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Natural feruloyl monoglyceride macrocycles as protecting factors against free-radical damage of lipidic membranes

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Abstract—Nine novel natural feruloyl monoglyceride (MGs) macrocycles have been isolated from the leaves of *Carex distachya*, an herbaceous plant growing in the Mediterranean maquis. All the structures have been elucidated on the basis of their spectroscopic features, especially two-dimensional NMR (DQ-COSY, TOCSY, NOESY, ROESY, HSQC, HMBC, HSQC–TOCSY) and ESI-MS. All the compounds have been assayed as protecting factors against the radical damage of the lipids by using different antioxidant tests.

Lipid peroxidation, which can cause extensive damage to subcellular organelles and biomembranes has been demonstrated to occur in isolated mitochondria, lysosomes and microsomes.[1] The destruction of unsaturated fatty acids which occurs in lipid peroxidation has been linked with altered membrane structure and enzyme inactivation. In addition to lipid hydroperoxides and lipid radicals, lipid peroxidation generates activated oxygen species, such as hydroxyl radicals and radical superoxide anions. Also, oxidative degradation of polyunsaturated membrane lipids leads to a variety of toxic carbonyl products such as hydroxyalkenals and other products.[2] Malondialdehyde (MDA) is a characteristic product of lipid peroxidation; it not only allows reaction with cellular nucleophiles but leads to self-condensation to form MDA oligomers. MDA is mutagenic and carcinogenic.[3] Hence, agents with the ability to protect against these reactive species may be therapeutically useful. In line with this hypothesis is the widely accepted view that the positive health effects of polyphenols, as cinnamic acids and flavonoids, can be attributed to their antioxidant activity. Ferulic acid is a potent ubiquitous plant antioxidant. Recent studies indicate the synergistic protective effect of ferulic acid and ascorbic acid on lipid peroxidation and antioxidant defense system during induced myocardial infarction and associated oxidative stress in rats.[4] In the search of new secondary metabolites from natural sources with antioxidant and radical scavenging activities, we studied *Carex distachya*,[5] an herbaceous plant belonging to the Cyperaceae family. From organic extract of the plant we recently reported the isolation and the characterization of new antioxidant metabolites.[6,7]

Continuing the phytochemical study of the plant[8] we isolated nine new feruloyl monoglyceride macrocycles showing a strong antioxidant activity against reactive oxygen species and an inhibition of the MDA synthesis.

The extract of the fresh leaves of *Carex distachya* in ethyl acetate was chromatographed on silica gel and HPLC to effort nine new compounds **1**–**9**.[9]

Compound **1** (Fig. 1) showed a molecular formula $C_{32}H_{50}O_7$ calculated on the basis of the elemental analysis and the ESI-MS spectrum that showed the pseudomolecular peak at *m/z* 547. This formula indicated the presence of eight unsaturations in the molecule.

The ^{1}H NMR spectrum showed five protons in the olefinic region. There was a 1,3,4-trisubstituted aromatic ring, as two doublets at δ 7.18 (J = 1.8 Hz) and 6.80 (J = 8.4 Hz), a double doublet at δ 7.06 (J = 8.4 and 1.8 Hz), and a double bond conjugated with a carbonyl group as two doublets at δ 6.35 (J = 15.9 Hz) and 7.59

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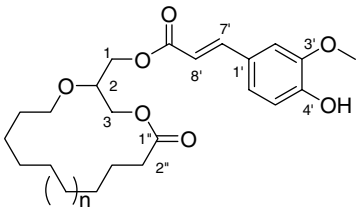


1 n = 10
2 n = 12
3 n = 15
4 n = 16
5 n = 18
6 n = 20
7 n = 21

Figure 1. Chemical structures of feruloyl MGs **1**–**7** from *Carex distachya*.

