Supplementary Materials

A characterization of the antimalarial activity of the bark of *Cylicodiscus gabunensis* Harms

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Materials and methods

Preparative High Pressure Liquid Chromatography (HPLC)

HPLC purification was carried out through a C-18 reversed phase column (Waters 5 µm, 19 mm × 100 mm) on a preparative Agilent 1260 HPLC system coupled with G1315D Diode Array Detector. Solvent A (0.1% glacial acetic acid in water) and solvent B (0.1% glacial acetic acid in methanol) were used for elution at flow rate of 16 ml/min. The gradient was as follows: 0-5 min 5%B, 5-25 min 60% B, 25-26 min 100% B, 31 min 100%B, and 31-32 min 5%B.

Gas Chromatography Mass Spectrometry (GC-MS) analysis

GC-MS was carried out on an Agilent 7890A Gas Chromatograph, connected to an Agilent MS model 5975C MSD with triple axis detector (Agilent Technologies, USA), the system was equipped with a HP5-MS column 5 % phenyl-methylpolysiloxane, 30 m × 0.25 mm× 0.25 µm (Agilent Technologies, USA). The temperature was programed to provide 60°C for 2 min, to reach 300°C at 10°C/min and kept at 300°C for 4 min. The retention indices (RI) were calculated using the alkanes C_8-C_{20} injected under the same GC-MS conditions, each sample was ran three different times and the standard deviations (SD) of the relative quantities of the metabolites calculated.

Hexane extract (CGH) was prepared in hexane (5 µg/µl), and 1 µl was injected for GC-MS analysis while for the polar extracts (CGEEA and CGEBU) and fractions trimethylsilyl (TMSi) derivatives were prepared as follows. 1 mg of each extract or fraction was incubated with 10 µL of pyridine and 50 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 60 °C for 2-6 hrs (Li and Barz, 2006) and 1 µL was injected into the GC-MS system.

Liquid chromatography Mass spectrometry (*LC-MS) analysis*

2 µL of the sample (~0.1 mg/ml) was injected onto a C8 reversed phase column (50x2.1mm, 5µm particle size, Thermo Fisher Scientific, UK) using an Agilent Infinity 1260 series liquid chromatography system. Compounds were separated using a linear gradient method utilizing mobile phase A (Milli-Q water with 0.1% formic acid) and mobile phase B (acetonitrile with 0.1% formic acid). The gradient consisted of A: B (95:5), acetonitrile increase A: B (5:95) at min 10, acetonitrile kept at 95% for 8 min. The flow rate was maintained at 0.5 mL/min throughout. The outlet from the chromatography system was directly coupled to an electrospray ion source on a 6530 Accurate-Mass Q-TOF detector. The MS method utilized a full-MS experiment in a positive ion mode and a scan range of *m/z* 100-1700 and scan rate of 1.0 spectrum/sec. Mass Hunter workstation software was used for qualitative analysis of data.

Analysis of the oligosaccharides composition

The CGEBU-F10-7 fraction was hydrolyzed using hydrochloric acid as described (Tene et al., 2011) with the following modifications. 2 mg of the CGEBU-F10-7 were mixed with 2 mL of 2 M HCl in 50 % methanol and heated in an ultrasonic bath at 50 °C for 4 hrs, before an overnight incubation, at 90 °C. After centrifugation at 10000 rpm for 10 min using Eppendorf Centrifuge 5418R, the supernatant was dried and the resultant residue (1 mg) was derived using BSTFA and pyridine for GC-MS analysis as described above. 1 mg of each standard monosaccharide including α-D-Glucose, L-(+)-Arabinose, and L-Rhamnose was dissolved in 150 µL of deionized water and kept overnight at 100°C. Water was removed using a Rota evaporator at 45 °C and the residue was derived into its TMSi derivatives and subjected to GC-MS analysis as described above.

Nuclear Magnetic Resonance (NMR) spectroscopic analysis

^{1}H and ^{13}C NMR spectra for the synthetic gallate derivatives were obtained on a Bruker 400 and 101 MHz instrument, respectively. Chemical shifts were reported in δ (ppm) using the solvent (CD_3OD) standard and coupling constants (J) were measured in Hertz. ^{1}H NMR spectrum of CGEBU-F10-7 (CD_3COCD_3:D_2O (1:1)) was analyzed on a Bruker AVIII 700 MHz instrument equipped with a TCI cryoproble.

