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Ericifolin: An eugenol 5-*O*-galloylglucoside and other phenolics from *Melaleuca ericifolia*

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Abstract

Ericifolin, an eugenol 5-*O*-β-(6′-*O*-galloylglucopyranoside) possessing the naturally unknown phenolic moiety, 5-hydroxyeugenol, together with the two new phenolics, 2-*O*-*p*-hydroxybenzoyl-6-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose and 3-methoxyellagic acid 4-*O*-rhamnopyranoside have been isolated from the antibacterial leaves extract of *Melaleuca ericifolia*. In addition, 19 known phenolics were also separated and characterized. All structures were elucidated on the basis of analysis of ^1H, ^{13}C NMR, HMQC, HMBC and FTMS spectral data.

Keywords: Myrtaceae; Antimicrobial; *Melaleuca ericifolia*; Phenolics; Ericifolin; 2-*O*-*p*-hydroxybenzoyl-6-*O*-galloyl glucose; 3-Methoxyellagic acid 4-rhamnoside

1. Introduction

Despite the valuable discoveries made through studies of constitutive phenolics of several Myrtaceous species (Moharram et al., 2003; El-Toumy et al., 2001; Seligmann and Wagner, 1981), many of these plants remain virtually unexplored from a chemical stand point of view. Thus, we have initiated studies of two of these plants, namely, *Eugenia edulis* Vell. and *Eugenia jambos* L. as potential sources of new bioactive phenolics (Hussein et al., 2002; Hussein et al., 2003). Among the Myrtaceous genera, the genus *Melaleuca*, also known as bottle-brush comprises over 250 species of Australian trees and shrubs (Baily, 1958). They provided extracts which possess antiseptic, analgesic, vermifuge and antioxidant activities (Seligmann and Wagner, 1981; Bouchet et al., 1998). *Melaleuca ericifolia* Sm. in particular produces essential oil which possesses antimicrobial, antifungal and antiviral activities. The oil also exhibited a marked antioxidant effect that improve the parameters of vitamins E and C as well as superoxide dismutase enzyme, thus can be used as a free radical scavenger (Farag et al., 2004). As long as the available literature is concerned this plant has not been subjected to any previous phytochemical investigation of its constitutive phenolics. Due to our interest in biological activity as well as the diverse phenolic metabolites production of the Myrtaceous plants, investigations of the activity against multiresistant gram-positive and gram-negative bacteria and of the chemistry of the phenolic constituents in *M. ericifolia* were undertaken during the course of the present work. The plant is a large shrub or a small tree of 20 feet height. Its leaves are narrowly linear or nearly cylindrical of 1/2 in. or less long, while the flowers are yellowish white in cylindrical spikes of

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1/2 to 1 in. long. In the present study, chemical studies of the aqueous alcohol extract of leaves of this plant led to the isolation and structure determination of 22 phenolic compounds including three new phenolics, namely, eugenol 5-*O*-β-*(6′*-*O*-galloylglucopyranoside) which we named ericifolin (**6**), 2-*O*-*p*-hydroxybenzoyl-6-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose (**11**) and 3-methoxyellagic acid 4-*O*-α-L-rhamnopyranoside (**17**).

2. Results and discussion

During evaluation of its biological activity (Cooksey et al., 1993; Sleigh and Timburg, 1981), *M. ericifolia* showed a capacity [minimal inhibitory concentrations (MIC), see Section 3] to inhibit the growth of several gram-positive and gram-negative bacteria, among them *Staphylococcus aureus*. The test bacterial strains were selected because of their resistance properties and of their medical relevance. Besides, the activity of the pure new compounds **6**, **11** and **17** have also been evaluated (Table 1), whereby compound **17** has shown moderate inhibiting effects against the multiresistant bacterial strain *S. aureus* Norddeutscher Epidemiestamm. The remarkable antimicrobial effect of the extract could be due to the existing combination of constituents or it could be attributed to other compound(s) than **6**, **11** and **17.**

Following column chromatographic fractionation of the antibacterial extract obtained by extraction of the leaves of *M. ericifolia* by aqueous alcohol, 22 compounds (**1**–**22**) were isolated. Conventional and spectral analysis mainly by NMR spectroscopy and by mass spectrometry indicated that three of these compounds are new natural products (**6**, **11** and **17**).