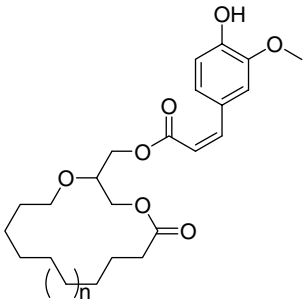
(J = 15.9 Hz). The coupling constant value (J = 15.9 Hz) was in good accordance with a trans geometry. The methoxyl signal as a singlet at δ 3.89 suggested the presence of a trans feruloyl group. In the carbinolic region of the proton magnetic resonance spectrum, three methylene protons as a triplet at δ 4.17, and two double doublets at δ 3.56/3.53 and 4.14/4.05, besides a methinic proton as multiplet at δ 3.82, were evident. In the upfield region of the spectrum, three methylene protons at δ 2.34, 1.67 and 1.33 and further 26 overlapped protons centered at δ 1.29 were present. The ^{13}C NMR showed 10 carbon signals of the ferulate. The comparison of the ^{13}C NMR with a DEPT experiment indicated the presence, in the carbinol region, of three methylenes at δ 66.5, 65.6 and 64.1 and of a methine at δ 71.2. The remaining signals resulted in methylene groups resonating between 26.0 and 34.9 ppm besides the carbonyl group at δ 175.5. The HSQC experiment showed all the one-bond proton/carbon correlations, and these were used to assign the carbon shifts to the corresponding protons (Table 1). The HSQC–TOCSY experiment allowed the proton–carbon connectivities to be determined: the proton at δ 3.82 attached to the carbon at δ 71.2, showed correlations to protons resonating at δ 3.56/3.53 and 4.14/4.05. All these protons showed correlations to the carbon atoms resonating at δ 64.1 and 66.5. These data together with the chemical shift of the methylene protons suggested the presence of a diacyl glycerol moiety. The HMBC experiment confirmed this hypothesis, showing correlations between the protons at δ 3.56/3.53 with the carboxyl group at δ 169.4, which showed, in turn, cross peaks with the olefinic protons at δ 6.35 and 7.59. The protons at δ 4.14/4.05 were correlated to the carboxyl at δ 175.5 which was in turn correlated with the methylene protons at δ 2.34 and 1.62. The methine proton at δ 3.82, besides the correlations with the methylene carbons of the glycerol, showed cross peak with the methylene at δ 65.6. This carbon was correlated with the protons at δ 4.17 in the HSQC experiment which was correlated, in the HSQC–TOCSY experiment, with the methylene protons ad δ 1.67, 1.33 and 1.29, which were correlated to the carbon atoms resonating at δ 29.8, 27.1, and 30.7 ppm. These data suggested the presence of a glycerol esterificated to the C-1 carbon with ferulic acid and to the C-3 carbon with a C-19 fatty acid. This latter acyl closed a C-23 members macrocycle through an ether bridge among its C-19 carbon and the C-2 carbon of the glycerol moiety.

The ESI-MS spectrum confirmed this hypothesis. In fact, besides the peaks corresponding to the molecular ion and to the lost of the methoxyl (m/z 516) and the

Table 1. NMR data[a] of feruloyl MG **1**

	δ_H	COSY	δ_C	DEPT	HMBC (H → C)
1	3.56 dd (10.9, 6.6 Hz) 3.53 dd (10.9, 5.7 Hz)	2	64.1	CH_2	2, 9′, 19″
2	3.82 m	1, 3	71.2	CH	1, 3, 19″
3	4.14 dd (11.4, 4.5 Hz) 4.05 dd (11.4, 6.0 Hz)	2	66.5	CH_2	1, 2, 1″
1′	—		127.7	C	—
2′	7.18 d (1.8 Hz)	6′	111.7	CH	3′, 4′, 6′
3′	—		149.4	C	—
4′	—		150.6	C	—
5′	6.80 d (8.4 Hz)	6′	116.5	CH	1′, 3′, 4′, 6′
6′	7.06 dd (8.4, 1.8 Hz)	2′, 5′	124.1	CH	1′, 4′, 5′
7′	7.59 d (15.9 Hz)	8′	146.7	CH	1′, 6′, 8′
8′	6.35 d (15.9 Hz)	7′	115.6	CH	1′, 7′, 9′
9′	—		169.4	C	—
OMe	3.89 s		56.4	CH_3	3′
1″	—		175.5	C	—
2″	2.34 t (7.5 Hz)	3″	34.9	CH_2	1″, 3″
3″	1.62 m	2″, 4″	26.0	CH_2	1″, 2″, 5″
4″–16″	1.29 ov		30.2–30.7	CH_2	
17″	1.33 m	16″, 18″	27.1	CH_2	18″
18″	1. 67 m	17″, 19″	29.8	CH_2	17″, 19″
19″	4.17 t (6.6 Hz)	18″	65.6	CH_2	2, 18″

[a]Data were recorded in CD_3OD on Varian Mercury 300 MHz (^{1}H, ^{13}C) spectrometer (DQ-COSY, TOCSY, HSQC, HMBC, ROESY, NOESY and HSQC–TOCSY); chemical shifts (δ) were expressed in parts per million with reference to the signal of CD_3OD (δ 3.31 ppm) for ^{1}H, and to the center peak of the signal of CD_3OD (δ 49.0 ppm) for ^{13}C, respectively. d, doublet; dd, double doublet; m, multiplet; ov, overlapped; t, triplet.



