

Analysis by High-Performance Liquid Chromatography Diode Array Detection Mass Spectrometry of Phenolic Compounds in Fruit of *Eucalyptus globulus* Cultivated in Algeria

Lila Boulekbache-Makhlouf,[†] Emmanuelle Meudec,^{*,‡} Mohamed Chibane,[†] Jean-Paul Mazauric,[‡] Sakina Slimani,[†] Max Henry,[§] Veronique Cheynier,[‡] and Khodir Madani[†]

[†]Faculty of Nature and Life Sciences, 3bs Laboratory, A. Mira University, Bejaia 06000, Algeria,
 [‡]INRA, UMR1083 Sciences pour l'œnologie, Montpellier, France, and [§]Structure et réactivité des systèmes moléculaires complexes (SRSMC), UMR 7565, Nancy-Université, CNRS, 5 rue Albert Lebrun, B.P. 80403, 540001 Nancy cedex, France

A method based on high-performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS) following fractionation by chromatography on a Sephadex LH-20 column has been developed to determine the phenolic composition of fruit of *Eucalyptus globulus* growing in Algeria. The presence of 18 gallotannins, 26 ellagitannins, and 2 flavonols was established. Tentative identification is provided for these compounds on the basis of UV-visible spectra and mass spectrometry data. Most compounds described in this study have not previously detected in fruit of *E. globulus*. Moreover, this is the first report of methyl digalloyl diglucose, 3,3'-*O*-dimethylellagic acid 4-*O*- β -glucopyranoside, ellagic acid hexose, methyl ellagic acid pentose, methyltetragalloylglucose, and valoneic acid isomers (sanguisorbic, flavogallic acid dilactone) in the genus *Eucalyptus*. Quantitatively, ellagic acid and its derivatives, including ellagitannins, are largely predominant.

KEYWORDS: *Eucalyptus globulus* fruit; phenolic compounds; Sephadex LH-20 column; HPLC-DAD; HPLC-ESI-MS

INTRODUCTION

The genus Eucalyptus is indigenous to Australia, comprises more than 523 species and 138 varieties, and new species and varieties are still being described (1). Among all of these species, *Eucalyptus globulus* is the most widely cultivated in subtropical and Mediterranean regions (2). It was introduced in Algeria in 1854 by Ramel. There are extensive eucalypt plantations in Algeria: 30000 ha in 1990 and 39000 ha in 1995 (3). Its leaves, roots, and fruit have been used as traditional remedies for treatment of various diseases such as pulmonary tuberculosis (4), influenza (5, 6), asthma (7), and diabetes (8-10). E. globulus is well-known for the volatile terpenoid constituents of its essential oil (5, 7, 11-13), and the phenolic compounds in its leaves and wood have been widely studied (6, 8, 11, 14-17). According to the published works, the major phenolic compounds of this plant are hydroxybenzoic acids (vanillic, gallic, protocatechuic, ellagic, and gentisic acids) (14-16, 18-20); hydroxycinnamic acids (caffeic, ferulic, p-coumaric, and chlorogenic acids) (18, 16, 20); flavonols (kaempferol, quercetin, quercetin 3-O-rhamnoside, and quercetin 3-O-rutinoside) (14-16); a methyl flavone (naringenin) (7, 15, 11); a flavan-3-ol ((+)-catechin) (5); flavones (luteolin and apigenin) (15); hydrolyzable tannins (tellimagrandin, eucalbanin C, eucaglobulin, and pentagalloylglucose) (5, 14, 15); and condensed tannins (proanthocyanidins) (14, 19-21). Phytochemical analysis has established that *E. globulus* contains monoterpene glycosides conjugated with gallic acid (6, 5) and phloroglucinolsesquiterpene-coupled compounds (5). Up to now, little has been done on the phenolic composition of the fruit of this plant. It has been reported to contain gallic acid (22, 23), cypellocarpin C, macrocarpal A, macrocarpal B (23), and other ellagic acid derivatives (3,4,3'-O-trimethylellagic acid, 3-O-methylellagic acid, $4'-O-(2''-O-acetyl)-\alpha-L$ -rhamnopyranoside, 3-O-methylellagic acid) (23).

Reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with diode array detection (DAD) and electrospray ionization mass spectrometry (ESI-MS) is frequently used for the separation, detection, and characterization of phenolic compounds from plants (24). UV-visible spectra allow identification of the class of compound, whereas ESI-MS data are useful for the structural determination of phenolic compounds (24). ESI is a gentle ionization method in MS, generating mainly deprotonated molecules $[M - H]^-$ of the compounds analyzed, when used in the negative ion mode (25). Further structural confirmation can be accomplished by tandem mass spectrometry carried out with an ion-trap (IT) mass analyzer, through analysis of the characteristic fragmentation patterns obtained by successive fragmentations of the parent and daughter ions (24).

Although a number of studies have been devoted to the identification of eucalypt phenolics, as described above, all of

^{*}Corresponding author (e-mail meudec@supagro.inra.fr; phone 04-99-61-25-84; fax 04-99-61-28-57).

them have focused only on specific families of compounds. To our knowledge, no exhaustive profiling has been published about eucalypt fruits.

The recovery of the fruits of this plant to obtain compounds with pharmaceutical or chemical applications, such as phenolic acids, flavonoids, and tannins, could be commercially interesting. The purpose of this study is to enhance knowledge of the phytochemical extract of the fruits of this plant and to highlight specific tracers for the future use of this extract in the industry as was the case for its leaves, which are used as a natural food additive in Japan (26). Therefore, we have developed a methodology that consists of combining fractionation using low-pressure chromatography on Sephadex LH-20 with HPLC-DAD-ESI-IT-MS analysis for comprehensive qualitative characterization of the phenolic composition of E. globulus fruits. Chromatography on a column filled with Sephadex LH-20 has been widely used for the fractionation of plant extracts (24, 27). Phenolic compounds are separated by gel permeation (molecule sieve effect of the gel) or adsorption (hydrogen bonding), depending on the type of solvent used (24). Using column chromatography on Sephadex LH-20 in adsorption mode, nonpolymeric phenols, including phenolic acids, lower molecular weight gallotannins (27, 28), flavonoids (29-32), and procyanidin oligomers (24) are eluted from the gel by ethanol and methanol, whereas hydrolyzable tannins with higher molecular weight are recovered by aqueous acetone (60-70%) (33, 34). Using this approach, in total, 55 compounds have been identified or tentatively identified in E. globulus fruits, including phenolic acids, ellagitannins, and flavonols. Among them, 21 compounds had been identified earlier in eucalypt, but had not been described in the fruits, and 7 had never been detected in eucalypt species. Quantitative data on the major families are also provided.

MATERIALS AND METHODS

Chemicals. *E. globulus* fruits, Myrtaceae family, were obtained from their natural habitats. They were collected from the arboretum of Darguinah, Bejaia, in northeastern Algeria, in February 2008. The plant was identified on the basis of its morphological characteristics, and a specimen has been deposited in the 3bs Laboratory (University of Bejaia). Formic acid, tannic acid, and ellagic acid were from Sigma-Aldrich (St. Louis, MO). Gallic acid and isoquercitrin (quercetin 3-*O*-glucoside) were from Extrasynthese (Genay, France). Methanol, ethanol, acetone, hexane, and acetonitrile (HPLC grade) were purchased from Merck (Darmstadt, Germany). Chlorhydric acid (HCl) and ellagic acid were purchased from Fluka (Buchs, Switzerland). Sephadex LH-20 was purchased from GE Heathcare (Uppsala, Sweden). Pure Milli-Q water was delivered by a water purification system from Millipore (Bedford, MA).

Sample Preparation. The sample was cleaned with tap water, dried in the drying oven at 40 °C during 5 days, and reduced to thin powder; 1 g was extracted with 100 mL of acetone/water (70:30, v/v) containing 0.5% acetic acid to prevent oxidation. The process of extraction continued for a week at room temperature in a dark place, using a magnetic blender. The extract was filtered through Whatman filter paper no. 4 and concentrated to dryness under reduced pressure by rotary evaporation at 40 °C. The obtained residue was defatted with hexane (25 mL \times 3) to remove lipids, concentrated under reduced pressure, and lyophilized to obtain *E. globulus* fruit extract (EGFE).

Column Chromatographic Fractionation of Crude Extract. Sephadex LH-20 gel was used for fractionation by column chromatography. Crude extract was dissolved in aqueous ethanol (75%); after sonication during 20 min, the mixture was applied to a column filled with Sephadex LH-20 (length = 30 cm, internal diameter = 1.6 cm) and fractionated by consecutive elution with ethanol, methanol, and aqueous acetone (60%), at a flow rate of 1.7 mL/min. Five fractions (5 mL each) were eluted with ethanol, two fractions (10 mL each) were eluted with methanol, and eight fractions (10 mL each) were collected after elution by aqueous acetone (60%). After evaporation of solvents under vacuum at 40 °C,



Figure 1. Sephadex LH-20 column chromatography profile of EGFE.

all fractions were reconstituted with ethanol, and then their absorbance was measured at 280 nm using an Agilent spectrophotometer. On the basis of the absorbance data, subfractions were pooled in three fractions (F1–F3). Solvent was evaporated to dryness under vacuum at 40 °C. Dried fractions were dissolved in 1 mL of ethanol/water/formic acid (75:24.5:0.5, v/v/v) and analyzed by HPLC-DAD-MS.