Ericifolin (**6**), an off-white amorphous powder, showed chromatographic properties and color reactions (positive $FeCl_3$ and KIO_3 tests) specific for galloyl esters (Haddock et al., 1982). Normal acid hydrolysis of **6** (2 N aqueous HCl, 2 h, 100 °C) yielded glucose, gallic acid and a third compound **6a**, which was separated by preparative paper chromatography (Prep. PC). The parent compound **6** was recovered unchanged after being incubated with β-glucosidase enzyme (lyophilized chromatographically pure, salts free enzyme from Almond, BDH) for 24 h, at 37 °C, but it was partially hydrolysed (0.1 N aqueous HCl, 1/4 h, 100 °C) to 6-*O*-galloyl glucose (Nawwar and Hussein, 1994a) and **6a**). The latter presented UV maxima which were reminiscent of a phenyl propene system and exhibited an $[M]^+$ molecular ion at *m/z* 180 in its EI-MS spectrum. The ^1H NMR spectrum of **6a** was in consistence with a 3,4,5-trisubstituted phenyl propene structure (Table 2). These data suggested that **6a** is the 5-hydroxy derivative of eugenol. In the ^{13}C NMR spectrum of **6a**, the propenyl side chain was recognized from the resonances at δ 39.9 (C-7), 137.5 (C-8) and 115.4 (C-9). The aromatic resonances were assigned (Table 2) through the substituent additive rules applied when introducing a hydroxyl substituent at C-5 of the eugenol molecule. Final confirmation of the identity of **6a** as 5-hydroxy eugenol was achieved through synthesis (Rao et al., 1949). The parent compound, ericifolin (**6**) had the molecular formula $C_{23}H_{26}O_{12}$ as indicated by ESIFTMS ($[M-H]^-$: 493.1361, calc.: 493.1351). In the ^1H NMR spectrum of **6** the resonance of the aromatic proton H-6 at δ ppm 6.43, when compared with the corresponding resonance in the spectrum of free 5-hydroxyeugenol (Table 2) was found to be shifted downfield ($\Delta\delta = 0.13$ ppm), thus confirming *O*-glucosidation, at OH-5. The spectrum also showed a distinct anomeric glucose proton resonance at δ 4.7 and a pair of glucose proton resonances at δ 4.45 and 4.39 assignable to the two methylenic glucose protons whose geminal hydroxyl group is galloylated. Direct correlations observable in the HMQC and HMBC spectra allowed unambiguous assignment of the protonated carbons and enabled determination of site of attachments of the different moieties in the molecule of **6** (see Table 2). Among the ^3J correlations recognized in the HMBC spectrum of **6**, one was found correlating the methoxyl proton signal at δ 3.69 to the aromatic carbon signal of C-3 at δ 148.0, another correlated the anomeric glucose proton at δ 4.7 to the aromatic carbon signal of C-5 at δ 145.6 and a third correlated the methylenic glucose protons at δ 4.45 to the carbonyl carbon C-7″ at δ 165.9. These and the above

Table 1
Activity of the aqueous alcohol leaf extract and compounds **6**, **11** and **17**) of *Melaleuca ericifolia* against multiresistant bacteria

Bacterial strains	Inhibition zones (mm, including paper disc) against				
Test sample	*Staphylococcus aureus* Norddeutscher Epidemiestamm	*S. aureus* 34289	*S. epidermidis* 847	*S. hämolyticus* 535	*Pseudomonas aeruginosa* 595
Melaleuca ericifolia (2 mg/disc)	20	14	16	8	10
Compound **6** (2 mg/disc)	8	r	r	r	r
Compound **11** (2 mg/disc)	8	r	r	r	r
Compound **17** (2 mg/disc)	14	10	12	r	8
Control (30 μg/disc)	Amikacin 22	Amikacin 22	Amikacin 20	Amikacin 18	Doxycyclin 16

r, no activity.