8 n = 16
9 n = 25

Figure 2. Chemical structures of feruloyl MGs **8** and **9** from *Carex distachya*.

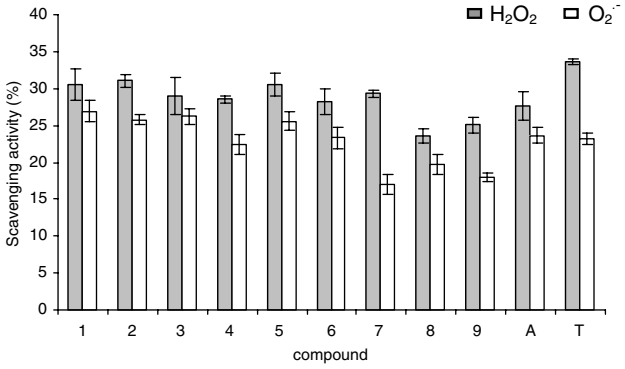


Figure 3. Scavenging activities of feruloyl MG **1**–**9** from *Carex distachya* on pro-oxidant hydrogen peroxide and anion superoxide radical. A, ascorbic acid; T, α-tocopherol.

hydroxyl (*m*/*z* 530) fragments, it showed a signal at *m*/*z* 177 due to the feruloyl fragment.

The NMR data of compounds **2**–**5** (Fig. 1) were superimposable to those showed by the previous described metabolite. The differences were evidenced by the ESI-MS spectra which showed pseudo molecular peaks which differed for multiple 14 uma, indicating the presence of superior homologues molecules having C-21, C-24, C-25, C-27, C-29 and C-30 acyl chains, respectively.

Compounds **8** and **9** (Fig. 2) showed similar ^{1}H NMR data of previously compounds. The main difference regarded the NMR values of the double bond of the feruloyl moiety, which was in accordance with the cis isomer to the 7′–8′ carbons.

The ESI-MS spectra allowed to determine the C-25 and C-30 chain length of the fatty acids for **8** and **9**, respectively.

The new feruloyl monoglyceride macrocycles were tested for their scavenging activity against the pro-oxidant hydrogen peroxide[10] and the anion superoxide radical[11] (Fig. 3) and for antioxidant activity by inhibition of TBARS (thiobarbituric acid reactive species) in rat liver microsomes[12,13] (Fig. 4). The registered activities

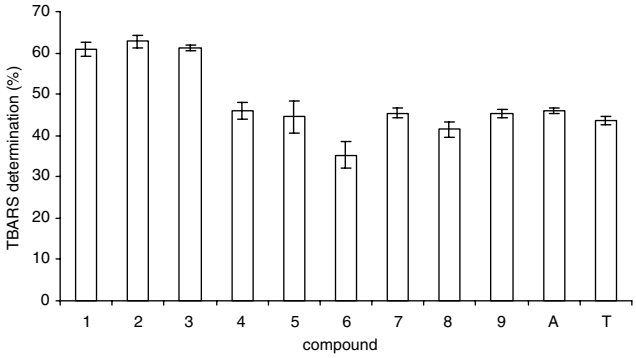


Figure 4. Inhibition of the formation of TBARS substances by feruloyl MG **1**–**9** from *Carex distachya*. A, ascorbic acid; T, α-tocopherol.

were compared to those exercised by the natural antioxidants ascorbic acid and α-tocopherol. The results confirm the efficacy of all the compounds as anti-lipoperoxidants. A comparison of inhibitory activities of the compounds with structural features reveals that the chain length and the geometry of the olefinic bond of ferulate moiety appear to have a role in the observed effects.