Synthesis of alkyl and alkenyl 3, 4, 5-trihydroxybenzoates (gallic acid derivatives)

To a solution of gallic acid (3.00 mmol; 510 mg, MW 170.12) and anhydrous alcohol (4.00 mmol) in tetrahydrofuran (THF) (6 ml) mixed in 50 ml dry flask and cooled in ice bath at 0°C, a solution of N,N’-dicyclohexylcarbodiimide (DCC) (3.50 mmol; MW 206.33, 720 mg) was added in THF (6 mL). The mixture was allowed to stir for 24 hrs with the reaction vessel connected to a Rotavapor to evaporate the solvent. The product, a dark yellow residue, was extracted in cold ethyl acetate three times and filtered. The filtrates were combined and evaporated until dry. The synthesized esters were purified by column chromatography on silica gel (35-75 µm; 4×30 cm), hexane: ethyl acetate 7: 3, isocratic, 3 ml/min. The eluates were subjected to thin layer chromatography (SiO2, ethyl acetate: hexane 7: 3) an illuminated using a UVGL-25 compact UV lamp (254/365 nm UV/4- Watt) under iodine vapour. Yields were as follows: 534 mg (83%) of propan-2-yl 3,4,5-trihydroxybenzoate (**1**); 583 mg (85%) of 2-methylpropyl 3, 4, 5-trihydroxybenzoate (**2**); 597 mg (82%) of 2-methylbutyl 3, 4, 5-trihydroxybenzoate (**3**); 606 mg (84%) of 3-methylbut-2-en-1-yl 3,4, 5-trihydroxybenzoate (**4**); 604 mg (83%) of 3-methylbutyl 3, 4, 5-trihydroxybenzoate (**5**) (Figure S4). The positive-ion ESI-MS of **1-5** generated the following pseudo-molecular ions at *m/z*: 227.0917; 241.1073; 239.0922; 241.1071 $[M+H]^+$ and 235.0576 $[M+Na]^+$, respectively.

The molecular peaks of the trimethylsilyl derivatives of the synthesized compounds (**1-5**) on GC-MS were detected at *m/z*: 442.3, **1-TMS**; 456.3, **2-TMS**; 454.2, **3-TMS**; 456.3, **4-TMS**; and 428.2, **5-TMS**. ^{1}H and ^{13}C NMR data of the five synthetic compounds are listed in Table S3 and S4, respectively.

Results

Bioassay guided fractionation of Cyclodiscus gabunensis bark

Figure S1: Preparative HPLC illustrating the eight fractions isolated from CGEBU-F10 through a C-18 reversed phase column (Waters 5 µm, 19 mm × 100 mm).

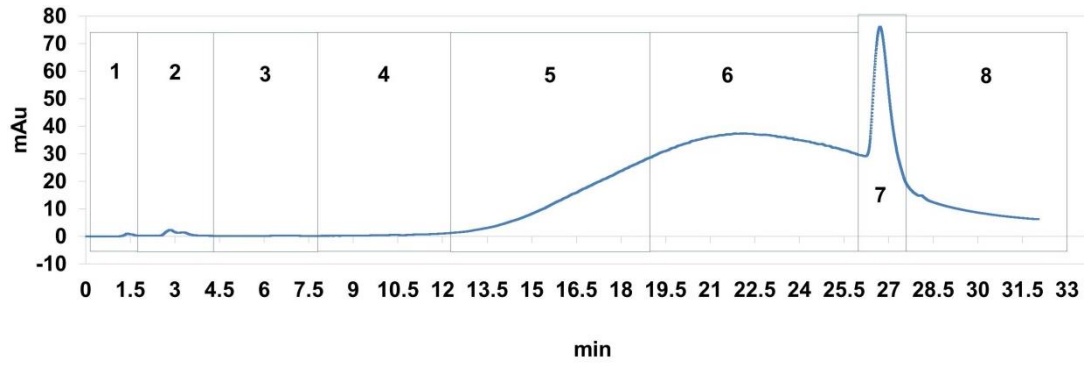


Figure S2: GC-MS chromatogram of TMSi derivatives of the *C.gabunensis* CGEEA fraction. Compounds (1-19) identified in the chromatogram are listed in Table S1.