HPLC-DAD and ESI-MS Analysis. The HPLC analysis was carried out on a Waters 2690 HPLC system equipped with a Waters 996 DAD (Waters Corp., Milford, MA) and Empower software (Waters). The separation was performed at 30 °C using a 1.6 cm diameter, 30 cm long column. The solvents were 2% aqueous formic acid (solvent A) and acetonitrile/water/formic acid (80:18:2; solvent B). Gradient conditions were as follows: from 0 to 2% B in 3 min, from 2 to 3% B in 10 min, from 3 to 8% B in 10 min, from 8 to 20% B in 15 min, from 20 to 25% B in 5 min, from 25 to 65% B in 30 min, and then to 80% B in 7 min at a flow rate of 0.25 mL/min. The injection volume was $10 \,\mu$ L, and detection was carried out between 210 and 650 nm. After passing through the flow cell of the DAD, the column eluate was directed to an LCQ ion trap mass spectrometer fitted with an electrospray interface (Thermo Finnigan, San Jose, CA). Experiments were performed in negative ion mode. Scan range was 100-2000. The desolvation temperature was 300 °C. High spray voltage was set at 5000 V. Nitrogen was used as the dry gas at a flow rate of 75 mL/min. MS was carried out using helium as target gas, and collision energy was set at 30%. Identifications were achieved on the basis of the molecular ion mass, fragmentation, UV-visible spectra, and relative retention times or co-injection with standards.

Quantities were evaluated from peak areas in the HPLC profile, using external calibration curves established with gallic acid (at 280 nm), for gallic acid and gallotannins, ellagic acid (at 253 nm) for ellagic acid and its derivatives, and quercetin 3-*O*-glucoside (at 360 nm) for flavonols. Quantities are thus expressed as gallic acid, ellagic acid, and quercetin 3-*O*-glucoside equivalents for each class of compounds in milligrams per gram of powder of initial extract. For gallotannins and ellagitannins, quantities were estimated by calculating the concentration in moles of gallic acid (or ellagic acid) equivalent, dividing by the number of gallic (or ellagic) units in the molecule, and multiplying by the molecular weight.

RESULTS AND DISCUSSION

Sephadex LH-20 Column Chromatography of Crude Extract. Fractionation of EGFE was performed by column chromatography on Sephadex LH-20. Elution with ethanol, methanol, and aqueous acetone (60%), successively, yielded 15 fractions that were pooled in 3 fractions (labeled F1–F3), according to the absorbance readings at 280 nm (**Figure 1**).

Identification of Chromatographic Peaks. HPLC-DAD-MS analysis of each of these fractions showed a large number of compounds eluting throughout the chromatographic profiles. The profiles of the three fractions appeared to be quite different, and most of the compounds were recovered in only one of them (Figure 2). Moreover, some compounds eluting at the same retention time in the three fractions showed different UV-visible



Figure 2. HPLC chromatogram at 280 nm of the three fractions recovered after Sephadex LH-20 chromatography of EGFE.

and MS spectra (e.g., peaks eluted at 5.89, 5.77, and 5.87 min, respectively, in F1, F2, and F3).

The UV-visible spectra, recorded with DAD, allowed three groups of compounds to be distinguished. The two major groups showed spectra resembling those of ellagic acid (with two absorbance maxima around 253 and 366 nm) and gallic acid (single maximum around 272 nm), respectively. The third group is minor and showed spectra that can correspond to those of flavonol derivatives (with two absorbance maxima 250-260 and 250-360 nm). These three groups were thus tentatively attributed to ellagitannins, gallotannins, and flavonol derivatives, respectively. Ellagitannins and gallotannins represent families of hydrolyzable tannins based on a sugar unit (usually D-glucose) multiply acylated with hexahydroxydiphenic acid (releasing ellagic acid (1) upon acid hydrolysis) or gallic acid (2) (releasing gallic acid upon acid hydrolysis), respectively (Figure 3a). Flavonols (e.g., quercetin (3)) are a group of flavonoid pigments, often found as glycosides. The λ_{max} values of flavonol derivatives and ellagitannins are the same, but the relative signal intensities at 250 and 360 nm are different, the second maximum being much less intense in the case of ellagitannins (14).

Each compound was further analyzed by mass spectrometry. Identifications were confirmed by comparison of the retention time and spectral data with those of reference compounds when available (i.e., for quercetin 3-O-glucoside, gallic acid, and ellagic acid). Data obtained for all peaks, including retention times, UV-visible spectra, molecular ion, and fragmentations obtained by MS² experiments, are given in **Table 1**. Several minor compounds could not be tentatively identified from their LC-DAD-MS data and are thus not presented.

 MS^2 analysis provided $[M - H]^-$ molecular ions and fragmentation patterns that are characteristic of each group of compounds, namely, loss of galloyl groups (-152 amu) and galloyl groups plus water (-170 amu) for gallotannins and/or loss of hexahydroxydiphenoyl (HHDP) groups (-302 amu) for ellagitannins, as described earlier (36). Other characteristic fragmentations include loss of hexoside (-162 amu), pentoside (-132 amu) (37), rhamnoside (-145 amu) (24), and carboxylic function (-44 amu) (36). Tentative identifications of all compounds are provided below for each fraction.

Fraction 1. Eight compounds (F1-1-F1-8) were detected in fraction 1, which was eluted with ethanol from the Sephadex LH-20 column. The most polar one was gallic acid (2), identified from the comparison of its retention time, UV-visible spectrum, and MS signal (m/z 169) with those of an authentic standard. Gallic acid anion has been previously detected in fruits of *E. globulus* (22, 23) and in its leaves and wood (5, 15, 19), in leaves of *E. camaldulensis* and *E. rudis* (14), and in bark and wood of *E. regnans* (20).

All other peaks were attributed to gallic acid derivatives on the basis of their UV-visible spectra and further characterized by mass spectrometry. Peaks F1-3 and F1-5 detected at m/z 499 and 535, respectively, were confirmed to be gallotannins on the basis of their characteristic fragment ions (313, 211, and 169). The ion at m/z 169 corresponds to gallic acid anion and the ion at m/z 313 to a galloylglucose group [M - H - 18, loss of water] (37). The daughter ion at m/z 211 has also been previously described among fragment ions formed from mono- through pentagalloylglucose (24). Mass spectrometric analysis of compound F1-7 revealed a molecular anion at m/z 659 that gave rise to monogalloyl diglucose (m/z 493) after loss of 166 amu, corresponding to a methylgalloyl group (24); this fragmentation is characteristic of a methyldigalloyldiglucose that, to our knowledge, has never been reported in *Eucalyptus*. Two compounds (F1-4 and F1-6) detected at m/z 497 provided fragment ions at 169 and 313 amu that can be interpreted as a gallic acid anion and a galloylglucose group, as explained above. They both gave rise to a neutral loss of 184 amu characteristic of an oleuropeic acid moiety (6). These MS spectra correspond to those of eucaglobulin (Figure 3b, 4) (5, 6)and globulusin B (5) (6), which have been already detected in leaves of E. globulus (5, 6). This is the first report of these compounds in the fruits of *E. globulus*. These phenol glucosides could be differentiated by their UV absorbance maximum at λ_{max} 291 and 218-279 nm, for globulusin B (6) and eucaglobulin (5), respectively. The signal detected at m/z 519 (F1-8), showing the loss of an oleuropeic acid (M - 184), could be attributed to

Boulekbache-Makhlouf et al.





Figure 3. Chemical structures of phenolic compounds detected in EGFE.

cypellocarpin C (6), already reported in the leaves of E. cypellocarpa (38) and fruits of E. globulus (22).

The mass spectrum of the compound F1-2 showed a molecular anion at m/z 483, which may correspond to digalloylglucose. However, its UV spectrum (λ_{max} 220 and 276 nm) allowed this hypothesis to be ruled out. According to its UV spectrum and MS data, fragments at m/z 331 (M – 152, loss of gallic acid) and at m/z 313 (M - 170, loss of monoterpene moiety: hydroxyl-1,8cineole or a galloyl group plus water)(39), this compound is tentatively identified as globulusin A (7, 2-hydroxy-1,8-cineole $2-O-\beta-D-(6'-galloyl)$ glucopyranoside), previously detected in leaves of E. globulus (6).

