorhydric vanillin, and sulfuric catechin [0.4% catechin in acetone/water/sulfuric acid (50:37.5:12.5 v/v/v)].

Analysis of Anthocyanins (Andersen and Francis, 1985). Two-dimensional chromatography with cellulose MN300 was carried out in the solvents water/acetic acid/hydrochloric acid (75:22:3 v/v/v) and *n*-amyl alcohol/acetic acid/water (20:10:10 v/v/v). Detection of the spots was made by visible light.

(5) HPLC Analysis. The equipment was from Waters Associates and consisted of a U6K universal injector, a 6000E pump system controller, and a 991 photodiode array detector, for the analysis of low-molecular-weight phenolic compounds and flavonols. On the other hand, in the analysis of anthocyanins, two pumps (M-510 and M-45) and a model 440 UV−vis detector were used; these also came from Waters Associates.

Analysis of Low-Molecular-Weight Phenolics and 3-Flavanols (Fernández de Simón et al., 1990). A stainless steel C_{18} Nova-Pack column (300 × 3.9 mm) was used. Solvent A was 2% acetic acid and solvent B methanol/acetic acid/water (30:2:68 v/v/v). Detection was performed simultaneously at 280 and 340 nm. The gradient was as follows:

time (min)	flow (mL/min)	% A	% B
0	1.0	100	0
55	1.0	20	80
57	1.2	10	90
70	1.2	10	90

gradient curve no. 5

Analysis of Flavonol Aglycons (Fernández de Simón et al., 1990). A stainless steel C_{18} Nova-Pack column (150 × 3.9 mm) was used. The solvent used was water/methanol/acetic acid (57.5:37.5:5 v/v/v), and the flow rate was 0.7 mL/min. Detection was at 360 and 254 nm.

Analysis of Flavonol Glycosides (Fernández de Simón et al., 1990). A stainless steel C_{18} Nova-Pack column (150 × 3.9 mm) was used. The solvent was a mixture, in 65:35 (v/v) proportions, of 2.5% acetic acid and tetrahydrofuran/water/acetic acid (50:47.5:2.5 v/v/v). The flow rate was 0.7 mL/min, and detection was at 365 and 254 nm.

Table 1. Quantitation in Milligrams per Gram of Dry Sample of the Polyphenolic Compounds' Family from *V. mocanera* L.f.

	procyanidins	catechins	anthocyanins	total polyphenols	low-polymerization polyphenols
leaves	3.07	0.10	0.07	22.71	7.13
fruits	39.86	40.91	1.26	30.80	6.38

Analysis of Anthocyanins (González-San José et al., 1988). A stainless steel C_{18} Nova-Pack column (5 μm) was used. The solvents used were acetic acid/water (10:90 v/v) (A) and acetic acid/methanol/water (10:45:45 v/v) (B), and the flow rate was 1.0 mL/min. The detection was at 546 nm (visible) and 313 nm (UV). The gradient was linear, from 25 to 80% solvent B in 30 min, followed by 10 min of isocratic treatment in 80% solvent B. The sensitivity was 0.2 AUFS.

(6) Identification of the Compounds. Identification of the compounds by HPLC analysis was achieved by cochromatography with authentic standards from Fluka, Aldrich, and Sarsynthèse, for polymers of procyanidins with authentic samples from grape seeds of *Vitis vinifera* var. *Airen*, comparing the retention times and UV absorbance ratios with those of the standards. Identification for procyanidins and related compounds was made by comparison of the spectral parameters obtained using photodiode-array detection (Bartolomé *et al*., 1996) with those of the standards. In TLC analysis, identification was carried out by comparison of R_f values and colors given with the spraying reagents and under UV light with those of the standards.

RESULTS AND DISCUSSION

(1) Total Quantitation of Phenolic Compounds. The results obtained in the quantitative study of the phenolic compounds, in leaves and fruits of *V. mocanera* L.f., are shown in Table 1. They indicate that the fruits are richer than leaves in these types of compounds with 4% procyanidins, 4% catechins, and 3% total polyphenols, the lowest content being in anthocyanins (0.1%) and polyphenols of low polymerization (0.6%).

(2) Identification of the Compounds by TLC and HPLC. The mocan fruits are rich in phenolic compounds, most of which were procyanidins or condensed tannins. This particular analysis of these compounds by TLC and HPLC allowed the identification of monomers as (+)-catechin, (−)-epicatechin, and polymers with different numbers of units. Using the method of Bartolomé *et al*. (1996), based on the study of spectral parameters for analysis of procyanidins by HPLC, the presence of dimers, trimers, and tetramers of (+)-catechin and (−)-epicatechin could be detected. The existence of gallocatechins was also detected (Figure 1).