Recently it was reported that ferulic acid derivatives could be strong antioxidant than acid by self. Studies on caffeic acid, dihydrocaffeic acid and some of their corresponding esters showed that their antioxidant activity in biological systems depends also on the ester chain, suggesting the importance on this modification.[14]

Acknowledgments

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8. Fresh leaves of *Carex distachya* (6.0 kg) were extracted with EtOAc for 5 days, to obtain 67.0 g of residual material. The EtOAc extract was chromatographed on silica gel, with hexane and EtOAc solutions. The fraction eluted with hexane–EtOAc (2:3) was rechromatographed on Sephadex LH-20® eluting with hexane-$CHCl_3$–MeOH (3:1:1) to obtain one fraction which, purified initially by flash-column SiO_2 with $CHCl_3$–Me_2CO (17:3) and then by HPLC using an RP-8 preparative column eluting with MeOH–MeCN–H_2O (5:4:1), gave pure compounds **1**–**9**.
9. Feruloyl MG (**1**): 1.0 mg, colorless oil, $[\alpha]_D^{25} + 17.1$, UV λ_{max} (log ε) (MeOH) 323 (4.02), 231 (3.91), 219 (3.97) nm; ESI-MS *m/z* 547 $[M+H]^+$ (100), 530 $[M+H–OH]^+$, 516 $[M+H–OMe]^+$. ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 1 Anal. Calcd for $C_{32}H_{50}O_7$: C, 70.30; H, 9.22. Found: C, 70.27; H, 9.24.
Feruloyl MG (**2**): 1.3 mg, colorless oil, $[\alpha]_D^{25} + 14.0$, UV λ_{max} (log ε) (MeOH) 325 (4.01), 235 (3.88), 217 (3.92) nm; ESI-MS *m/z* 575 $[M+H]^+$ (100), 558 $[M+H–OH]^+$, 544 $[M+H–OMe]^+$. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.55 (dd, *J* = 10.9 and 6.6 Hz, H-1a); 3.53 (dd, *J* = 10.9 and 5.6 Hz, H-1b); 3.83 (m, H-2); 4.15 (dd, *J* = 11.2 and 4.6 Hz, H-3a); 4.03 (dd, *J* = 11.2 and 5.8 Hz, H-3b); 7.18 (d, *J* = 1.8 Hz, H-2′); 6.80 (d, *J* = 7.8 Hz, H-5′); 7.05 (dd, *J* = 7.8 and 1.8 Hz, H-6′); 7.59 (d, *J* = 15.9 Hz, H-7′); 6.35 (d, *J* = 15.9 Hz, H-8′); 3.89 (s, OCH_3); 2.34 (t, *J* = 7.5 Hz, H-2″); 1.62 (m, H-3″); 1.28 (ov, H-4″–H-18″); 1.37 (m, H-19″); 1.70 (m, H-20″); 4.17 (t, *J* = 6.6 Hz, H-21″); ^{13}C NMR (75 MHz, CD_3OD) δ: 64.1 (C-1); 71.3 (C-2); 66.4 (C-3); 127.5 (C-1′); 111.7 (C-2′); 149.4 (C-3′); 150.7 (C-4′); 116.3 (C-5′); 124.1 (C-6′); 146.6 (C-7′); 115.6 (C-8′); 169.3 (C-9′); 56.4 (OCH_3); 175.6 (C-1″); 34.7 (C-2″); 26.1 (C-3″); 30.3–30.7 (C-4″–C-18″); 27.1 (C-19″); 29.6 (C-20″); 65.8 (C-21″). Anal. Calcd for $C_{34}H_{54}O_7$: C, 71.05; H, 9.47. Found: C, 71.07; H, 9.44.
Feruloyl MG (**3**): 2.4 mg, colorless oil, $[\alpha]_D^{25} + 24.6$, UV λ_{max} (log ε) (MeOH) 323 (3.88), 232 (3.76), 217 (3.99) nm; ESI-MS *m/z* 617 $[M+H]^+$ (100), 600 $[M+H–OH]^+$, 586 $[M+H–OMe]^+$. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.55 (dd, *J* = 10.9 and 6.6 Hz, H-1a); 3.53 (dd,*J* = 10.9 and 5.6 Hz, H-1b); 3.83 (m, H-2); 4.13 (dd, *J* = 11.4 and 4.6 Hz, H-3a); 4.03 (dd, *J* = 11.4 and 6.0 Hz, H-3b); 7.18 (d, *J* = 1.8 Hz, H-2′); 6.80 (d, *J* = 7.8 Hz, H-5′); 7.05 (dd, J = 7.8 and 1.8 Hz, H-6′); 7.59 (d, *J* = 15.9 Hz, H-7′); 6.35 (d, *J* = 15.9 Hz, H-8′); 3.89 (s, OCH_3); 2.34 (t, *J* = 7.5 Hz, H-2″); 1.62 (m, H-3″); 1.28 (ov, H-4″–H-21″); 1.37 (m, H-22″); 1.70 (m, H-23″); 4.17 (t, *J* = 6.6 Hz, H-24″); ^{13}C NMR (75 MHz, CD_3OD) δ: 64.1 (C-1); 71.3 (C-2); 66.4 (C-3); 127.5 (C-1′); 111.7 (C-2′); 149.4 (C-3′); 150.7 (C-4′); 116.3 (C-5′); 124.1 (C-6′); 146.6 (C-7′); 115.6 (C-8′); 169.3 (C-9′); 56.4 (OCH_3); 175.6 (C-1″); 34.7 (C-2″); 26.1 (C-3″); 30.3–30.7 (C-4″–C-21″); 27.1 (C-22″); 29.6 (C-23″); 65.8 (C-24″). Anal. Calcd for $C_{37}H_{60}O_7$: C, 72.04; H, 9.80. Found: C, 72.06; H, 9.78.
Feruloyl MG (**4**): 5.9 mg, colorless oil, $[\alpha]_D^{25} + 4.1$, UV λ_{max} (log ε) (MeOH) 325 (4.04), 235 (3.88), 219 (3.95) nm; ESI-MS *m/z* 631 $[M+H]^+$ (100), 614 $[M+H–OH]^+$, 600 $[M+H–OMe]^+$. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.57 (dd, *J* = 10.9 and 6.6 Hz, H-1a); 3.53 (dd, *J* = 10.9 and 5.6 Hz, H-1b); 3.83 (m, H-2); 4.14 (dd, *J* = 11.2 and 4.7 Hz, H-3a); 4.03 (dd, *J* = 11.2 and 5.6 Hz, H-3b); 7.18 (d, *J* = 1.8 Hz, H-2′); 6.80 (d, *J* = 8.4 Hz, H-5′); 7.05 (dd, *J* = 8.4 and 1.8 Hz, H-6′); 7.59 (d, *J* = 15.6 Hz, H-7′); 6.35 (d, *J* = 15.6 Hz, H-8′); 3.89 (s, OCH_3); 2.34 (t, *J* = 7.5 Hz, H-2″); 1.62 (m, H-3″); 1.27 (ov, H-4″–H-22″); 1.36 (m, H-23″); 1.68 (m, H-24″); 4.17 (t, *J* = 6.6 Hz, H-25″); ^{13}C NMR (75 MHz, CD_3OD) δ: 64.1 (C-1); 71.3 (C-2); 66.4 (C-3); 127.5 (C-1′); 111.7 (C-2′); 149.4 (C-3′); 150.7 (C-4′); 116.3 (C-5′); 124.1 (C-6′); 146.6 (C-7′); 115.6 (C-8′); 169.3 (C-9′); 56.3 (OCH_3); 175.6 (C-1″); 34.7 (C-2″); 26.1 (C-3″); 30.3–30.7 (C-4″–C-22″); 27.1 (C-23″); 29.6 (C-24″); 65.8 (C-25″). Anal. Calcd for $C_{38}H_{62}O_7$: C, 72.34; H, 9.91. Found: C, 72.37; H,9.88.