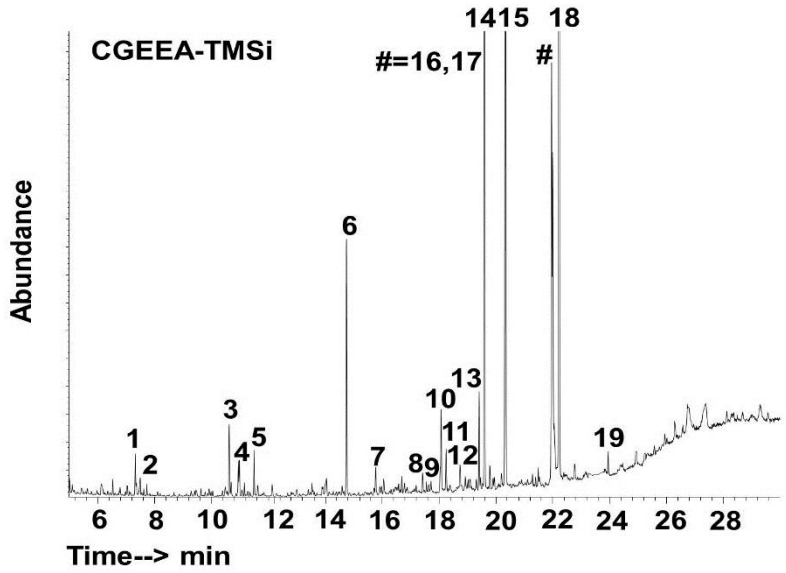


Figure S3: GC-MS chromatogram of TMSi derivatives of *C.gabunensis* CGEBU fraction. Compounds (1-12) identified in the chromatogram are listed in Table S2.

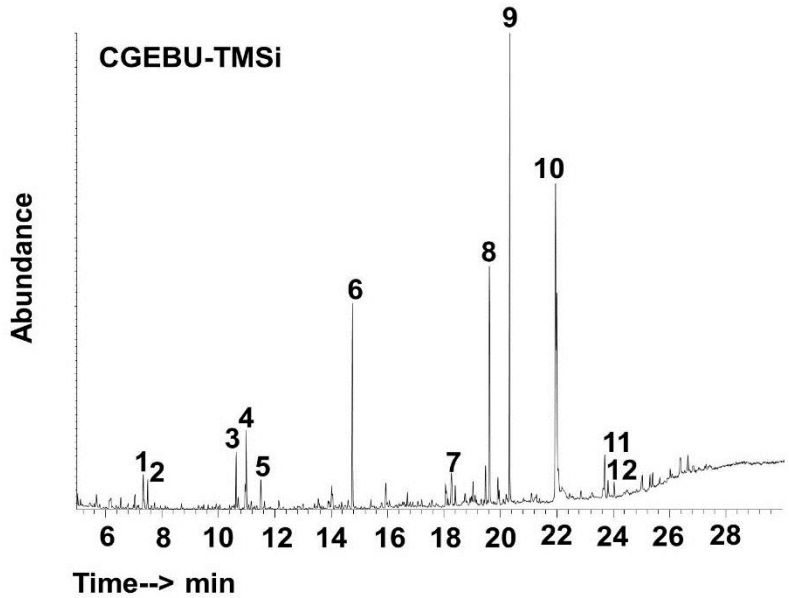
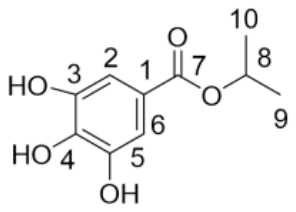
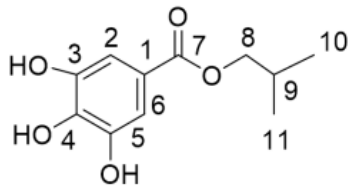


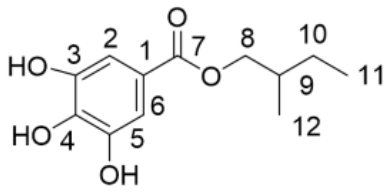
Figure S4: Structures of the synthesized 3,4,5-trihydroxybenzoates.