In the analysis of flavonols, the mocan fruits showed the presence of both free and glycoside substances identifying the aglycons quercetin, myricetin, and kaempferol and the mono- and diglycosides isorhamnetin 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, quercetin 3-*O*-rutinoside, quercetin 3-*O*-rhamnoside, and quercetin 3-*O*-galactoside, the majority of compounds being quercetin and their glycosides. Other minor glycosides still unidentified were also detected.

On the other hand, when the content in this type of compound was studied in the leaves, the chromatogram showed a very high complexity, and the compounds have not yet been identified.

With respect to low-molecular-weight phenols, three benzoic acids (*p*-hydroxybenzoic, protocatechuic, and gallic), three benzoic aldehydes (*p*-hydroxybenzoic, vanillic, and syringic), and the cinnamic acids *p*-coumaric and ferulic have been identified in leaves and fruits.

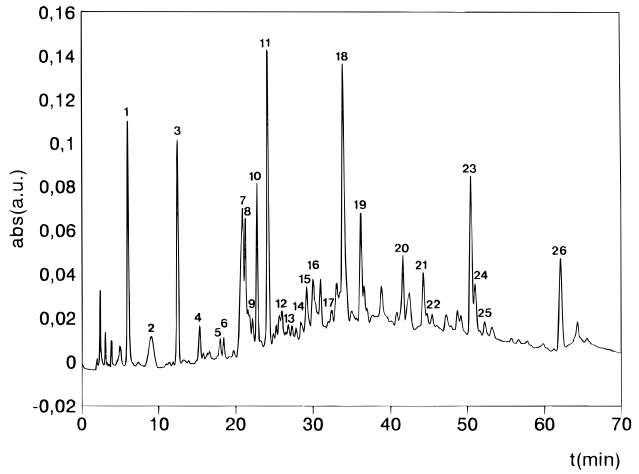

Figure 1. Chromatogram of low molecular weight phenols and 3-flavonols (280 nm) from mocán fruits: (1) gallic acid, (2) procyanidin trimer, (3) protocatechuic acid, (4, 9, 12, 14, 15, and 18) gallocatechins, (5) caffeic acid derivative, (6) protocatechuic aldehyde, (7, 8, and 13) procyanidin dimers, (10, 17, and 22) *p*-coumaric derivatives, (11) catechin, (16) *p*-hydroxybenzoic aldehyde, (19) *p*-coumaric acid, (20, 21, and 23−25) flavonol glycosides, and (26) chalcone glycoside.

The identification of anthocyanin compounds in fruits gave as a result the presence of glycosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, the majority of compounds being glycosides of cyanidin (cyanidin 3-glucoside and cyanidin 3-galactoside) (Figure 2). There is also a high proportion of diglycosides and polymers of anthocyanins still unidentified under the chromatographic conditions used. The anthocyanin fraction from fruit is very complex, indicating a very developed fruit, similar to wine grape seeds. This fact was proven making an acid hydrolysis (breaking of glycosidic bonds in position 3) and afterward analyzing the hydrolyzed fraction by HPLC. The result was a more complex chromatogram than initially supposed, detecting further to aglycons from glycosides, with other glycosides unidentified for the moment.

On the other hand, the presence of anthocyanins in leaves is low or null.

Unluckily, the complexity of the chromatograms of flavonols extracted from the leaves, and of the diglycosides and polymers of anthocyanins extracted from the fruit, has not allowed us to determine their precise composition under the working conditions used. A more detailed study is now being carried out.

In view of the results obtained, we can say that mocan fruits are rich in polyphenol compounds, having found a large variety of these as benzoic and cinnamic acids, aglycons and glycosides of quercetin, glycosides of cyanidin, and compounds more or less polymerized as procyanidins and polymers of anthocyanins.

The presence of these substances is important because they contribute to the sensory quality of the fruits, such as color, astringency, bitterness, and flavor, besides giving body to the mocan wine which was very appreciated by the forebears the guanches. It is worthwhile to mention the fixed stains that these fruits show, not being removed with habitual detergent substances; this aspect could be useful in the food and textile industry as a new source of coloring substances.

In the same way, some of these compounds also have important pharmacological properties (Huang *et al.*, 1992; Rider *et al.*, 1992; Bruneton, 1993). In this sense, we can say that the presence of procyanidins or condensed tannins could justify the intestinal astringent and hemostatic activities found in the fraction corresponding to these substances (Hernández-Pérez *et al.*, 1995a) and, thus, the presence of phenolic acids and flavonoids in the antimicrobial, peripheral analgesic, antiinflammatory, and slightly antipyretic activities which are found (Hernández-Pérez *et al.*, 1994a,b).

Figure 2. Chromatogram of anthocyanidins from mocán fruits: (1) delphinidin 3-glucoside, (2) cyanidin 3-galactoside, (3) cyanidin 3-glucoside, (4) petunidin 3-glucoside, (5) peonidin 3-glucoside, and (6) malvidin 3-glucoside.

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