Feruloyl MG (**5**): 2.0 mg, colorless oil, $[\alpha]_D^{25} + 10.8$, UV λ_{max} (log ε) (MeOH) 322 (4.01), 235 (3.86), 215 (4.02) nm; ESI-MS *m/z* 659 $[M+H]^+$ (100), 642 $[M+H–OH]^+$, 628 $[M+H–OMe]^+$. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.55 (dd, *J* = 10.9 and 6.6 Hz, H-1a); 3.53 (dd, *J* = 10.9 and 5.6 Hz, H-1b); 3.83 (m, H-2); 4.12 (dd, *J* = 11.3 and 4.8 Hz, H-3a); 4.05 (dd, *J* = 11.3 and 6.0 Hz, H-3b); 7.18 (d, *J* = 1.8 Hz, H-2′); 6.80 (d, *J* = 7.8 Hz, H-5′); 7.05 (dd, *J* = 7.8 and 1.8 Hz, H-6′); 7.59 (d, *J* = 15.9 Hz, H-7′); 6.35 (d, *J* = 15.9 Hz, H-8′); 3.89 (s, OCH_3); 2.34 (t, *J* = 7.5 Hz, H-2″); 1.62 (m, H-3″); 1.28 (ov, H-4″–H-24″); 1.37 (m, H-25″); 1.70 (m, H-26″); 4.17 (t, *J* = 6.6 Hz, H-27″); ^{13}C NMR (75 MHz, CD_3OD) δ: 64.1 (C-1); 71.3 (C-2); 66.4 (C-3); 127.5 (C-1′); 111.7 (C-2′); 149.4 (C-3′); 150.7 (C-4′); 116.3 (C-5′); 124.1 (C-6′); 146.6 (C-7′); 115.6 (C-8′); 169.3 (C-9′); 56.3 (OCH_3); 175.6 (C-1″); 34.7 (C-2″); 26.1 (C-3″); 30.3–30.7 (C-4″–C-24″); 27.1 (C-25″); 29.6 (C-26″); 65.8 (C-27″). Anal. Calcd for $C_{40}H_{66}O_7$: C, 72.91; H, 10.10. Found: C, 72.89; H, 10.08.
Feruloyl MG (**6**): 3.0 mg, colorless oil, $[\alpha]_D^{25} + 3.0$, UV λ_{max} (log ε) (MeOH) 323 (4.02), 235 (3.94), 215 (3.96) nm; ESI-MS *m/z* 687 $[M+H]^+$ (100), 670 $[M+H–OH]^+$, 656 $[M+H–OMe]^+$. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.55 (dd, *J* = 10.9 and 6.6 Hz, H-1a); 3.53 (dd, *J* = 10.9 and 5.6 Hz, H-1b); 3.83 (m, H-2); 4.14 (dd, *J* = 11.2 and 4.5 Hz, H-3a); 4.04 (dd, *J* = 11.2 and 6.0 Hz, H-3b); 7.18 (d, *J* = 1.8 Hz, H-2′); 6.80 (d, *J* = 7.8 Hz, H-5′); 7.05 (dd, *J* = 7.8 and 1.8 Hz, H-6′); 7.59 (d, *J* = 16.2 Hz, H-7′); 6.35 (d, *J* = 16.2 Hz, H-8′); 3.89 (s, OCH_3); 2.34 (t, *J* = 7.5 Hz, H-2″); 1.61 (m, H-3″); 1.28 (ov, H-4″–H-26″); 1.35 (m, H-27″); 1.66 (m, H-28″); 4.17 (t, *J* = 6.6 Hz, H-29″);^{13}C NMR (75 MHz, CD_3OD) δ: 64.1 (C-1); 71.3 (C-2); 66.4 (C-3); 127.5 (C-1′); 111.7 (C-2′); 149.4 (C-3′); 150.7 (C-4′); 116.3 (C-5′); 124.1 (C-6′); 146.6 (C-7′); 115.6 (C-8′); 169.3 (C-9′); 56.2 (OCH_3); 175.6 (C-1″); 34.7 (C-2″); 26.1 (C-3″); 30.3-30.7 (C-4″–C-26″); 27.1 (C-27″); 29.6 (C-28″); 65.8 (C-29″). Anal. Calcd for $C_{42}H_{70}O_7$: C, 73.43; H, 10.27. Found: C, 73.41; H, 10.25.
Feruloyl MG (**7**): 2.0 mg, colorless oil, $[\alpha]_D^{25} + 11.4$, UV λ_{max} (log ε) (MeOH) 323 (4.01), 235 (3.98), 217 (3.89) nm; ESI-MS *m/z* 701 $[M+H]^+$ (100), 684 $[M+H–OH]^+$, 670 $[M+H–OMe]^+$. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.55 (dd, *J* = 10.9 and 6.6 Hz, H-1a); 3.53 (dd, *J* = 10.9 and 5.6 Hz, H-1b); 3.83 (m, H-2); 4.14 (dd, *J* = 11.2 and 4.5 Hz, H-3a); 4.05 (dd, *J* = 11.2 and 5.8 Hz, H-3 b); 7.18 (d, *J* = 1.8 Hz, H-2′); 6.80 (d, *J* = 7.8 Hz, H-5′); 7.05 (dd, *J* = 7.8 and 1.8 Hz, H-6′); 7.59 (d, *J* = 15.9 Hz, H-7′); 6.35 (d, *J* = 15.9 Hz, H-8′); 3.89 (s, OCH_3); 2.34 (t, *J* = 7.5 Hz, H-2″); 1.62 (m, H-3″); 1.28 (ov, H-4″–H-27″); 1.37 (m, H-28″); 1.70 (m, H-29″); 4.17 (t, *J* = 6.6 Hz, H-30″); ^{13}C NMR (75 MHz, CD_3OD) δ: 64.1 (C-1); 71.3 (C-2); 66.4 (C-3); 127.5 (C-1′); 111.7 (C-2′); 149.4 (C-3′); 150.7 (C-4′); 116.3 (C-5′); 124.1 (C-6′); 146.6 (C-7′); 115.6 (C-8′); 169.3 (C-9′); 56.4 (OCH_3); 175.6 (C-1″); 34.7 (C-2″); 26.1 (C-3″); 30.3–30.7 (C-4″–C-27″); 27.1 (C-28″); 29.6 (C-29″); 65.8 (C-30″). Anal. Calcd for $C_{43}H_{72}O_7$: C, 73.67; H, 10.35. Found: C, 73.64; H, 10.33.
Feruloyl MG (**8**): 1.5 mg, colorless oil UV λ_{max} (log ε) (MeOH) 324 (3.94), 232 (3.84), 218 (3.75) nm; ESI-MS *m/z* 631 $[M+H]^+$ (100), 614 $[M+H–OH]^+$, 600 $[M+H–OMe]^+$. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.54 (dd, *J* = 10.7 and 6.4 Hz, H-1a); 3.52 (dd, *J* = 10.7 and 5.6 Hz, H-1b); 3.83