Fraction 2. Twenty-three different compounds were detected in fraction 2, eluted with ethanol and then methanol. They could be

Table 1. HPLC-DAD-ESI-MS Data for Phenolic Compounds in EGFE

| nock | t (min) |) (pm) | [M – H] ⁻ / | MS^2 ions | neutral | phenolic compound | earlier reports in |
|----------------|------------------|----------------|------------------------|-----------------------|------------------------|---|---|
| реак | $l_{\rm R}$ (mm) | λ (nm) | [M — 2H] | (111/2) | IOSS | hypothesis (structure no.) | Eucarypius species |
| F1 | | | | | | | |
| F1-1 | 5.89 | 232—272 | 169 | ND ^o | ND | gallic acid (2) (co-injection) | E g F (22, 23), E g L B W (5, 15, 19), E cam, rud L (14), E rgn B W (20) |
| F1-2 | 31.6 | 220-276 | 483 | 331-271-313-439 | 152-212-170-44 | globulusin A (7) | E g L (6) |
| F1-3 | 45.76 | 282 | 499 | 439-313-211-169 | 60-126-288-330 | gallotannin | |
| F1-4 | 47-48 | 218-279 | 497 | 169-211-313-437 | 328-286-184-60 | eucaglobulin (4) | E g L (5,6) |
| F1-5 F1-6 | 49.50 50.7 | 239-279 201 | 232 497 | 169-479-313-137 | 222-300 | dobulusin B (5) | E g L (6) |
| F1-7 | 60.45 | 229-279 | 659 | 493 | 166 | methyl digallovl diglucose | first report in genus E |
| F1-8 | 63.48 | 298-318sh | 519 | 335-353-233 | 184-166-286 | cypellocarpin C (6) | E g F (6), E cyp L (38) |
| F2 | | | | | | | |
| F2-1 | 3.8 | 255-300 | 481 | 301-275 | 180-206 | HHDP glucose | E nit W (36), E alb F (41) |
| F2-2 | 5.87 | 232-272 | 633 | 301-463-257-275 | 170-376-358 | HHDP galloylglucose | E nit W (<i>36</i>), con L (<i>40</i>) |
| F2-3 | 7.84 | 262 | 633 | 301-249-275-(481-463) | 332-384-358-(152-1/0) | HHDP galloylglucose | E nit W (36), con L (40) |
| F2-4 F2-5 | 12 27 | 265 | 633 | 301-339-421-481-249 | 332-294-212-152-384 | HHDP galloyigiucose | E NIT W (36), E CON L (40) E pit W (36), E con L (40) |
| F2-5 F2-6 | 27 | 200 | 483 | 271-331-313 | 212—170 212—152—170 | digallovlalucose | E nit W (36), E con L (40) E nit W (36), E con L (40) |
| F2-7 | 35 | 263 | 635 | 483-465-423-248 | 152-170-212-387 | trigallovlglucose | E nit W (36), E ros L (14), E con L (40) |
| F2-8 | 36.75 | 269 | 635 | 483-465-423-248 | 152-170-212-387 | trigalloylglucose | E nit W (36), E ros L (14), E con L (40) |
| F2-9 | 37.17 | 269 | 635 | 483-465-423-248 | 152-170-212-387 | trigalloylglucose | E nit W (36), E ros L (14), E con L (40) |
| F2-10 | 38.56 | 269 | 635 | 483-465-423-248 | 152-170-212-387 | trigalloylglucose | E nit W (36), E ros L (14), E con L (40) |
| F2-11 | 42.59 | 253-364 | 463 | 301 | 162 | acid ellagic hexoside | first report in genus E |
| F2-12 | 43.16 | 255-355 | 463 | 301 | 162 | quercetin 3-O-glucoside | E g, cam, rud L (15), E g, B (44), |
| F0 10 | 4.4 | 051 004 | 477 | 015 | 100 | (co-injection) | E gun H (45) |
| F2-13 | 44 | 251-364 270 | 4// 787 | 315 | 162 170—152 | totragallovi glucoso | E g F (23) E pit W (36) E vim L (40) |
| F2-14 F2-15 | 44.2 47 | 253-366 | 301 | 229 | 170—152 79 | ellagic acid (1) (co-injection) | $E \sin(15, 26)$, $E \sin(16, 40)$ |
| 12 10 | 77 | 200 000 | 001 | LLU | 12 | | $E \operatorname{sp}(70, 20), E \operatorname{gand}$ |
| F2-16 | 47.1 | 253-355 | 477 | 415-301 | 62-176 | quercetin 3-O-glucuronide | E sp L (15, 26), E con L (40) |
| F2-17 | 48.98 | 246 - 370 | 491 | 329-313 | 162-178 | 3,3'-O-dimethylellagic acid | first report in genus E |
| F2-18 | 50 | 223-242- | 629 | 477-315-301 | 152-314-328 | callovl ester of a | E g L (16) |
| 12.10 | 00 | 255-366 | 020 | | | methylellagic acid hexoside | |
| F2-19 | 51.7 | 251-364 | 447 | 315 | 132 | methylellagic acid pentose | first report in genus E |
| F2-20 | 51.98 | 246-363 | 315 | ND | ND | methylellagic acid | E g F (23) |
| F2-21 | 53.19 | 279 | 649 | 497-479 | 152-170 | methyl trigalloylglucose | E nit W (36) |
| F2-22 | 54.33 | 239-279 | 649 | 479 | 170 | methyl trigalloylglucose | E nit W (<i>36</i>) |
| F2-23 | 57 | 220-277 | 801 | 631-649 | 1/0-152 | methyl tetragalloylglucose | first report in genus E |
| F3 F3-1 | 3 77 | 265 | 783 | 301-481-275-765 | 482-302-508-18 | casuariin (8)/ desgalloylstachyurin | F nit W (36) F con L (40) |
| F3-2 | 4.53 | 267 | 951 | 907 - (783 - 301) | 44 - (168 - 650) | trisgallovI HHDP glucose isomer | E nit W (36), E vim L (40) |
| F3-3 | 6.11 | 269 | 783 | 481-765-721-301 | 302-18-62-482 | casuariin (8)/desgalloyIstachyurin | E nit W (36), E con L (40) |
| F3-4 | 8-13 | 265 | 783 | 301-481 | 482-302 | pedunculagin (12) | E nit W (36), E con, vim L (40) |
| F3-5 | 8-13 | 265 | 783 | 301-481 | 482-302 | pedunculagin anomer | E nit W (36), E con, vim L (40) |
| F3-6 | 14.2 | 267 | 951 | 907 | 44 | trisgalloyl HHDP glucose isomer | E nit W (36), E vim L (40) |
| F3-7 | 20 | 267 | 951 | 907 | 44 | trisgalloy HHDP glucose isomer | E nit W (<i>36</i>), E vim L (<i>40</i>) |
| F3-8 | 22.59 | 265 | 1567/783 | /65-935 | 802-632 | oenothein B (13) | E sp (40, 26), E alb F (41) |
| F3-9 F3-10 | 25 27 4 | 207 | 951 | 907 | 44 | casuarinin (14) | E fill W (36), E VIII L (40) E pit W (36), E alb E (41) |
| E3-11 | 28.01 | 203 | 935 | 917-633-783-301 | 18-302-152-634 | casuarinin (14) | $L \operatorname{Int} W (00), L \operatorname{and} L (47)$ |
| F3-12 | 34 | 270 | 785 | 301-483-615 | 484-302-170 | tellimagrandin (10) | E a L (5). E ros (14). E nit W (36). |
| | • | | | | | | E alb F (41), E con, vim L (40) |
| F3-13 | 34.1 | 258-366 | 1085 | 765-633-473 | 320-452-612 | cornusiin B (17) or | E alb F (41) or E nit W (36) |
| E3-1/ | 35.7 | 265 | 785 | ND | ND | HHDP digallov/glucose isomer | E nit W (36) E con L (40) |
| E3-15 | 36.65 | 258-366 | 1085 | 765-783-633 | 320-302-452 | cornusiin B (17) or | E alb E (41) E nit W (36) |
| 1010 | 00.00 | 200 000 | 1000 | 100 100 000 | | eucalbanin A (16) | |
| F3-16 | 37.8 | 253-371 | 469 | 425 | 44 | valoneic acid dilactone (18) or | first report in genus E |
| | | | | | | one of its isomers | |
| F3-17 | 38.38 | 270 | 785 | ND | ND | HHDP digalloylglucose isomer | E nit W (<i>36</i>) |
| F3-18 | 39.33 | 266 | 935 | 633-301-451 | 302-634-484 | casuarictin (15) | E nit W (36) |
| F3-19 | 39.99 | 255-366 | 469 | 425 | 44 | valoneic acid dilactone (18) or one of its isomers | tirst report in genus E |
| F3-20 | 43.5 | 280 | 937 | 767-(741)-465-301 | 170-(196)-472-636 | tellimagrandin II (11) | E nit W (36), E vim L (40) |
| F3-21 | 44.21 | 279 | 787 | 617-635 | 170-152 | tetragalloylglucose | E nit W (36), E vim L (40) |
| F3-22 | 45.6 | 280 | 787 | 617-635 | 170-152 | tetragalloylglucose | E nit W (36), E vim L (40) |
| F3-23 | 47.7 | 239-279 | 939 | /69-787 | 1/0-152 | pentagalloyiglucose (9) | EgL (5), E nit W (36), E alb F (41) |
| ⊢ 3-24 | 52.78 | 239-279 | 2182/1091 | 939-1285-1787-1935 | 244-898-396-248 | enagitannin | |

^{*a*}Key to simple nomenclature: E, eucalyptus; g, globulus; alb, alba; cam, camaldulensis; con, consideniana; gun, gunnii; nit, nitens; rud, rudis; ros, rostra; rgn, regnans; sp, species; vim, viminalis; cyp, cypellocarpa; L, leaves; , bark; F, fruit; W, wood; H, hook. ^{*b*}ND, not identified.

divided into three groups, corresponding respectively to gallotannins, ellagitannins, and flavonols, on the basis of their UV-visible spectra.