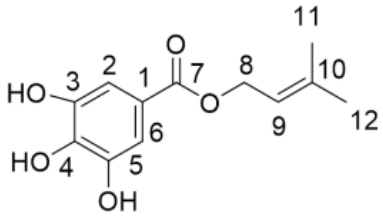
Propan-2-yl 3,4,5-trihydroxybenzoate



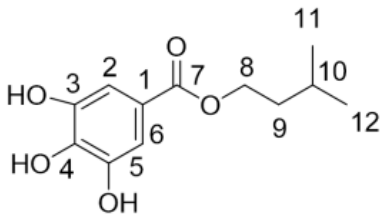
2-Methylpropyl 3, 4, 5-trihydroxybenzoate



2-Methylbutyl 3, 4, 5-trihydroxybenzoate



3-Methylbut-2-en-1-yl 3, 4, 5-trihydroxybenzoate



3-Methylbutyl 3, 4, 5-trihydroxybenzoate

Figure S5: Positive ESI-MS of compounds picked as masses from CGEBU-F10-7 by qualitative analysis mass hunter work station software. Masses detected are 1: 1260.5497; 2: 1436.6116 and 1406.6074; 3: 1044.5522; 4: 1244.5487

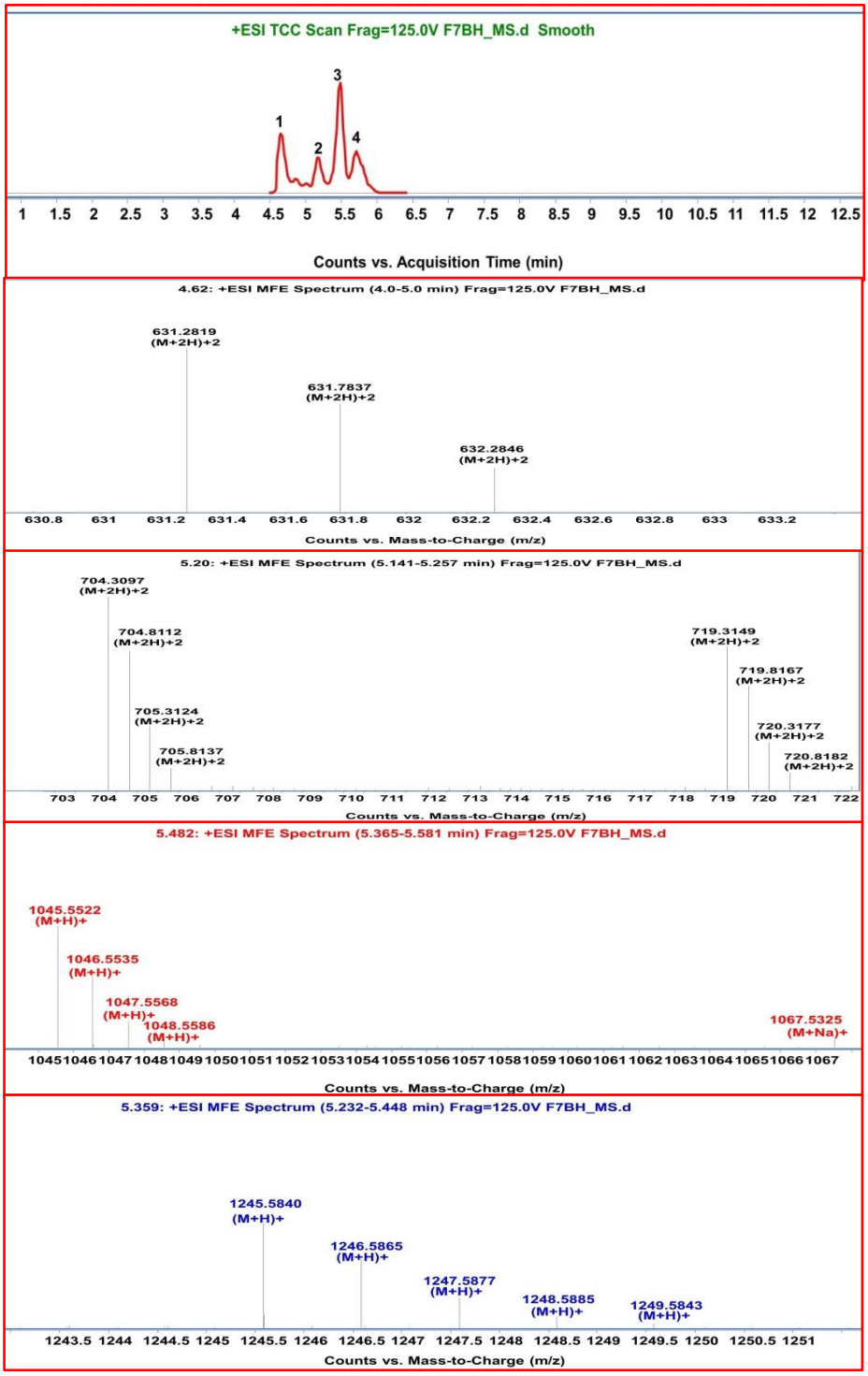


Figure S6: GC-MS chromatogram of acid hydrolysed CGEBU-F10-7 after TMSi derivation. Peaks from left to right are a1: α-L-arabinose-TMS; r1: α-L-rhamnose-TMS; a2: β-L-arabinose-TMS; r2: β-L-rhamnose-TMS; g1: α-D-glucose-TMS; g2: β-D-glucose-TMS; G: gallic acid. The assignment of these peaks was based on the NIST library and comparison with standard compounds.

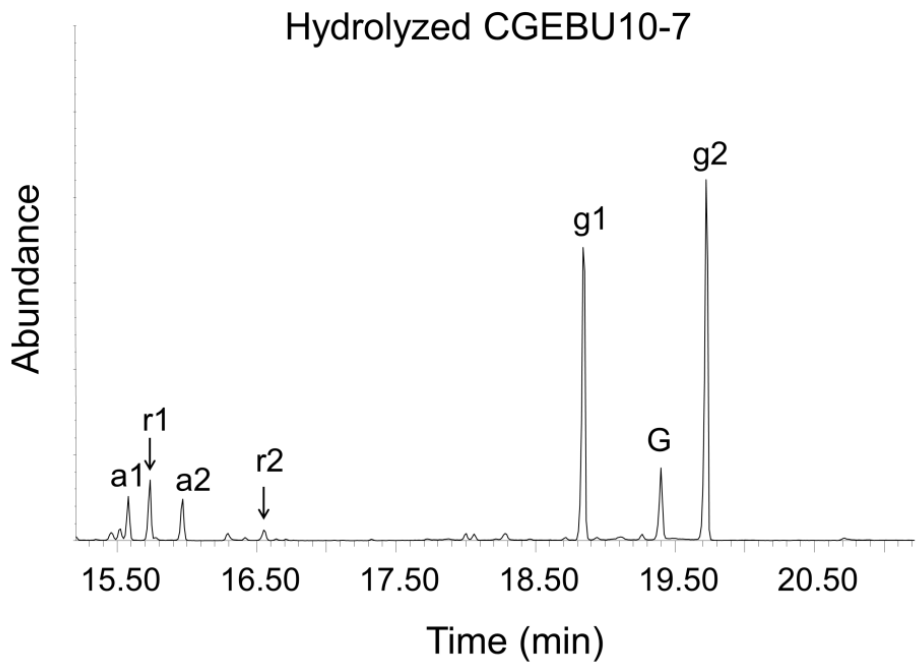


Figure S7: ^{1}H NMR (700M Hz) spectrum of CGEBU-F10-7 in CD_3COCD_3:D_2O (1:1).