(m, H-2); 4.15 (dd, J = 11.4 and 4.5 Hz, H-3a); 4.05 (dd, J = 11.4 and 5.8 Hz, H-3b); 7.18 (d, J = 1.8 Hz, H-2′); 6.80 (d, J = 7.8 Hz, H-5′); 7.05 (dd, J = 7.8 and 1.8 Hz, H-6′); 6.84 (d, J = 13.0 Hz, H-7′); 5.77 (d, J = 13.0 Hz, H-8′); 3.89 (s, OCH_3); 2.34 (t, J = 7.5 Hz, H-2″); 1.62 (m, H-3″); 1.28 (ov, H-4″–H-22″); 1.37 (m, H-23″); 1.70 (m, H-24″); 4.17 (t, J = 6.6 Hz, H-25″); ^{13}C NMR (75 MHz, CD_3OD) δ: 64.1 (C-1); 71.3 (C-2); 66.4 (C-3); 127.5 (C-1′); 111.7 (C-2′); 149.4 (C-3′); 150.7 (C-4′); 116.3 (C-5′); 124.1 (C-6′); 146.6 (C-7′); 115.6 (C-8′); 169.3 (C-9′); 56.4 (OCH_3); 175.6 (C-1″); 34.7 (C-2″); 26.1 (C-3″); 30.3–30.7 (C-4″–C-22″); 27.1 (C-23″); 29.6 (C-24″); 65.8 (C-25″). Anal. Calcd for $C_{38}H_{62}O_7$: C, 72.34; H, 9.91. Found: C, 72.37; H, 9.88.