The first group contained 14 compounds, showing a λ_{max} around 270 nm and fragment ions characteristic of gallotannin derivatives. Among them, 9 were galloylglucose derivatives, whereas 5 also contained one or two HHDP groups. Compound F2-6 showed a λ_{max} at 269 nm and a molecular anion at m/z 483, yielding fragment ions corresponding to the loss of a galloyl group (-152), of a galloyl group plus a water molecule (-180), and of another fragment of 212 amu, characteristic of galloylglucose derivatives, as explained above. It could thus be assigned to digalloylglucose previously detected in E. nitens wood (36) and E. consideniana leaves (40). Similarly, the mass signals at m/z 635 (F2-7–F2-10) with their characteristic fragment ions at m/z 483 and 465 corresponded to trigalloylglucose isomers, detected earlier in E. nitens wood (36), leaves of E. consideniana (40), and E. rostra leaves (14). F2-14, present in a small amount in fraction 2, could be attributed to a tetragalloylglucose. This compound was more abundant in fraction 3 (F3-21) and will be described below along with the other tetragalloylglucose isomers present in this fraction.

The ions detected at m/z 801 and 649 (F2-21, F2-22, and F2-23) also showed fragments corresponding to losses of 152 and 170 amu, indicating gallotannin structures. The MS data (+ 14 amu compared to the molecular ions of tri- and tetragalloylglucose) suggest that these compounds are methylated tetragalloylglucose and trigalloylglucose, respectively. The latter has been previously detected in *E. nitens* wood (*36*).

The $[M - H]^-$ at m/z 481 (F2-1) with absorbance maxima 255–300 nm and fragment ions at m/z 301 (loss of 180, loss of a galloyl group plus water) has been assigned to an HHDP glucose, reported earlier in the wood of *E. nitens* (36) and identified as 2,3-(*S*)-HHDP-D-glucose in *E. alba* fruits (41).

The signals detected at m/z 633 (F2-2, F2-3, F2-4, and F2-5) were attributed to HHDP galloylglucose isomers on the basis of their molecular ion and fragment ions at m/z 301 (loss of 332, which indicated the presence of a galloylglucose unit) (42), corresponding to HHDP, and at 463 (loss of a galloyl group plus water). These compounds have been previously detected in *E. nitens* wood (one isomer) (36) and *E. consideniana* leaves (three isomers) (40).

Seven mass signals detected in fraction 2 could be attributed to ellagic acid and its derivatives. The presence of free ellagic acid (1) in fraction F2 was confirmed by its retention time (47 min) and MS data (*m*/*z* 301) (F2-16). The peak eluted at 51.98 min (F2-20) with m/z 315 and UV-visible spectrum (λ_{max} 246 and 363 nm), characteristic of ellagic acid, was tentatively identified as methylellagic acid, which has been previously detected in fruits of E. globulus and identified as 3-O-methylellagic acid (23). The two peaks F2-13 and F2-19, showing similar UV-visible spectra with two absorbance maxima at 251 and 364 nm, were also postulated to be ellagic acid derivatives. MS analysis of the former showed an intense molecular anion at m/z 477, which vielded a fragment ion at m/z 315 attributed to methylellagic acid, through loss of a hexosyl group $(m/z \ 162)$. This compound is thus a methylellagic acid hexoside, presumably 3-O-methylellagic acid 4'-O-a-L-rhamnopyranoside identified earlier in E. globulus fruits (23). The peak at m/z 463 (F2-11) with a fragment at m/z301 has been assigned to ellagic acid hexoside. The peak at m/z447 (F2-19) yielded a fragment ion at m/z 315 through loss of 132 amu, corresponding to a pentose residue. It could thus be assigned to methylellagic acid pentoside as described in raspberry fruits (37). To our knowledge, this is the first report of these two compounds in the genus Eucalyptus. Another compound detected at m/z 629 (F2-18) showed fragments at m/z 477 (methylellagic acid hexoside, loss of 152 amu, corresponding to a galloyl group) and at m/z 315 (methyl ellagic acid, loss of 314 amu, corresponding to a galloylhexoside) and was thus identified as a galloyl ester of a methylellagic acid hexoside. This compound has been already detected in E. globulus leaves (16). Finally, a compound at m/z 491 (F2-17) and its fragment at m/z 329 [M - H - 162]⁻, which corresponds to loss of a hexose, can be interpreted as dimethylellagic acid hexose (43). This is the first report of this compound in the genus Eucalyptus. Several methylellagic acid derivatives, namely, 3-O-methylellagic acid, 3,4,3'-O-trimethylellagic acid, 3-O-methylellagic acid 4'-O-α-L-rhamnopyranoside, and 3-Omethylellagic acid 4'-O-(2"-O-acetyl)-α-L-rhamnopyranoside, have been identified earlier in the fruits of E. globulus (23). Methylellagic acid derivatives have also been previously detected in eucalypt species such as leaves of E. globulus, E. camaldulensis, and E. rudis (15) and stem bark of E. globulus (20, 44).

The last group of compounds detected in this fraction was flavonols, showing the same fragment ion at m/z 301, which could be attributed to quercetin. One of them, detected at m/z 463 (F2-12) (i.e., 301+162 amu, corresponding to a hexoside residue), can be identified as a quercetin hexoside, presumably quercetin 3-O-glucoside, the presence of which has been reported in the leaves of E. globulus, E. camaldulensis, and E. rudis (14-16, 26), in the bark of E. globulus, and in the hook E. gunnii (45) and E. consideniana (40). Further argument in favor of this identification is provided by coelution with the corresponding standard. The other compound, detected at m/z477 (F2-16) and yielding the aglycone fragment ion at m/z 301 through loss of a glucuronide substituent (-176 amu), was tentatively identified as quercetin 3-O-glucuronide, which has been already described in *Eucalyptus* species (15, 40, 45). This is the first report of flavonols in E. globulus fruits.

Fraction 3. Twenty-four compounds were detected in this fraction. They could be classified as gallotannins and ellagitannins on the basis of their UV spectra and characteristic mass fragmentations.

Compounds at m/z 787 (F3-21 and F3-22) are assigned to tetragalloylglucose isomers, which are characterized by fragment ions at m/z 635 and at m/z 617, corresponding to loss of a galloyl residue (M – H – 152) and of a gallic acid group (M – H – 170), respectively, as described by Barry and co-workers (*36*). These compounds have been previously detected in wood of *E. nitens* (*36*) and in *E. viminalis* leaves (*40*).

The compound at m/z 939 (F3-23) was identified according to its UV-visible and mass spectra data (loss of a galloyl residue [M - H - 152] and of gallic acid [M - H - 170]) as pentagalloylglucose (9), yielding fragment ions at m/z 787 and at m/z 769, respectively (46). Pentagalloylglucose has been described in wood of *E. nitens* (36) and in *E. alba* fruits (41).

Other compounds present in the fraction showed a loss of 302 amu, characteristic of ellagitannin structures (36). The mass signal detected at m/z 785 (F3-12, F3-14, and F3-17), with fragment ions at m/z 301 (loss of digalloylglucose) and m/z 483 (loss of HHDP), correspond to digalloylglucose, presumably tellimagrandin I (10), which has been previously identified in the leaves of E. globulus and the wood of E. nitens (5, 36), in E. rostra leaves (14), in E. alba fruits (41), and in E. consideniana leaves (40). The signal at m/z 937 (F3-20), yielding fragment ions at m/z 767 (M - 170, loss of gallic acid) and m/z 465 (losses of HHDP and gallic acid groups) can correspond to tellimagrandin II (11), which has been detected earlier in *E. nitens* wood (36) and E. viminalis leaves (40). This compound differs from tellimagrandin I by the presence of one additional galloyl unit at the anomeric center of the glucopyranosyl core. Tellimagrandin II is produced by enzymatic transformation of pentagalloylglucose (47).

Article

Another group of compounds with λ_{max} around 270 nm showed mass signals characteristic of HHDP glucose derivatives, with molecular ions detected at m/z 783 (F3-1, F3-3, F3-4, and F3-5), yielding a fragment ion at m/z 301, which corresponds to ellagic acid (M - 482, loss of HHDP glucose) as well as loss of ions at m/z 481, which correspond to deprotonated HHDP glucose (M - 302, loss of HHDP). These compounds can correspond to di-HHDP glucose. F3-1 and F3-3 vielded a fragment at m/z 765 (M – 18, loss of water). An abundant loss of 18 amu is characteristic of C-glycosidic ellagitanins (36), which are presumably casuariin (Figure 3c, 8), and its anomer desgalloylstachyurin. Casuariin has been previously detected in E. nitens wood (36) and E. alba fruit (41). F3-4 and F3-5 can correspond to pedunculagin anomers (2,3;4,6-di-HHDP glucose, 12) or pedunculagin isomers, as reported by Barry and co-workers (36). Three isomers of this compound have been previously detected in E. nitens wood (36), whereas only one was found in E. consideniana leaves (40).

