

LOW MOLECULAR WEIGHT POLYPHENOLS IN WOOD AND BARK OF *EUCALYPTUS GLOBULUS*

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ABSTRACT

Polyphenols (mainly flavonoids and phenolic acids and aldehydes) were studied in wood and bark samples of *Eucalyptus globulus*. Gallic and ellagic acids, vanillin, syringaldehyde, sinapaldehyde, and quercetin were identified in wood; and gallic, protocatechuic, vanillic and ellagic acids, protocatechuic aldehyde, taxifolin, eriodictyol, quercetin, and naringenin in bark. Ellagitannins and some unidentified flavonols were also detected in both samples.

Keywords: Wood, bark, *Eucalyptus globulus*, polyphenols, flavonoids, phenolic acids, phenolic aldehydes.

INTRODUCTION

The information on minor components, and especially on polyphenols, in wood and bark of the genus *Eucalyptus* is spare. Cyanidin and delphinidin glycosides were identified in bark of *E. globulus* (Sharma and Crowden 1974). Catechin, gallic acid, chlorogenic and ellagic acids, and glycosides of 3-O-methyl ellagic acid were also detected in wood and bark of this species (Yazaki and Hillis 1976). In a recent study, ellagic and gallic acids contents were quantified in extracts of wood of *E. globulus* (Charrier et al. 1992).

The aim of this study is to contribute to knowledge about the composition of low molecular weight polyphenols of wood and bark of *E. globulus*, with emphasis on the analysis of ether-extractable flavonoids and phenolic acids and aldehydes.

MATERIAL AND METHODS

Samples

Wood from a branch of about 15-cm diameter (mainly composed of sapwood) and stem outer bark were collected from a 24-year-old *E. globulus* tree, grown in "Bodegones"

TABLE 1. Calibration functions obtained from commercial standards.

Component	Concentration range (μM)	Equation $y = ax + b$	Correlation coefficient (r^2)
Protocatechuic acid	320-6490	$y = 0.55x - 105$	0.996
Protocatechuic aldehyde	140-7240	$y = 0.45x - 41$	0.996
Vanillin	60-140	$y = 0.33x - 63$	0.996
Gallic acid	110-3530	$y = 0.56x - 168$	0.991
Ellagic acid	5-3950	$y = 0.11x$	0.999
Quercetin	160-3310	$y = 0.16x - 208$	0.998
Naringenin	30-3680	$y = 0.33x - 60$	0.996
Eriodictyol	170-3470	$y = 0.44x - 17$	0.996
Taxifolin	30-3290	$y = 0.35x - 64$	0.990

arboretum, located in Southwestern Spain (Huelva).

Extraction

Samples were ground in a hammermill, using small quantities of sample and short cycles of grinding, in order to avoid heating. The ground samples were extracted with methanol-water (80:20), at room temperature for 24 h. The suspension was filtered, and methanol was removed by vacuum distillation. The aqueous solution (solution I) was then extracted with ethyl ether, in order to obtain a fraction with a high concentration of low molecular weight phenolics. The dried ether extract was redissolved in methanol and analyzed by HPLC and TLC.

Content of total phenols

In solution I, the content of total phenols was determined according to the method of Folin-Ciocalteu (Singleton and Rossi 1965), using quercetin as standard.

TABLE 2. Extraction yields and total phenols contents of wood and bark samples of *E. globulus*.

	Extraction MeOH-H ₂ O (80:20) %	Extraction Et ₂ O %	Total phenols mg/g *
Wood	3.4	0.21	9.4
Bark	7.8	0.83	32.4

* = expressed in quercetin.

TABLE 3. HPLC evaluation of ethyl ether extract components of *E. globulus* wood.

Num.	Component	Retention time (min)	Semi-quantitative evaluation	Quantitative evaluation	
				μM	ppm*
1	Gallic acid	3.50	+	54	2.5
2	Vanillin	10.22	+	13	0.5
3	Syringaldehyde	10.78	+		
4	Sinapaldehyde	13.80	++		
5	Ellagic acid	16.63	+++	433	36
6	Ellagitannin	18.14	++		
7	Flavonol	18.24	t		
8	Ellagitannin	19.18	+		
9	Ellagitannin	19.59	+		
10	Quercetin	20.63	t	t	t
11	Ellagitannin	21.01	+		
12	Ellagitannin	22.31	+		
13	Ellagitannin	25.22	+		

* = related to dried matter; t = traces.

Identification of polyphenols

Identifications were carried out by comparing the UV spectra and the chromatographic behavior (HPLC, TLC) of the unknown compounds with those of standards, and also with those reported in literature. Identification of gallic and ellagic acids, syringaldehyde, and vanillin was confirmed by GC-MS.