Table 2
NMR spectral data of ericifolin (**6**), 5-hydroxyeugenol (**6a**), 3-methoxyellagic acid 4-*O*-α-L-rhamnopyranoside (**17**) and 3-methoxyellagic acid (**17a**)

	6			**6a**	
	δ_H (*J*, Hz)	δ_C	HMBC[a]	δ_H (*J*, Hz)	δ_C
1		130.0 *s*			130.7
2	6.45 (*br s*, $\Delta\nu_{1/2} = 4$)	109.3 *d*	4, 6, 7, 2, 3, 5_{weak}	6.46 (*br s*,*m* $\Delta\nu_{1/2} = 4$)	103.5
3		148.0 *s*			147.3
4		134.34 *s*			131.4
5		145.6 *s*			143.8
6	6.43 (*br s*, $\Delta\nu_{1/2} = 4$)	107.3 *d*	2, 4, 6, 7, 1_{weak}, 5_{weak}	6.30(*br s*, $\Delta\nu_{1/2} = 4$)	108.9
7	3.02 (8)	39.2 *t*	2, 6, 9, 1_{weak},	3.26(8)	39.9
8	5.72 (*m*)	137.9 *d*		5.92(*m*)	137.5
9_a	4.9 (14)	115.4 *t*	7	5.09(15)	115.4
9_b	4.8 (*br s*, $\Delta\nu_{1/2} = 4$)			4.96 (*br s*, $\Delta\nu_{1/2} = 4$)	
1′	4.7 (7.5)	102.5 *d*	5		
2′	3.30–3.70 (*m*)	74.11 *d*			
3′	3.30–3.70 (*m*)	75.6 *d*			
4′	3.30–3.70 (*m*)	70.00 *d*			
5′	3.30–3.70 (*m*)	73.4 *d*			
$6'_a$	4.45 (12)	63.58 *dd*	7″		
$6'_b$	4.39 (12 and 4.5)				
1″		119.3 *s*			
2″	6.96 *s*	108.7 *d*	4″, 6″, 2″, 7″		
3″		145.8 *s*			
4″		138.8 *s*			
5″		145.8 *s*			
6″	6.96 *s*	108.7 *d*	2″, 4″, 6″, 7″		
7″		165.9 *s*			
OMe	3.69	55.87 *q*	3	3.81	55.3
	17			**17a**	
	δ_H (*J*, Hz)	δ_C and ^1J (C · H)	HMBC[a]	δ_H (*J*, Hz)	δ_C
1		114.1 *s*			112.36
2		139.98 *s*			141.98
3		146.36 *s*			140.64
4		141.6 *s*			148.63
5	7.69 *s*	111.47 *d* (168 Hz)	1, 3, 7, $4_{(weak)}$	7.47 *s*	111.84
6		111.13 *s*			112.66
7		158.54 *s*			159.40
1′		112.8 *s*			112.98
2′		135.94 *s*			136.63
3′		141.3 *s*			140.21
4′		152.5 *s*			152.63
5′	7.47 *s*	111.35 *d* (165 Hz)	1′, 3′, 7′, $4'_{(weak)}$, $6'_{(weak)}$	7.42 *s*	110.83
6′		106.8 *s*			107.85
7′		158.50 *s*			159.30
OMe	4.03	60.84 *q* (146 Hz)	3	4.00	61.45
1″	5.46 (*br s*, $\Delta\nu_{1/2} = 4$)	100.02 *d* (173 Hz)	4		
2″		69.83 *d* (142 Hz)			
3″		70.00 *d* (145 Hz)			
4″		71.7 *d* (146 Hz)			
5″		69.76 *d* (141 Hz)			
Me	1.25 (*d*, $J = 6$ Hz)	17.75 *q* (128 Hz)			

[a] H–C.

given data finally confirmed the structure of compound **6** to be eugenol 5-*O*-β-(6′-galloylglucopyranoside) or ericifolin, a new phenolic, which has not been previously reported to occur in nature.