Compound F3-8 yielded two ions at m/z 1567 and 783, corresponding to the monocharged and doubly charged ions of a compound with a molecular weight of 1568. Fragment ions at m/z 765, loss of 802 amu, which is reported to be ellagitannin (42), and 935 ($[M - H]^-$ ion of galloyl-bis-HHDP-glucopyranose), allowed assignment to a dimer of tellimagrandin I, linked by two valoneoyl groups, oenothein B (13) identified in leaves of eucalyptus species (26, 40) and in fruits of *E. alba* (41).

On the basis of molecular weight and MS/MS data, the four peaks F3-2, F3-6, F3-7, and F3-9 with m/2 951 would appear to be ellagitannins. Loss of 44 amu from the $[M - 1]^{-1}$ ion is consistent with a free carboxyl group. These compounds could be assigned to trigalloyl HDDP glucose isomers, which have been already detected in *E. nitens* wood (36). These consist of an HDDP glucose and a trisgalloyl group such as valoneic acid dilactone (18) (46) or one of its isomers (tergallic acid dilactone (19)), sanguisorbic acid, or flavogallic acid dilactone. Three peaks at m/z 935 (F3-10, F3-11, and F3-18) are also detected; they could be attributed to casuarinin (14) and its anomer and casuarictin (15); these two isomers could be distinguished by their retention times and also by their MS/MS spectra. The MS/MS spectra of casuarinin and of its anomer showed an ion resulting from a loss of water, owing to its open glucose ring structure. Casuarinin and casuarictin have been already detected in E. nitens wood (36). Compounds detected at m/z 1085 (F3-13 and F3-15) with fragment ion at 633 corresponding to the HHDP group (M - 452, loss oftrigalloyl group) were assigned to eucalbanin A (16) or its isomer cornusiin B (17), which has been already detected in the fruit extract of E. alba (41).

The peak at m/z 469 (F3-16 and F3-19) with UV-visible absorption spectra similar to that of ellagic acid gives a fragment ion at m/z 425 (valoneic acid dilactone with loss of CO₂). This compound was tentatively identified as valoneic acid dilactone (18) (46) or one of its isomers (tergallic acid dilactone (19), sanguisorbic acid, or flavogallic acid dilactone). This is the first report of this compound in *Eucalyptus* species. Valoneic acid dilactone can be a product of hydrolysis of cornusiin B or of oenothein B and tergallic acid dilactone a product of its isomer eucalbanin A (46).

Co-occurrence of most compounds detected in this fraction can be explained by the proposed biosynthetic pathway for the formation of the hydrolyzable tannins in oak leaves (48). Consecutive galloylation steps of glucose lead to the formation of pentagalloylglucose. Oxidative coupling between galloyl groups at C-4 and C-6 produces tellimagrandin II, which yields casuarictin after oxidative coupling between galloyl groups at C-2 and C-3. Cleavage of the galloyl group at C-1 of this compound leads to the formation of casuarinin. Furthermore, it has been suggested that the C-glycosidic ellagitannins are formed from pedunculagin, through formation of casuarinin as intermediate.

HPLC-DAD-ESI-MS/MS enabled detection of a series of gallotannins and ellagitannins and of two flavonols in the fruits of *E. globulus*. Fractionation by low-pressure chromatography on Sephadex LH-20 proved to be efficient to separate lower and higher molecular weight compounds and enabled separation of compounds coeluted in the HPLC procedure.

The concentrations of the major compounds present in EFGE were evaluated from the HPLC profiles of each fraction (**Table 2**). It should be emphasized that these values are indicative, as they are calculated using external calibration with a limited number of standards. Moreover, some changes induced in the relative amounts of the various compounds may have occurred during the extraction and fractionation procedures, because hydrolyzable tannins can be modified by desiccation or thermal treatments and easily undergo partial hydrolysis under acidic conditions.

Most of the phenolic compounds were recovered in F3 (109.4 mg/g of EGFE) and F2 (97.5 mg/g of EGFE), whereas F1 contained only small amounts (7.3 mg/g of EGFE) (**Table 2**).

F1, eluted with ethanol, contained gallic acid, terpenyl derivatives of galloylglucose (tentatively identified to eucaglobulin, globulisin B) and of noreugenin glucoside (tentatively identified as cypellocarpin C), and derivatives of lower molecular weight gallotannins in small amounts (**Table 2**).

F2 contained high levels of ellagitannins (HHDP glucose, HHDP galloylglucose, trigalloylglucose, methyltrigalloylglucose, and methyltetragalloylglucose), ellagic acid, and ellagic acid derivatives (methylellagic acid, ellagic acid hexose, methylellagic acid hexose and pentose) (**Table 2**), which is in agreement with previous studies reporting that ellagic acid and its derivatives are the major phenolic class that characterizes *E. globulus* fruits (22, 23). However, the large amount of ellagic acid and HHDP glucose could be due to hydrolytic reaction during the extraction procedure. Gallotannins and flavonols (presumably quercetin 3-*O*-glucoside and quercetin 3-*O*-glucuronide) were present only as minor components in this fraction (**Table 2**).

F3, eluted with aqueous acetone, contains tetra- and pentagalloylglucose and higher molecular weight ellagitannins, as expected from the literature (24, 25, 36, 46). Di-HHDP glucose (casuariin or desgalloylstachyurin) is particularly abundant in this fraction but can result from hydrolysis of ellagitannins during the extraction procedure.

Fractionation on Sephadex LH-20 followed by HPLC-DAD-MS analysis proved to be efficient for the identification of phenolic compounds in eucalypt fruit. Ellagic acid derivatives were the major phenolic compounds in the fruit extract, ellagic acid and its smaller molecular weight derivatives being recovered in fraction F2, whereas ellagitannins were distributed in fractions F2 and F3. Among the wide range of phenolic compounds (including structural isomers) detected in this study, gallic acid, ellagic acid derivatives, and cypellocarpin C had been previously identified in the fruits of E. globulus (22, 23), whereas quercetin derivatives, globulusins A and B, and hydrolyzable tannins were described for the first time in this material. Among hydrolyzable tannins, methyltrigalloylglucose, di-HHDP glucose, HHDP glucose, HHDP galloylglucose, digalloylglucose, trigalloylglucose, tetragalloylglucose, trisgalloyl HHDP glucose, casuarinin, casuarictin, cornusiin B, eucalbanin A, and tellimagrandin II are reported here for the first time in E. globulus plants. These compounds have been detected earlier in the wood of *E. nitens* (36). Methyltetragalloylglucose, methyldigalloyldiglucose, methylellagic acid pentose, and valoneic acid dilactone are described for the first time in the genus Eucalyptus. It should be emphasized

