# 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, gallocatechin, 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"

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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-$H_2O$ (80:20) % | Extraction $Et_2O$ % | 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 | Quantitative evaluation 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 × 4 mm i.d.), protected with a precolumn of the same material. Two solvents were employed for elution—A: Methanol-$H_3PO_4$ (999:1), and B: $H_2O$-$H_3PO_4$ (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 microcristaline plates were used. Two-dimensional developments were carried out with *n*-buthanol-acetic acid-$H_2O$ (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 (polyethylenglycol) 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 × 0.22 mm i.d. 0, 33 μm film thickness) were used.

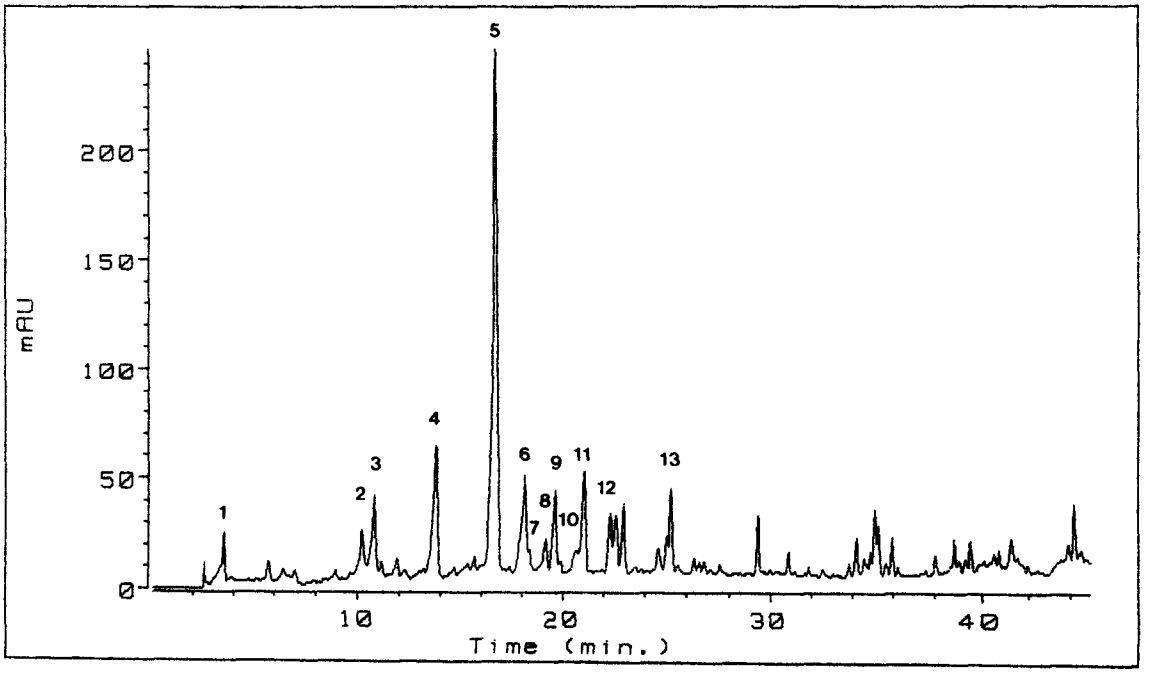


FIG. 1. HPLC chromatogram of the ether extract of wood. Detection was made at 325 ± 75 nm.

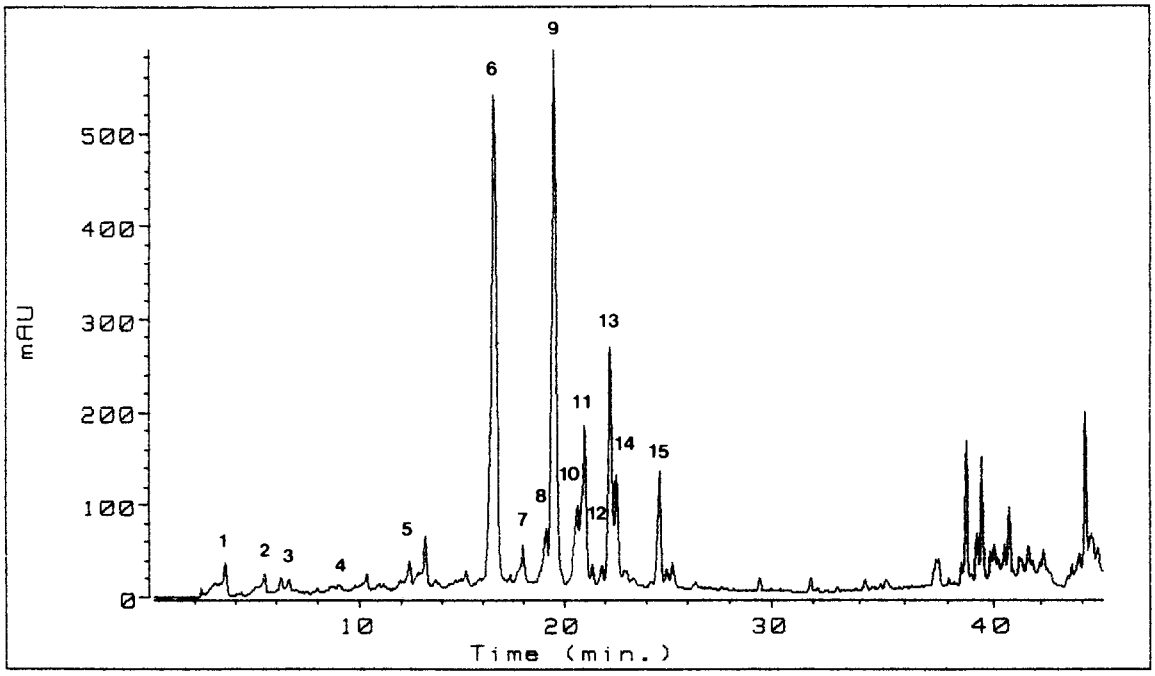


FIG. 2. HPLC chromatogram of the ether extract of bark. Detection was made at 325 ± 75 nm.

### *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-$H_2O$ (80:20) and ethyl ether of wood and bark from a tree of *E. globulus* and the total phenols contents of MeOH-$H_2O$ 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 ($UV_{\lambda max}$ (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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