Compound **11** was obtained as an off-white amorphous powder and showed color reactions indicative of galloyl esters (Haddock et al., 1982). It exhibited an $[M-H]^-$ molecular ion in its ESIFTM mass at $m/z = 451.0877$ (calc.: 451.0871) corresponding to a M_r of 452 and to a molecular formula of $C_{20}H_{20}O_{12}$. On normal acid hydrolysis **11** yielded glucose, gallic and *p*-hydroxybenzoic acid (Hussein et al., 2006), but it was partially hydrolysed to 6-*O*-galloyl glucose (Nawwar and Hussein, 1994a) and *p*-hydroxybenzoic acid, thus suggesting that **11** is a mono-*O*-*p*-hydroxybenzoyl-6-*O*-galloyl glucose. The ^{1}H spectrum of **11** (DMSO-d_6, room temp.) revealed two distinct patterns of proton signals belonging to di-*O*-substituted α- and β-glucose anomers. In this spectrum, a pair of

doublets, centered at δ 5.0 ($J = 3.5$ Hz) and at δ 4.5 ($J = 8$ Hz) were assigned to the α- and β-glucose anomeric protons, respectively. The pair of downfield glucose proton signals at δ 4.4 and 4.52 were attributed to the glucose H-2 proton in the α- and β-glucose anomers, respectively. Consequently, the *p*-hydroxybenzyol moiety is present in the molecule of **11** attached to the glucose carbon C-2. In addition, the values of the above given coupling constants indicate that the glucose core in **11** is adopting a $^4C_1$-conformation. The weight of evidences given above, confirmed that compound **11** is 2-*O*-*p*-hydroxybenzoyl-6-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose. The ^{13}C NMR spectrum of **11** contained essentially double signals for most of the glucose, galloyl and *p*-hydroxybenzoyl carbons. Signals were assigned by comparison with the ^{13}C NMR data reported for 2,6-di-*O*-galloyl glucose (Nawwar and Hussein, 1994a). Attachment of the *p*-hydroxybenzoyl moiety to the glucose carbon C-2 was evidenced by the β-upfield shift recognized for the signals of the vicinal carbons, C-1 and C-3 [all in comparison with the chemical shifts of the corresponding signals in the spectrum of free α/β glucopyranose] (Nawwar et al., 1984a). In both anomers, C-2 was found resonating downfield (α-effect) at δ 74.7 (C-2α) and 76.3 (C-2β), thus confirming the final structure of **11** to be 2-*O*-*p*-hydroxybenzoyl-6-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose, which represents, to the best of our knowledge, a new natural product.

Compound **17** was obtained as a white amorphous powder. The molecular ion peak at *m/z* 461.0712 $[M-H]^-$ (calc.: 461.0715) observed by FTMS and the ^{1}H, ^{13}C and DEPT NMR data suggested the molecular formula $C_{21}H_{17}O_{12}$. The characteristic chromatographic properties (weak mauve spot on PC under UV light) and UV absorption maxima in methanol suggested the presence of ellagic acid derivative in **17**. The pronounced red shift of the absorption maxima at 249 and 273 (shoulder) nm of the aromatic chromophors in the molecule of (**17**), observed on addition of $NaOAC + H_3BO_3$ (see Section 3) might be attributed to the presence of free di-*ortho*-hydroxyl groups in the aromatic ring(s). Normal acid hydrolysis of **17** yielded rhamnose (Co-PC), and compound **17a**. The latter was also released on incubating **17** at 37 °C for 24 h, together with hesperidinase enzyme [α-L-rhamnosidase (Ec 3.2.1.40), lypholized powder from *Penicillium* species, Sigma]. Compound **17a** was extracted by EtOAc from the 2 N acidic hydrolysate. This has a molecular weight of 316 as established by EIMS ($[M]^+$ at $m/z = 316$), corresponding to a molecular formula of $C_{15}H_8O_8$. Chromatographic properties (yellowish buff spot on PC under UV light) and UV absorption maxima, together with the EIMS data suggested that **17a** is a monomethoxy-ellagic acid. Comparison of the 1D NMR data of **17a** with those of free ellagic acid (Nawwar et al., 1994b), and of 3,3′-dimethoxyellagic acid (Nawwar et al., 1982) indicated that the methoxyl function in **17a** is attached at C-3. Analysis of the ^{13}C spectrum of **17a**, reported here for the first time (see Table 2), was aided by comparison with the ^{13}C data reported for 3,3′-dimethoxyellagic acid (Sato, 1987) and for ellagic acid (Nawwar et al., 1994b) as well. These data confirmed the structure of **17a** to be 3-methoxyellagic acid. Consequently, the parent compound **17** is 3-methoxyellagic acid mono-α-*O*-L-rhamnopyranoside.