12622 J. Agric. Food Chem., Vol. 58, No. 24, 2010

Table 2. Contents of Phenolic Compounds in EGFE

| Compands in F1 gelic acid 5.24 rd 170 rd 5.24 rd globulari A 0.01 rd 44 rd 0.01 rd tepprny derivatives: 175 globulari B 1.34 rd 44 rd 0.01 rd tepprny derivatives: 175 globulari B 1.34 rd 44 rd 0.01 rd tepprny derivatives: 175 globulari B 1.34 rd 420 rd 0.34 rd 520 rd populacerpin C 0.34 rd 520 rd 0.34 rd 520 rd organolis IF 2 0.01 rd 0.02 rd 121 0.04 rd diguloglobuce 0.02 rd 212 0.01 rd 121 trigglobyloce 0.02 rd 212 0.01 rd 121 0.68 rd trigglobyloce 0.01 rd 212 0.02 rd elagianoms: 50.6 Elf HHOP globyloce 0.71 rd 211.33 0.24 rd elagic acid and derivatives: 45.8 dilloi acid 0.05 rd 452 0.25 rd 452 0.25 rd dilloi acid 0.05 rd 452 0.25 rd | | concn (mg/g) | correction factor ^a | corrected concn ^b (mg/g) | concn (mg/g) per family |
|---|-------------------------------------|---------------------------|--------------------------------|-------------------------------------|------------------------------------|
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| | trigalloyiglucose | 0.13° | 212 | 0.04 | |
| Inglite/glucose 0.02* 212 0.01* Inglite/glucose 0.09* 197 0.02* Integrate/glucose 0.09* 200.5 0.27* IHDP glucose 95.99* 240.5 44.09* ellagitannin:: 50.6 IHDP glucogicose 6.77* 211.33 2.28* ellagitannin:: 50.6 IHDP glucogicose 0.71* 211.33 0.24* ellagita colt hexase 0.24* ellagita colt hexase 0.24* 464 0.24* ellagita colt hexase 0.24* ellagita colt hexase 0.24* 464 0.24* ellagita colt hexase 0.24* ellagita colt hexase 0.24* 464 0.25* 464 0.5* quercein 3-O-glucoside 0.25* 464 0.25* flavonols: 0.5 quercein 3-O-glucoside 0.25* 464 0.25* flavonols: 0.5 etargalo/glucose 1.39* 197 0.35* galotannins: 5.0 tetragalo/glucose 1.39* 197 0.35* galotannins: 5.0 tetragalo/glucose 1.40* 196 0.61* 195 | trigalloyiglucose | 0.32° | 212 | 0.11° | |
| Inglity glucose 0.16° 212 0.06° tritingal ly glucose 0.06° 197 0.02° methy letragal ly glucose 1.06° 200.5 0.27° HHDP glucy glucose 67.7° 211.33 0.24° ellagic acid hexose 0.24° 464 0.24° ellagic acid hexose 0.41° 473 0.41° ellagic acid hexose 0.52° 492 0.52° gluty ester on thrybelagic acid 0.55° 316 4.05° quercatin 3-Oglucosidi 0.25° 166 4.05° quercatin 3-Oglucosidi 0.25° 197 1.38° pertagaloglucose 7.55° 197 1.89° pertagaloglucose 7.55° 197 1.89° tartagaloglucose 7.55° 197 1.89° pertagaloglucose 1.39 | trigalloylglucose | 0.02° | 212 | 0.01° | |
| leftragaloylpucose 0.09 ^o 197 0.02 ^o HHDP glucose 1.06 ^o 200.5 0.27 ^o HHDP glucose 6.77 ^o 211.33 2.26 ^o HHDP glucose 0.71 ^o 211.33 0.24 ^d ellogic add hexose 0.24 ^d 464 0.24 ^d ellogic add hexose 0.41 ^d 478 0.41 ^d ellogic add hexose 0.25 ^d 482 0.25 ^d galdy ster of methylellogic add hexose 0.45 ^d 630 0.65 ^d methylellogic add hexose 0.24 ^d 464 0.25 ^d galdy ster of methylellogic add hexose 0.25 ^d 482 0.5 ^d galdy ster of methylellogic add 0.05 ^d 630 0.5 ^d uparceln 3-Oglucoside 0.25 ^d 464 0.25 ^d flavonols: 0.5 uparceln 3-Oglucoside 0.25 ^d 197 0.35 ^c galotannins: 5.0 tetragaloylplucose 7.55 ^d 197 1.89 ^d ellagitannins: 10.5 tetragaloylplucose 7.55 ^d 197 1.89 ^d ellagitannins: 10.5 tetragaloylplucose 1.77 ^d 19 | trigalloylglucose | 0.18° | 212 | 0.06 | |
| methyletragalloylgucose 1.06° 200.5 0.27^{5} HHDP glucose 96.99° 240.5 4.00° ellagitannins: 50.6 HHDP glucylgucose 0.71° 211.33 0.24° ellagitannins: 50.6 HHDP glucylgucose 0.71° 211.33 0.24° ellagita acid and derivatives: 45.8 ellagita acid acid x1 ^o 778 0.21° ellagita acid and derivatives: 45.8 ellagita acid persose $0.24^{\circ \circ}$ 464 $0.24^{\circ \circ}$ ellagita acid and derivatives: 45.8 ellagita acid persose $0.25^{\circ \circ}$ 492 $0.55^{\circ \circ}$ glucose $0.5^{\circ \circ}$ glucose acid persose $0.25^{\circ \circ}$ 448 $0.55^{\circ \circ}$ flavonols: 0.5 glucose quercetin 3-O-glucoside $0.25^{\circ \circ}$ 464 $0.25^{\circ \circ}$ flavonols: 0.5 glucose casuarin/desgalloy/slucose $1.39^{\circ \circ}$ 197 $0.35^{\circ \circ}$ gallotamins: 5.0 tetragalloy/glucose $1.39^{\circ \circ}$ 197 $0.35^{\circ \circ}$ gallotamins: 5.0 tetragalloy/glucose $1.22^{\circ \circ}$ 196 $0.33^{\circ \circ}$ ellagitannins: 101.5 pedurculagin <td< td=""><td>tetragalloylglucose</td><td>0.09°</td><td>197</td><td>0.02</td><td></td></td<> | tetragalloylglucose | 0.09° | 197 | 0.02 | |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$ | methyltetragalloylglucose | 1.06° | 200.5 | 0.27 | |
| HHDP palloyglucese 6.77° 211.33 $2.28°$ ellagic acid hexose 0.71° 211.33 0.24° ellagic acid hexose 0.44° 464 0.24° ellagic acid hexose 0.44° 478 0.41° ellagic acid hexose 0.44° 478 0.41° ellagic acid hexose 0.44° 478 0.52° galoy jester of methylellagic acid 0.05° 630 0.05° enthylellagic acid pentose 8.15° 448 8.15° enthylellagic acid 0.25° 464 0.25° quercetin 3-O-glucoside 0.21° 478 0.21° quercetin 3-O-glucoside 0.21° 478 0.21° total F2 149.69 96.80 0.05° compounds in F3 197 0.35° galolannins: 5.0 tetragaloydjucose 7.55° 197 1.89° galolannins: 101.5 pedmoculagin 2.24° 196 0.30° ellagitannins: 101.5 pedmoculagin 2.24° 196 0.31° casuarinin despaloydjucose isomer 0.17° 190.4 0.03° | HHDP glucose | 95.99 ^c | 240.5 | 48.09 ^c | ellagitannins: 50.6 |
| HHDP galogipucose 0.71° 211.33 0.24° elagic acid hexose 0.24^d 464 0.24^d elagic acid and derivatives: 45.8 methylelagic glucopyranese 0.52° 492 0.52° 0.05° galoy ester of methylelagic acid 0.05° 630 0.06° 0.06° methylelagic acid 0.05° 630 0.06° 0.05° 0.05° querotin $3-Oglucoside$ 0.25° 464 0.25° flavonols: 0.5 querotin $3-Oglucoside$ 0.25° 464 0.25° flavonols: 0.5 tetragaloy/glucose 1.39° 197 0.35° gallotannins: 5.0 tetragaloy/glucose 1.52° 197 1.89° elagitannins: 101.5 pedmoulagin 1.22° 196 0.30° elagitannins: 101.5 pedmoulagin 2.44° 196 0.30° elagitannins: 101.5 risigaloy/HHDP glucose isomer 0.17° 190.4 0.05° elagitannins: 101.5 risigaloy/HDP glucose isomer 0.31° 196.5 0.38° elagitann | HHDP galloylglucose | 6.77 ^c | 211.33 | 2.26 ^c | |
| ellagic acid hexose 0.24^d 464 0.24^d ellagic acid hexose 0.41^d 478 0.41^d ellagic acid 32.35^d 302 32.35^d 302 32.35^d galoy lester of methylellagic acid 0.05^d 630 0.05^d 316 4.05^d quercetin 3-O-glucoside 0.25^d 448 8.15^d 160^d 10^d quercetin 3-O-glucoside 0.25^d 464 0.25^g flavonols: 0.5 omptylellagic acid 0.25^d 464 0.25^g flavonols: 0.5 quercetin 3-O-glucoside 0.25^d 477 0.35^c galotannins: 5.0 tetragaloylglucose 1.39^c 197 0.35^c galotannins: 5.0 tetragaloylglucose 1.25^c 197 1.89^c ellagitannins: 101.5 pedunculagin 2.44^c 196 0.30^c ellagitannins: 101.5 10.42^c resultin/rescale/rescale/rescale 0.39^c 139.4 0.65^c 0.30^c ellagitannins: 101.5 pedunculagin 2.44^c 196 0.30^c el | HHDP galloylglucose | 0.71 ^c | 211.33 | 0.24 ^{<i>c</i>} | |