High pressure liquid chromatography (HPLC)

HPLC analysis was carried out in a chromatograph equipped with a diode array detector. The column used was a Hypersil ODS (200 \times 4 mm i.d.), protected with a precolumn of the same material. Two solvents were employed for elution—A: Methanol-H₃PO₄ (999:1), and B: H₂O-H₃PO₄ (999:1). The gradient profile was: 0-40 min, 20-100% A; 40-45 min, 100% A (isocratic). Flow rate was 1 ml/min, and the temperature of the chromatographic oven was 30 C. Detection was carried out at 325 nm with a bandwidth of 150 nm.

Thin layer chromatography (TLC)

Cellulose Sigmacell microcrystalline plates were used. Two-dimensional developments were carried out with *n*-butanol-acetic acid-H₂O (4:1:5, upper phase), for the first dimen-

TABLE 4. HPLC evaluation of ethyl ether extract components of *E. globulus* bark.

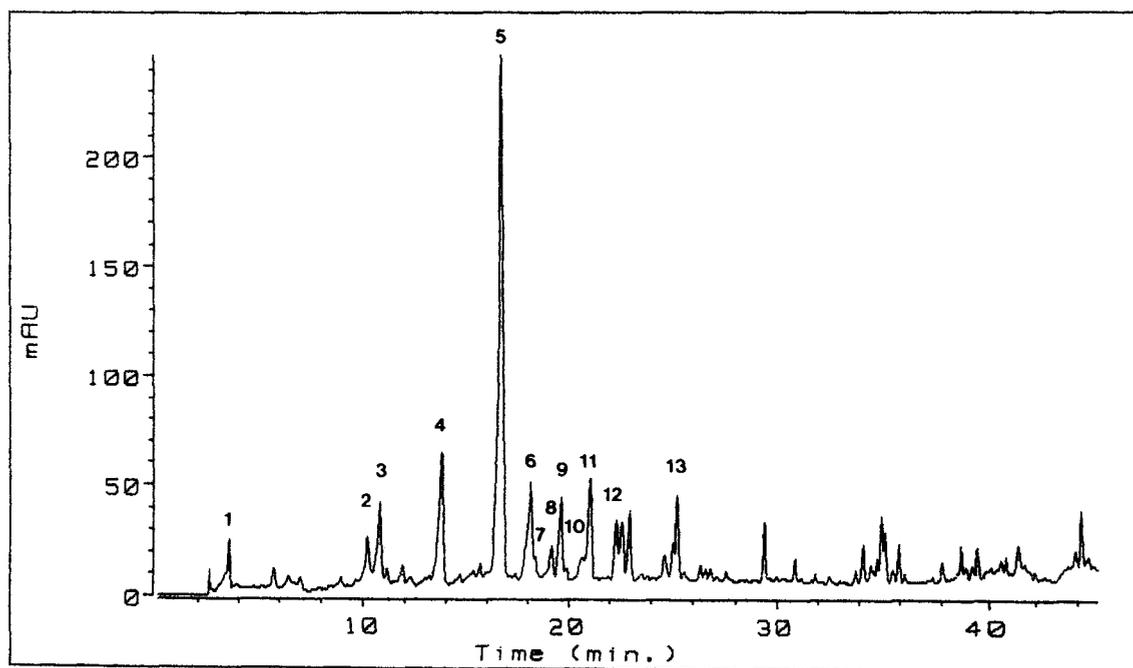
Num.	Component	Retention time (min)	Semiquantitative evaluation	Quantitative evaluation	
				μM	ppm*
1	Gallic acid	3.54	+	389	48
2	Protocatechuic acid	5.42	+	247	27
3	Protocatechuic aldehyde	6.65	+	60	8
4	Vanillic acid	9.04	+		
5	Taxifolin	12.40	+	159	35
6	Ellagic acid	16.57	++++	1123	243
7	Eriodictyol	18.03	+	526	109
8	Ellagitannin	19.12	+		
9	Ellagitannin	19.53	++++		
10	Qurecetin	20.65	++	75	16
11	Ellagitannin	21.02	++		
12	Naringenin	21.39	+	83	16
13	Flavonol	22.22	++		
14	Ellagitannin	22.59	+		
15	Ellagitannin	24.64	++		

* = related to dried matter.

sion, and 30% acetic acid for the second. For detection, a 0.6% solution of diphenylboric acid- β -aminoethylester (Reagent A) in methanol and a 2% solution of PEG (polyethyleneglycol) 1000 in methanol were used (Conde et al. 1992).

Gas chromatography/mass spectrometry (GC/MS)

A gas chromatograph fitted with EI-mass selective detector and a capillary column (methylsilicone, 12 m \times 0.22 mm i.d. 0, 33 μm film thickness) were used.

FIG. 1. HPLC chromatogram of the ether extract of wood. Detection was made at 325 ± 75 nm.