Comparison of the 1D NMR data proved that **17** contained a rhamnoside moiety which revealed its anomeric proton as a broad singlet at δ 5.46, of half-width ($\Delta\upsilon_{1/2} = 4$ Hz). This finding, together with the result of hydrolysis with hesperidinase enzyme proved the α-configuration of the existing rhamnose moiety. Besides, in a ^{1}H–^{13}C NMR spectrum of **17**, a high ^1J(C-1.H) of 173 Hz was measured from the signal of the proton coupled anomeric carbon, a coupling value which firmly establishing the α-configuration (Hansen, 1981) of the rhamnose moiety in **17**. In the ^{13}C NMR spectrum, the α-configuration was derived from the δ values of the recorded sugar resonances (Table 2) (Kalinowski et al., 1984). In the HMBC spectrum of **17**, a ^3J correlation of the anomeric rhamnose proton H-1″ (δ 5.46) to the aromatic carbon C-4 (δ 146.36) allowed positioning of this moiety at this carbon. The recognizable ^2J correlation of the downfield aromatic proton (δ 7.69) to the same C-4 carbon was in accordance with this conclusion. Correlations of the methoxyl protons (δ 4.02) to C-3 (δ 141.6) and of the same downfield aromatic proton (δ 7.69) to the same C-3 carbon confirmed that the site of attachment of the rhamnosyl moiety is at the C-4 position of the methoxy ellagic acid moiety. The complete structure of compound **17** was therefore determined to be 3-methoxyellagic acid 4-*O*-α-L-rhamnopyranoside. This is the first report of a 3-methoxyellagic glycoside bearing its sugar moiety at the same ring containing the methoxyl function (Kim et al., 2001; Yazaki and Hillis, 1976; Malhorta and Misra, 1981; Yang et al., 1998).

In addition, the known compounds, gallic acid (**1**), *p*-hydroxybenzoic acid (**2**), kaempferol 3-*O*-xylosyl-(1‴ 2″)-glucoside (**3**), quercetin 3-*O*-xylosyl-(1‴ 2″)-glucoside (**4**), myricetin 3-*O*-xylosyl-(1‴ 2″)-glucoside (**5**), 1,6-di-*O*-galloyl-β-glucose (**7**), 1-*O*-*p*-hydroxybenzoyl-6-*O*-galloyl-β-glucose (**8**), 2,3-di-*O*-galloyl glucose (**9**), 2,6-di-*O*-galloyl glucose (**10**), quercetin 3-*O*-galactoside (**12**), quercetin 3-*O*-glucoside (**13**), kaempferol 3-*O*-rhamnoside (**14**), quercetin 3-*O*-rhamnoside(**15**), myricetin 3-*O*-rhamnoside (**16**), 3,3′-dimethoxyellagic acid (**18**), ellagic acid (**19**), kaempferol (**20**), quercetin (**21**), and myricetin (**22**) were also isolated and identified by applying the conventional and spectral methods of analysis.

3. Experimental

3.1. General experimental procedures

^{1}H NMR spectra were measured by a Jeol ECA 500 MHz NMR spectrometer, at 500 MHz. ^{1}H chemical

shifts (δ) were measured in ppm, relative to TMS and ^{13}C NMR chemical shifts to DMSO-d_6 and converted to TMS scale by adding 39.5. Typical conditions: spectral width = 8 kHz for ^{1}H and 30 kHz for ^{13}C, 64 K data points and a flip angle of 45°. FTMS spectra were measured on a Finnigan LTQ-FTMS (Thermo Electron, Bremen, Germany) (Department of Chemistry, Humboldt-Universität zu, Berlin). UV recording were made on a Shimadzu UV–Visible-1601 spectrophotometer. Paper chromatographic analysis was carried out on Whatman No. 1 paper, using solvent systems: (1) H_2O; (2) 6 % HOAc; (3) BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, upper layer). Solvent 3 was used for PPC.