| methylelagic acid 0.4 if a 0.4 if a elagic acid 32.35 ² 302 32.35 ² galloy lester of methylelagic glucopyranose 0.52 ^d 492 0.52 ^d galloy lester of methylelagic acid 0.05 ^d 630 0.05 ^d methylelagic acid pentose 8.15 ^d 448 8.15 ^d methylelagic acid 4.05 ^d 316 4.05 ^d quercetin 3-O-glucoside 0.25 ^d 464 0.25 ^d quercetin 3-O-glucoside 0.21 ^d 478 0.21 ^d compounds in F3 tetragaloy/glucose 1.39 ^c 197 0.35 ^c galotannins: 5.0 tetragaloy/glucose 7.55 ^c 197 1.89 ^c elagitannins: 101.5 14.02 ^c casuarin/desgaloy/glucose 1.39 ^c 197 0.35 ^c galotannins: 101.5 15 pedmculagin 1.22 ^c 196 0.30 ^c elagitannins: 101.5 15 resultin/desgaloy/glucose 1.22 ^c 196 0.30 ^c elagitannins: 101.5 15 pedunculagin 1.22 ^c 196 0.30 ^c elagitannins: 101.5 15 | ellagic acid bexose | 0.24 ^d | 464 | 0 24 ^d | ellagic acid and derivatives: 45.8 |
| Interpretation Notice Noti | methylellagic acid hexose | 0.41 ^d | 478 | 0.41 ^d | |
| Initial constraint Calculation Calculation Calculation dimethyleligic acid pentose 0.52^{st} 492 0.52^{st} galoy lester of methyleligic acid 0.05^{st} 630 0.05^{st} methyleligic acid pentose 8.15^{st} 448 0.25^{st} flavonois: 0.5 quercetin 3-O-glucoside 0.25^{st} 464 0.25^{st} flavonois: 0.5 quercetin 3-O-glucoside 0.21^{st} 478 0.21^{st} flavonois: 0.5 total F2 149.69 96.80 compounds in F3 galotannins: 5.0 tetragaloy/glucose 7.55^{st} 197 0.35^{st} galotannins: 5.0 tetragaloy/glucose 7.55^{st} 197 8.95^{st} ellagitannins: 101.5 pedunculagin 1.22^{st} 196 9.30^{st} ellagitannins: 101.5 pedunculagin 2.44^{st} 196 0.30^{st} ellagitannins: 101.5 pedunculagin 2.24^{st} 196 0.30^{st} ellagitannins: 101.5 triggaloy/HDP glucose isomer 0.77^{st} 197.2 0.04^{st} 0.3^{st} ellagitannins: | ellanic acid | 32.35 ^d | 302 | 32 35 ^d | |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$ | dimethylellagic gluconyranose | 0.52 ^d | 492 | 0.52 ^d | |
| | calloyl ester of methylellagic acid | 0.02 0.05 ^d | 630 | 0.02 ^d | |
| Interpletagic add 0.13 4.05 4.05 quercetin 3-O-glucoside 0.25° 464 0.25° flavonols: 0.5 quercetin 3-O-glucouronide 0.21° 478 0.21° flavonols: 0.5 total F2 149.69 96.80 0.35° gallotannins: 5.0 tetragaloylglucose 1.39° 197 1.89° gallotannins: 5.0 tetragaloylglucose 7.55° 197 1.89° ellagitannins: 101.5 casuarin/desgalloylstachyurin 377.39° 196 94.35° ellagitannins: 101.5 pedunculagin 1.22° 196 0.30° ellagitannins: 101.5 pedunculagin 2.44° 196 0.61° trigaloyl HHDP glucose isomer 0.26° oenotherin B 18.43° 196 2.30° easuarinin 0.22° 137.2 easuarinin 0.22° 187.2 0.13° casuarinin 0.22° 137.2 0.36° ielimagrandin I 1.46° 196.5 0.36° 140.4° 146° 146.5 0.66° HHDP digaloylglucose isomer 0.31° 196.5 0.36° 147.2 | methylellagic acid pentose | 8.15 ^d | 448 | 8.15 ^d | |
| quercetin 3-O-glucoside quercetin 3-O-glucoronide 0.25° 0.21° 464 478 0.25° 0.21° flavonois: 0.5total F2149.6996.80compounds in F3 tetragalloy/glucose 1.39° 197 0.35° 197 gallotannins: 5.0tetragalloy/glucose $1.4.02^{\circ}$ 157.6 2.80° casuariin/desgalloy/stachyurin 377.39° 244° 196 94.35° 0.30° ellagitannins: 101.5casuariin/desgalloy/stachyurin 377.39° 244° 196 0.30° 0.61° ellagitannins: 101.5risgalloy/ HHDP glucose isomer 0.25° 0.25° 190.4 0.03° 0.30° ellagitannins: 101.5casuarinin 0.22° 0.22° 187.2 0.13° 0.33° ellagitannins: 101.5valoneix intin 0.25° 0.22° 187.2 0.44° 0.36° HHDP digalloy/glucose isomer 0.31° 0.33° 187.2 0.07° 0.36° HHDP digalloy/glucose isomer 3.70° 0.33° 187.2 0.07° 0.07° valoneic acid or isomer 0.11^{d} 0.49^{d} 470 0.07^{d} 0.11^{d} 0.07^{d} valoneic acid or isomer 0.11^{d} $0.7d'$ 470 0.07^{d} 0.07^{d} 0.07^{d} valoneic acid or isomer 0.11^{d} $0.7d'$ 470 0.07^{d} 0.07^{d} 0.07^{d} valoneic acid or isomer 0.11^{d} $0.7d'$ 470 0.07^{d} 0.07^{d} 0.07^{d} valoneic acid or isomer <td>methylellagic acid</td> <td>4.05^d</td> <td>316</td> <td>4.05^d</td> <td></td> | methylellagic acid | 4.05 ^d | 316 | 4.05 ^d | |
| quercetin 3-C-glucuronide 0.21^{o} 478 0.21^{o} total F2 149.69 96.80 compounds in F3 97 0.35° gallotannins: 5.0 tetragalloylglucose 7.55° 197 1.89° pentagalloylglucose 7.55° 197 1.89° casuariin/desgalloylstachyurin 377.39° 196 94.35° ellagitannins: 101.5 pedunculagin 122° 196 0.30° ellagitannins: 101.5 pedunculagin 2.44° 196 0.30° ellagitannins: 101.5 pedunculagin 2.24° 196 0.30° ellagitannins: 101.5 pedunculagin 2.24° 196 0.30° ellagitannins: 101.5 risgalloyl HHDP glucose isomer 0.26° 190.4 0.05° ellagitannin<: 101.5 casuarinin 0.65° 187.2 0.13° casuarinin 0.22° easuarinin Using alloyl glucose isomer 0.31° 187.2 0.32° easuarinin 0 | quercetin 3-O-glucoside | 0.25 ^e | 464 | 0.25 ^e | flavonols: 0.5 |
| total F2 149.69 96.80 compounds in F3 tetragalloylglucose 1.39° 197 0.35° gallotannins: 5.0 tetragalloylglucose 7.55° 197 1.89° gallotannins: 5.0 casuariin/desgalloylstachyurin 377.39° 196 9.435° ellagitannins: 101.5 pedunculagin 1.22° 196 0.30° ellagitannins: 101.5 pedunculagin 2.44° 196 0.30° ellagitannins: 101.5 pedunculagin 2.44° 196 0.30° ellagitannins: 101.5 trisgalloyl HDP glucose isomer 0.26° 190.4 0.05° ellagitannins: 101.5 trisgalloyl HDP glucose isomer 0.26° 190.4 0.05° ellagitannins: 101.5 casuarinin 0.65° 187.2 0.13° easuarinin 0.65° 0.38° HHDP digalloylglucose isomer 3.70° 196.5 0.92° easuarinin 0.33° 187.2 0.07° easuarinin 0.33° 187.6 0.60° </td <td>quercetin 3-O-glucuronide</td> <td>0.21</td> <td>478</td> <td>0.21</td> <td></td> | quercetin 3-O-glucuronide | 0.21 | 478 | 0.21 | |
| compounds in F3 139° 197 0.35° gallotannins: 5.0 tetragalloylglucose 7.55° 197 1.89° pentagalloylglucose 14.02° 157.6 2.80° casuariin/desgalloylstachyurin 377.39° 196 94.35° ellagitannins: 101.5 pedunculagin 1.22° 196 0.30° ellagitannins: 101.5 pedunculagin 2.44° 196 0.61° ellagitannins: 101.5 pedunculagin 2.44° 196 0.61° ellagitannins: 101.5 pedunculagin 2.26° 190.4 0.03° ellagitannins: 101.5 oenothein B 18.43° 196 2.30° easuarinin 0.65° 187.2 0.13° casuarinin 0.22° 187.2 0.04° easuarinin 0.22° 187.2 0.04° HHDP digaloylglucose isomer 0.31° 196.5 0.92° easuaritin easuaritin 0.33° 187.2 0.07° tellimagrandin I 1.46° 196.5 0.92° easuaritin easuaritin 0.49° cornusiin or eucalbanin 1.12° 1086 1.12° | total F2 | 149.69 | | 96.80 | |
| tetragalloylglucose 1.39° 197 0.35° gallotannins: 5.0 tetragalloylglucose 7.55° 197 1.89° casuariin/desgalloylstachyurin 377.39° 196 9.435° ellagitannins: 101.5 pedunculagin 1.22° 196 0.30° ellagitannins: 101.5 pedunculagin 2.44° 196 0.61° ellagitannins: 101.5 pedunculagin 2.44° 196 0.61° ellagitannins: 101.5 risgalloyl HHDP glucose isomer 0.17° 190.4 0.03° ellagitannins: 101.5 oenothein B 18.43° 196 2.30° casuarinin 0.65° 187.2 0.13° casuarinin 0.65° 187.2 0.04° 0.4° 14HDP digalloylglucose isomer 0.31° 196.5 0.08° HHDP digalloylglucose isomer 3.70° 196.5 0.92° casuaritin 0.49° 0.66° valoneic acid or isomer 0.31° 196.5 0.92° casuaritin 0.49° 0.66° 0.40° ormusin or eucalbanin 1.12° 00° 111° ellagic acid and derivatives: 2.9 valoneic acid or isomer <td>compounds in F3</td> <td></td> <td></td> <td></td> <td></td> | compounds in F3 | | | | |