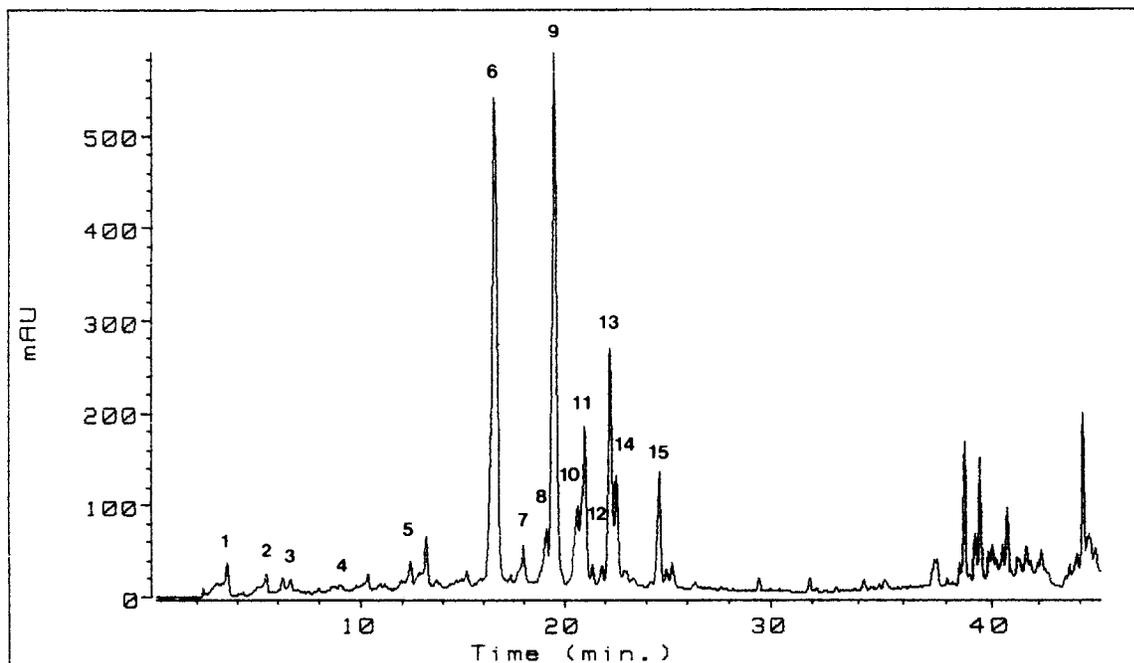


FIG. 2. HPLC chromatogram of the ether extract of bark. Detection was made at 325 ± 75 nm.

Semiquantitative and quantitative evaluation of polyphenolic compounds

Semiquantitative evaluation was carried out according to the areas of each chromatographic peak. Quantitative evaluation was made using the external standard method when commercial standards were available. Table 1 shows the calibration functions obtained from the commercial standards.

RESULTS AND DISCUSSION

Table 2 shows the extraction yields with MeOH-H₂O (80:20) and ethyl ether of wood and bark from a tree of *E. globulus* and the total phenols contents of MeOH-H₂O extracts. All these values are higher for bark than for wood. The greater total phenol content in bark is probably due to a high concentration of polymeric polyphenols, such as tannins.

The semiquantitative and quantitative HPLC evaluations of components of the ether-soluble fractions of wood and bark extracts are shown in Tables 3 and 4. The components are

numbered according to their elution from the HPLC column, as shown in Figs. 1 and 2, representing the HPLC chromatograms for wood and bark extracts, respectively.

Gallic and ellagic acids, vanillin, syringaldehyde, sinapaldehyde, and quercetin in wood; and gallic, protocatechuic, ellagic and vanillic acids, protocatechuic aldehyde, taxifolin, eriodictyol, quercetin, and naringenin in bark were identified by comparison of their UV spectra and chromatographic behavior (HPLC and TLC) with those of standards.

Compounds 6, 8, 9, 11–13 in wood, and 8, 9, 11, 14, and 15 in bark, were recognized as ellagitannins, because of the similarity of their UV spectra with that of ellagic acid (UV_{λmax} (methanol): 253, 365 nm). Compounds 7 in wood, and 13 in bark were recognized as flavonols, according to their UV spectra.

The major components in wood and bark extracts are ellagic acid and ellagitannins 6 in wood, and 9 in bark. The variety and quantity of flavonoids are greater in the bark.

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REFERENCES

- CHARRIER, B., M. MARQUES, AND J. HALUK. 1992. HPLC analysis of gallic and ellagic acids in European oakwood (*Quercus robur* L.) and eucalyptus (*Eucalyptus globulus*). *Holzforschung* 46:87–89.
- CONDE, E., E. CADAHÍA, AND M. C. GARCÍA-VALLEJO. 1992. Optimization of TLC for research on the flavonoids in wood and bark of the genus *Eucalyptus* L'Heritier. *Chromatographia* 33:418–426.
- SHARMA, D. S., AND R. K. CROWDEN. 1974. Anthocyanins in some *Eucalyptus* species. *Aust. J. Bot.* 22:623–627.
- SINGLETON, V. L., AND J. A. ROSSI. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16:144–158.
- YAZAKI, Y., AND W. E. HILLIS. 1976. Polyphenols of *Eucalyptus globulus*, *E. regnans* and *E. deglupta*. *Phytochemistry* 15:199–206.