3.2. Plant materials

Collection of the leaves of *M. ericifolia* was made at the Nile river bank near Faculty of Pharmacy, Cairo University, in April 2004. Authentication was performed by Dr. M. El-Gebali, former researcher of Botany at the National Research Centre (NRC) of Cairo, Egypt. Voucher specimen was deposited at the herbarium of the NRC.

3.3. Preparation of extract for antibacterial assay

A 20 g sample of the leaves of the plant was homogenized in EtO–H_2O (3:1) (three extractions each with 100 ml). The dried filtrate of the homogenate was then subjected to antibacterial assay.

3.3.1. Antibacterial assay

The disc-diffusion method (Cooksey et al., 1993; Sleigh and Timburg, 1981) was used to determine the inhibition zones of the extract and compounds **6**, **11** and **17** (see Table 1). The following multiresistant test strains were used: *Staphylococcus aureus* Norddeutscher Epidemiestamm (multiresistant reference strain, Smith Kline Beecham), *S. aureus* 34289 (Friedrich Löffler Institute of Medical Microbiology, University Greifswald), *S. epidermidis* 847, *S. hämolyticus* 535, and *Pseudomonas aeruginosa* 595 (Institute of Hygiene, Greifswald). Paper discs (6 mm diameter) containing the material were deposited on the surface of inoculated agar plates (10 cm diameter) and incubated for 16 h at 37 °C. Minimal inhibitory concentration (MIC) values were determined by standard serial broth microdilution assay. All test strains were grown in nutrition medium II (SIFIN) and inoculated for 16 h at 37 °C.

The MIC of the crude extract was found to be

1. 19.5 μg/ml against *S. aureus* (Norddeutscher Epidemiestamm);
2. 39 μg/ml against the strains *S. aureus* 34289 and *S. epidermidis* 847;
3. 312 μg/ml against *S. haemolyticus* 535;
4. 1250 μg/ml against *P. aeruginosa* 595.

3.4. Extraction, isolation and identification of phenolics from *M. ericifolia* leaves

The fresh *M. ericifolia* leaves (5 kg) were homogenized in EtOH–H_2O (3:1) mixture (three extractions each with 5 L). The dried filtrate (230 g) of the homogenate was applied to a polyamide 6s (1500 gm) (Riedel-De-Hän Ag, Seelze Hannover, Germany) column (150 × 7.5 cm) and eluted with H_2O followed by H_2O–MeOH mixtures of decreasing polarities to yield seven fractions (I– VII), which were individually subjected to 2D-PC. Compounds **1** (145 mg) and **2** (125 mg) were isolated pure from fraction I (eluted with 20%) by Column fractionation (CF) of 3.19 g material over 35 g Sephadex LH-20 using 2 l of H_2O for elution. Compounds **3** (88 mg), **4** (102 mg) and **5** (54 mg) were individually separated from 762 mg of fraction II (eluted with 30 %) by prep. PC, using BAW as solvent, while compounds **6** (75 mg), **7** (78 mg), **8** (63 mg), **9** (111 mg) were obtained from 908 mg of fraction III (eluted with 40 %) through precipitation from an acetone solution by ether (thrice), and the subsequent prep. PC of the precipitate, using BAW as solvent. Repeated CF of 2.65 g of fraction IV (eluted with 50%) on Sephadex LH-20, using *n*-BuOH saturated with H_2O yielded pure samples of **10** (122 mg); **11** (79 mg) **12** (134 mg) **13** (102 mg) and **14** (85 mg). Compounds **15** (134 mg) and **16** (62 mg) were isolated from 775 mg of fraction V (eluted with 70%) by repeated prep. PC using BAW as solvent. Compound **17** (185 mg) was obtained pure by crystallization from MeOH (thrice) of 604 mg of fraction VI (eluted with 80 %). Compounds **18** (41 mg), **19** (28 mg), **20** (19 mg) **21** (39 mg) and **22** (16 mg) were individually isolated from 792 mg of the last major column fraction VII (eluted by MeOH) through repeated prep. PC, using BAW as solvent.