| tetragalloylglucose 7.55° 197 1.89° pentagalloylglucose 14.02° 157.6 2.80° casuariin/desgalloylstachyurin 377.39° 196 94.35° ellagitannins: 101.5 pedunculagin 1.22° 196 0.30° if an interval inter | tetragalloylglucose | 1.39 ^{<i>c</i>} | 197 | 0.35 ^c | gallotannins: 5.0 |
| pentagalloylglucose 14.02^{c} 157.6 2.80^{c} casuariin/desgalloylstachyurin 377.39^{c} 196 94.35^{c} ellagitannins: 101.5 pedunculagin 1.22^{c} 196 0.30^{c} pedunculagin 2.44^{c} 196 0.61^{c} pedunculagin 2.44^{c} 196 0.61^{c} 0.61^{c} 0.61^{c} trisgalloyl HHDP glucose isomer 0.26^{c} 190.4 0.03^{c} 0.05^{c} oenothein B 18.43^{c} 196 2.30^{c} 0.05^{c} 0.04^{c} casuarinin 0.65^{c} 187.2 0.13^{c} 0.36^{c} 14.46^{c} 196.5 0.38^{c} HHDP digalloylglucose isomer 0.31^{c} 196.5 0.92^{c} 0.07^{c} 14.02^{c} 0.07^{c} HHDP digalloylglucose isomer 0.33^{c} 187.2 0.07^{c} 0.66^{c} 0.49^{d} 0.66^{c} 0.49^{d} 0.66^{c} 0.49^{d} 0.60^{c} 0.11^{c} 0.11^{c} 0.11^{c} 0.07^{c} 0.60^{c} | tetragalloylglucose | 7.55 [°] | 197 | 1.89 ^{<i>c</i>} | |
| casuariin/desgalloylstachyurin 377.39° 196 94.35° ellagitannins: 101.5pedunculagin 1.22° 196 0.30° pedunculagin 2.44° 196 0.61° trisgalloyl HHDP glucose isomer 0.17° 190.4 0.03° trisgalloyl HHDP glucose isomer 0.26° 190.4 0.05° centhein B 18.43° 196 2.30° casuarinin 0.65° 187.2 0.13° casuarinin 0.22° 187.2 0.04° tellimagrandin I 1.46° 196.5 0.86° HHDP digalloylglucose isomer 0.31° 196.5 0.92° casuaritin 0.33° 187.2 0.07° HHDP digalloylglucose isomer 0.31° 187.6 0.66° cornusiin or eucalbanin 0.49° 1086 $4.9q^{\circ}$ cornusiin or eucalbanin 0.49° 1086 1.12° valoneic acid or isomer 0.07° 470 0.07° ellagic acid 2.73° 302 2.73° total F3437.06199.42 213.25 | pentagalloylglucose | 14.02 ^{<i>c</i>} | 157.6 | 2.80 ^c | |
| pedunculagin 1.22^c 196 0.30^c pedunculagin 2.44^c 196 0.61^c trisgalloyl HHDP glucose isomer 0.17^c 190.4 0.03^c trisgalloyl HHDP glucose isomer 0.26^c 190.4 0.05^c oenothein B 18.43^c 196 2.30^c casuarinin 0.65^c 187.2 0.13^c casuarinin 0.22^c 187.2 0.04^c tellimagrandin I 1.46^c 196.5 0.36^c HHDP digalloylglucose isomer 0.31^c 196.5 0.92^c casuaritin 0.33^c 187.2 0.07^c tellimagrandin I 3.00^c 187.6 0.60^c cornusin or eucalbanin 0.49^d 1086 0.49^d cornusin or eucalbanin 1.12^d 1086 1.12^d valoneic acid or isomer 0.07^d 470 0.07^d ellagic acid 2.73^d 302 2.73^d total F3 437.06 109.42 213.25 | casuariin/desgalloylstachyurin | 377.39 ^c | 196 | 94.35 ^c | ellagitannins: 101.5 |
| pedunculagin 2.44° 196 0.61° trisgalloyl HHDP glucose isomer 0.17° 190.4 0.03° trisgalloyl HHDP glucose isomer 0.26° 190.4 0.05° oenothein B18.43^{\circ}196 2.30° casuarinin 0.65° 187.2 0.13° casuarinin 0.22° 187.2 0.04° tellimagrandin I 1.46° 196.5 0.36° HHDP digalloylglucose isomer 0.31° 196.5 0.92° casuarictin 0.33° 187.2 0.07° HHDP digalloylglucose isomer 3.70° 196.5 0.92° casuarictin 0.33° 187.2 0.07° HHDP digalloylglucose isomer 3.70° 196.5 0.92° casuarictin 0.33° 187.2 0.07° tellimagrandin II 3.00° 187.6 0.60° cornusiin or eucalbanin 0.49° 1.12° ellagic acid and derivatives: 2.9valoneic acid or isomer 0.11° 470 0.11° ellagic acid and derivatives: 2.9valoneic acid or isomer 0.07° 470 0.07° ellagic acid and derivatives: 2.9valoneic acid or isomer 0.11° 2.73° 302 2.73° total F3437.06 109.42 213.25 | pedunculagin | 1.22 ^c | 196 | 0.30 ^c | |
| trisgalloyl HDP glucose isomer 0.17^{c} 190.4 0.03^{c} trisgalloyl HHDP glucose isomer 0.26^{c} 190.4 0.05^{c} oenothein B18.43^{c}196 2.30^{c} casuarinin 0.65^{c} 187.2 0.13^{c} casuarinin 0.22^{c} 187.2 0.04^{c} tellimagrandin I 1.46^{c} 196.5 0.36^{c} HHDP digalloylglucose isomer 0.31^{c} 196.5 0.92^{c} casuaritin 0.33^{c} 187.2 0.07^{c} HHDP digalloylglucose isomer 3.70^{c} 196.5 0.99^{c} casuaritin 0.33^{c} 187.2 0.07^{c} tellimagrandin II 3.00^{c} 187.6 0.60^{c} cornusiin or eucalbanin 0.49^{d} 1086 1.12^{d} valoneic acid or isomer 0.07^{d} 470 0.11^{d} valoneic acid or isomer 0.07^{d} 470 0.07^{d} ellagic acid 2.73^{d} 302 2.73^{d} total F3437.06109.42total F1 + F2 + F3593.80213.25 | pedunculagin | 2.44 ^c | 196 | 0.61 ^{<i>c</i>} | |
| trisgalloyl HHDP glucose isomer 0.26° 190.4 0.05° oenothein B 18.43° 196 2.30° casuarinin 0.65° 187.2 0.13° casuarinin 0.22° 187.2 0.04° tellimagrandin I 1.46° 196.5 0.36° HHDP digalloylglucose isomer 0.31° 196.5 0.92° casuaritin 0.33° 187.2 0.07° HHDP digalloylglucose isomer 3.70° 196.5 0.92° casuaritin 0.33° 187.2 0.07° HHDP digalloylglucose isomer 0.33° 187.2 0.07° casuaritin 0.49^{d} 1086 0.49^{d} cornusiin or eucalbanin 0.49^{d} 1086 1.12^{d} valoneic acid or isomer 0.11^{d} 470 0.11^{d} valoneic acid or isomer 0.07^{d} 302 2.73^{d} total F3 437.06 109.42 total F1 + F2 + F3 593.80 213.25 | trisgalloyl HHDP glucose isomer | 0.17 ^c | 190.4 | 0.03 ^c | |
| oenothein B18.43°196 $2.30°$ casuarinin $0.65°$ 187.2 $0.13°$ casuarinin $0.22°$ 187.2 $0.04°$ tellimagrandin I $1.46°$ 196.5 $0.36°$ HHDP digalloylglucose isomer $0.31°$ 196.5 $0.08°$ HHDP digalloylglucose isomer $3.70°$ 196.5 $0.92°$ casuarictin $0.33°$ 187.2 $0.07°$ tellimagrandin I $3.00°$ 187.6 $0.60°$ cornusiin or eucalbanin $0.49°$ 1086 $0.49°$ cornusiin or eucalbanin $1.12°$ 1086 $1.12°$ valoneic acid or isomer $0.11°$ 470 $0.11°$ ellagic acid and derivatives: 2.9 $2.73°$ $2.73°$ total F3437.06109.42total F1 + F2 + F3593.80213.25 | trisgalloyl HHDP glucose isomer | 0.26 ^c | 190.4 | 0.05 ^c | |
| casuarinin 0.65° 187.2 0.13° casuarinin 0.22° 187.2 0.04° tellimagrandin I 1.46° 196.5 0.36° HHDP digalloylglucose isomer 0.31° 196.5 0.08° HHDP digalloylglucose isomer 3.70° 196.5 0.92° casuarictin 0.33° 187.2 0.07° tellimagrandin II 3.00° 187.6 0.60° cornusiin or eucalbanin 0.49^{d} 1086 0.49^{d} cornusiin or eucalbanin 1.12^{d} 1086 1.12^{d} valoneic acid or isomer 0.07^{d} 470 0.07^{d} ellagic acid 2.73^{d} 302 2.73^{d} total F3437.06 109.42 total F1 + F2 + F3 593.80 213.25 | oenothein B | 18.43 ^c | 196 | 2.30 ^c | |
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| total F1 + F2 + F3 593.80 213.25 | total F3 | 437.06 | | 109.42 | |
| | total F1 + F2 + F3 | 593.80 | | 213.25 | |

^a Correction factor: molecular weight/number of gallic (ellagic, flavonol) unit in the molecule. ^b Calculated using the correction factor. ^c Gallic acid equivalent. ^d Ellagic acid equivalent.

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that only tentative identification can be provided on the basis of UV-visible and MS spectra, even when associated with co-injection with standards. Further work should thus be performed to provide formal identification of phenolics tentatively characterized in this study.

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