Feruloyl MG (**9**): 1.0 mg, colorless oil UV λ_{max} (log ε) (MeOH) 322 (3.98), 233 (3.82), 217 (3.90) nm; ESI-MS m/z 701 $[M+H]^+$ (100), 684 $[M+H–OH]^+$, 670 $[M+H–OMe]^+$. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.55 (dd, J = 10.9 and 6.6 Hz, H-1a); 3.53 (dd, J = 10.9 and 5.6 Hz, H-1b); 3.83 (m, H-2); 4.14 (dd, J = 11.4 and 4.5 Hz, H-3a); 4.03 (dd, J = 11.4 and 6.0 Hz, H-3b); 7.18 (d, J = 1.8 Hz, H-2′); 6.80 (d, J = 7.8 Hz, H-5′); 7.05 (dd, J = 7.8 and 1.8 Hz, H-6′); 6.84 (d, J = 12.9 Hz, H-7′); 5.77 (d, J = 12.9 Hz, H-8′); 3.89 (s, OCH_3); 2.34 (t, J = 7.5 Hz, H-2″); 1.62 (m, H-3″); 1.28 (ov, H-4″–H-27″); 1.37 (m, H-28″); 1.70 (m, H-29″); 4.17 (t, J = 6.6 Hz, H-30″); ^{13}C NMR (75 MHz, CD_3OD) δ: 64.1 (C-1); 71.3 (C-2); 66.4 (C-3); 127.5 (C-1′); 111.7 (C-2′); 149.4 (C-3′); 150.7 (C-4′); 116.3 (C-5′); 124.1 (C-6′); 146.6 (C-7′); 115.6 (C-8′); 169.3 (C-9′); 56.4 (OCH_3); 175.6 (C-1″); 34.7 (C-2″); 26.1 (C-3″); 30.3–30.7 (C-4″–C-27″); 27.1 (C-28″); 29.6 (C-29″); 65.8 (C-30″). Anal. Calcd for $C_{43}H_{72}O_7$: C, 73.67; H, 10.35. Found: C, 73.65; H, 10.38.