3.4.1. Eugenol 5-*O*-β-(6′-galloylglucopyranoside), ericifolin (**6**)

Off-white amorphous powder, $[\alpha]_D^{25}$ + 77.85° ($c = 0.14$, MeOH), R_f-values: 0.33 (H_2O), 0.48 (HOAc), 0.65 (BAW). UV max nm in MeOH: 278. HRFIMS: m/z = 493.1352 $[M-H]^-$ ($C_{23}H_{26}O_{12}$). Normal acid hydrolysis gave glucose, gallic acid and 5-hydroxyeugenol (**6a**) (Co-PC). Gallic acid: R_f-values: 0.33 (H_2O), 0.55 (HOAc), 0.78 (BAW). UV max nm in MeOH: 272. ^{1}H NMR: δ ppm 6.96s. 5-hydroxyeugenol (**6a**): R_f-values: 0.43 (H_2O), 0.68 (HOAc), 0.82 (BAW). UV max nm in MeOH: $272_{inflection}$, $278,350_{shoulder}$. EIMS: $[M]^+$: $m/z = 180$. ^{1}H NMR (Table 2). ^{13}C NMR (Table 2). Partial acid hydrolysis yielded 6-mono-*O*-galloyl glucose and 5-hydroxyeugenol. 6-mono-*O*-galloyl glucose: R_f-values: 0.67 (H_2O), 0.75 (HOAc), 0.62 (BAW). UV max nm in MeOH: 273. ^{1}H NMR: δ ppm: α-glucose moiety: 5.10 (1H, *d*, $J = 3.5$ Hz, H-1); 3.50–3.90 (sugar protons overlapped with water protons); 3.92 (1H, *m*, H-5); 4.38 (1H, *d*, $J = 12.5$ Hz, H-6_a); 4.25 (1H, *dd*, $J = 12.5$ and 4.5 Hz, H-6_b); β-glucose moiety: 4.60 (1H, *d*, $J = 8$ Hz, H-1); 3.50–3.90 (*m*, sugar protons

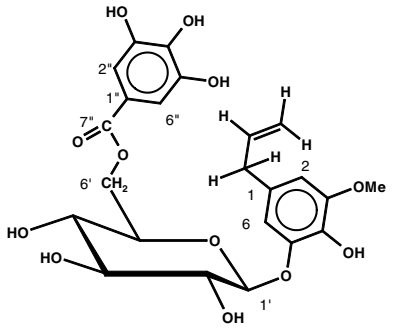
overlapped with water protons); 3.93 (1H, *m*, H-5); 4.42 (1H, *d*, $J = 12.5$ Hz, H-6_a); 4.30 (1H, *dd*, $J = 12.5$ and 4.5 Hz, H-6_b); galloyl moieties: 6.99s, 7.00s. NMR data of **6**: Table 2.

3.4.2. 2-*O*-p-hydroxybenzoyl-6-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose (*11*)