10. Bahorun, T.; Gressier, B.; Trotin, F.; Brunet, C.; Dine, T.; Luyckx, M.; Vasseur, J.; Cazin, M.; Cazin, J. C.; Pinkas, M. *Arzneimittel-Forschung* **1996**, *46*, 1086, Hydrogen peroxide scavenging activity was performed by the method of Pick and Keisari, modified by Bahorun et al.: 100 μl of water solution of each isolated compound (0.1 mg/ml) were added to 100 μl of 0.002% hydrogen peroxide. Phosphate buffer (700 μl, 0.1 M, pH 7.4) and sodium chloride (100 μl, 0.1 M) were added. The reaction mixture was incubated for 20 min at 37 °C. Then 1 ml of 0.2 mg/ml phenol red dye with 0.1 mg/ml horseradish peroxidase in 0.1 M phosphate buffer was added. After 15 min, 100 μl of NaOH 0.5 M were added and absorbance was measured at 610 nm using a UV-1700 Shimadzu spectrophotometer. The results were expressed as percentage of reduction of H_2O_2 adsorption by test compounds.
11. Nabasree, D.; Bratati, D. *Food Chem.* **2004**, *88*, 219, The assay of superoxide radical scavenging activity was based on the capacity of each isolated metabolite (0.1 mg/ml) to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the riboflavin–light–NBT system. Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 2 μM riboflavin, 100 μM EDTA, 75 μM NBT and 100 μl sample solution. The production was followed by monitoring the increase in absorbance at 560 nm after 10 min illumination from a fluorescent lamp.
12. Preparation of liver microsomes. Microsomes were isolated from male Wistar rats (250 g). Animals were killed by decapitation. The liver was quickly excised and washed several times with ice cold saline solution (0.15 M KCl, pH 7.4), then homogenized in the same saline solution. The homogenate was filtered and then centrifuged successively at 1600*g* for 10 min. The supernatants were followed by centrifugation at 20,000*g* for 10 min and the pellets were discarded. Microsomes were obtained from the 20,000 g supernatant by centrifugation at 105,000*g* for 1 h at 4 °C. The microsomal pellet was washed by suspension in cold ice Tris-buffer (pH 7.4) and then stored at −80 °C.
13. Markwell, M. A.; Haas, S. M.; Tolbertr, N. E.; Bieber, L. L. *Methods Enzymol.* **1981**, *72*, 296, The thiobarbituric acid assay was carried out according to the method reported by Markwell et al., with some modifications. The liver microsomes (10 μl), containing 100 μg protein, were emulsified with 15 mg of Tween 40 previously dissolved in 1 ml of phosphate buffer (0.2 M, pH 7.4). The emulsion was incubated for 3 h at 60 °C with 100 μl of water solution of test compounds (0.1 mg/ml). Then the reaction mixture was treated with 2 ml of TBA reagent consisting of 0.37% of thiobarbituric acid, 15% of trichloroacetic acid in 0.5 N HCl and placed in a boiling water bath for 1 h, cooled and centrifuged using a Beckman GS-15R centrifuge for 5 min at 3500*g*. The supernatant was measured at 532 nm. Inhibition of lipid peroxidation was measured as percentage vs blank containing no test compounds.
14. Anselmi, C.; Centini, M.; Andreassi, M.; Buonocore, A.; La Rosa, C.; Maffei Facino, R.; Sega, A.; Tsuno, F. *J. Pharm. Biomed. Anal.* **2004**, *35*, 1241.