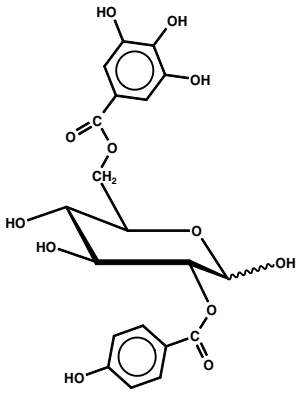
Off-white amorphous powder, $[\alpha]_D^{25} - 44.6^\circ$ ($c = 0.7$, MeOH), R_f-values: 0.45 (H_2O), 0.56 (HOAc), 0.41 (BAW). UV max nm in MeOH: 262. HRFIMS: $m/z = 451.0877$ $[M-H]^-$ ($C_{20}H_{19}O_{12}$). Normal acid hydrolysis gave glucose, gallic acid and *p*-hydroxybenzoic acid. *p*-hydroxybenzoic acid: R_f-values: 0.54 (H_2O), 0.72 (HOAc), 0.88 (BAW). UV max nm in MeOH: 253. ^{1}H: δ ppm 6.8 (2H, *d*, $J = 8$ Hz, H-3 and H-5); 7.75 (2H, *d*, $J = 8$ Hz, H-2 and H-6). ^{13}C NMR: δ ppm 167.5 (C=O), 123.0 (C-1), 132.2 (C-2 and C-6), 115.5 (C-3 and C-5), 161.3 (C-4). Partial acid hydrolysis yielded 6-mono-*O*-galloyl glucose and *p*-hydroxybenzoic acid (Co-PC). ^{1}H NMR of **11**: δ ppm: α-glucose moiety: 5.02 (1H, *d*, $J = 3.5$ Hz, H-1); 4.44 (1H, *dd*, $J = 8$ and 3.5 Hz, H-2); 3.67 (1H, *t*, $J = 8$ Hz- H-3); 3.6–3.3 (*m*, sugar protons overlapped with water protons); 3.84 (1H, *m*, H-5); 4.34 (1H, *d*, $J = 12.5$ Hz, H-6_a); 4.15 (1H, *dd*, $J = 12.5$ and 4.5 Hz, H-6_b); β-glucose moiety: 4.53 (1H, *d*, $J = 8$ Hz, H-1); 4.52 (1H, *t*, $J = 8$ Hz, H-2); 3.30–3.60 (sugar protons overlapped with water protons); 3.92 (1H, *m*, H-5); 4.37 (1H, *d*, $J = 12.5$ Hz, H-6_a); 4.17 (1H, *dd*, $J = 12.5$ and 4.5 Hz, H-6_b); galloyl moieties: 6.87s, 6.82s; *p*-hydroxybenzoyl moieties: 6.74 and 6.75 (each as a doublet, $J = 8$ Hz, H-3 and H-5 in each anomer); 7.69 and 7.70 (each as a doublet, $J = 8$ Hz, H-2 and H-6 in each anomer). ^{13}C NMR data of **11**: δ ppm: α-glucose moiety: 89.91 (C-1), 74.7(C-2), 71.2(C-3), 70.9(C-4), 70.8 (C-5), 64.17 (C-6); β-glucose moiety: 95.3 (C-1), 76.3 (C-2), 74.7 (C-3), 71.9 (C-4), 74.4 (C-5), 64.2 (C-6); galloyl moieties: 120.8, 119.9 (C-1), 109.26, 109.37 (C-2 and C-6), 146.03 (C-3 and C-5), 138.9, 139.0 (C-4), 166.05, 166.10 (C=O); *p*-hydroxybenzoyl moieties: 120.78, 120.80 (C-1), 132.0 (C-2 and C-6), 115.89 (C-3 and C-5), 162.85, 162.609 (C=O).

3.5. 3-Methoxyellagic acid 4-*O*-α-rhamnopyranoside (*17*)

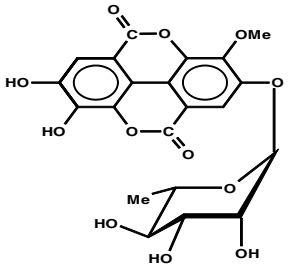
White amorphous powder, $[\alpha]_D^{25} - 14.15^\circ$ ($c = 0.077$, MeOH), R_f-values: 0.23 (H_2O), 0.38 (HOAc), 0.50 (BAW). UV max nm in MeOH 249,$273_{shoulder}$, $335_{shoulder}$, 363; in MeOH + NaOAC + H_3BO_3: 254, $315_{shoulder}$, 354. HRFIMS: $m/z = 461.0715$ $[M-H]^-$ ($C_{21}H_{25}O_{12}$). Normal acid hydrolysis gave rhamnose and 3-methoxyellagicacid (**17a**) (Co-PC). 3-Methoxyellagicacid (**17a**): R_f-values: 0.03 (H_2O), 0.10 (HOAc), 0.76 (BAW). UV max nm in MeOH 251, $348_{shoulder}$, 369; in MeOH + NaOAC + H_3BO_3 257, $315_{shoulder}$, 375 nm. EIMS: $[M]^+$at $m/z = 316$. NMR data: Table 2. 3-Methoxyellagic acid 4-*O*-α-rhamnopyranoside (**17**): NMR data: Table 2.



Compound (**6**): Eugenol-5-*O*-β-(6'-*O*-galloylglucopyranoside)



Compound (**11**): 2-*O*-*p*-hydroxybenzoyl-6-*O*-galloyl-α/β-$^4C_1$-glucopyranose



Compound (**17**): 3-methoxyellagic acid 4-*O*-α-rhamnopyranoside

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