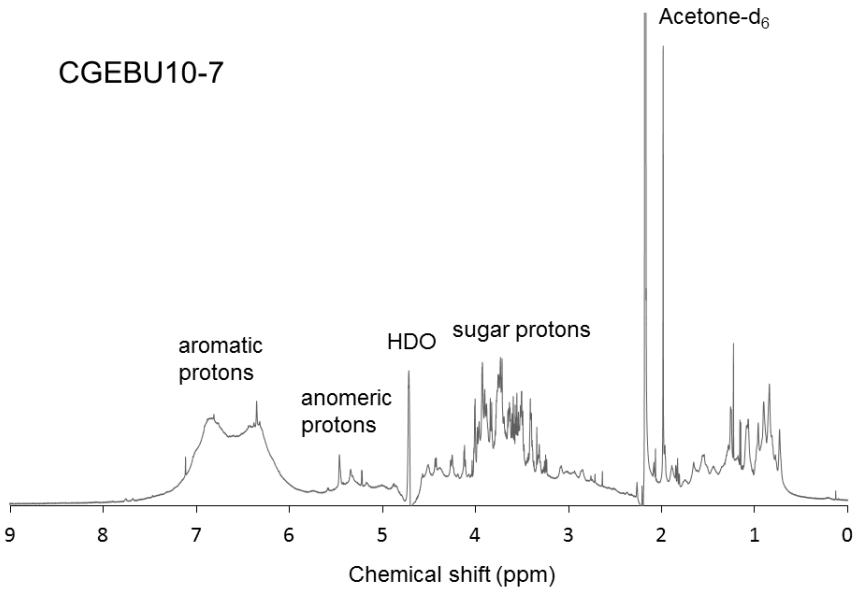


Table S1: Compounds detected from CGEEA by GC-MS

no.	RT	Compound detected	The name of the compound before derivatisation	percentages ±SD (n=3)	RI
1	7.48	Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	2-hydroxy-propanoic acid or lactic acid	1.30±0.08	1068
2	7.73	Acetic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	2-hydroxy acetic acid or glycolic acid	0.29±0.03	1082
3	10.60	1,3-bis(1,1-dimethylethyl)- benzene	1,3-bis(1,1-dimethylethyl)- Benzene	1.42±0.14	1261.
4	10.98	Glycerol, tris(trimethylsilyl) ether	Glycerine	0.54±0.01	1285
5	11.50	Butanedioic acid, bis(trimethylsilyl) ester	Succinic acid	0.88±0.07	1319
6	14.75	Trimethyl(2,6 ditert.-butylphenoxy)silane	2,6-ditert-butylphenol	5.4±0.60	1556
7	15.73	Benzoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	4-hydroxy-benzoic acid	0.42±0.01	1634
8	17.43	Benzoic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	3-methoxy-4-hydroxy-benzoic acid or vanillic acid	0.44±0.06	1778
9	17.70	Azelaic acid, bis(trimethylsilyl) ester	Azelaic acid	0.38±0.01	1804
10	18.08	Benzoic acid, 3,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	3,4-dihydroxy-benzoic acid or protocatechuic acid	2.18±0.07	1836
11	18.26	Tetradecanoic acid, trimethylsilyl ester	Tetradecanoic acid	1.05±0.13	1852
12	18.90	Trimethylsilyl 3,5-dimethoxy-4-(trimethylsilyloxy)benzoate	3,5-dimethoxy-4-hydroxy-benzoic acid or syringic acid	0.47±0.04	1914
13	19.40	3,4,5-Trihydroxybenzoic acid ethyl ester, tris(O-trimethylsilyl)-	3,4,5-trihydroxybenzoic acid ethyl ester or ethyl gallate)	1.88±0.10	1960
14	19.60	Benzoic acid, 3,4,5-tris(trimethylsilyloxy)-, trimethylsilyl ester	3,4,5-trihydroxy benzoic acid or gallic acid	13.24±0.10	1973

15	20.30	Hexadecanoic acid, trimethylsilyl ester	Hexadecanoic acid	29.55±2.50	-
16	21.96	9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester	(Z,Z)-9,12-octadecadienoic acid	8.52±0.45	-
17	21.99	11-trans-Octadecenoic acid, trimethylsilyl ester	(11-trans-octadecenoic)	8.95±0.43	-
18	22.22	Octadecanoic acid, trimethylsilyl ester	Octadecanoic acid	15.58±0.93	-
19	23.96	Eicosanoic acid, trimethylsilyl ester	Eicosanoic acid	0.36±0.01	-

RT = Retention time, SD= Standard deviation, n= Number of experiment replication, RI= Retention index

Table S2: Compounds detected in CGEBU by GC-MS

no.	RT	Compound detected	The name of the compound before derivatisation	percentages ±SD (n=3)	RI
1	7.5	Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	2-hydroxy-propanoic acid or lactic acid	1.41±0.15	1069
2	7.7	Acetic acid, [(trimethylsilyl)oxy]-, trimethylsilyl ester	2-hydroxy-acetic acid or glycolic acid	0.32±0.01	1083
3	10.63	Benzene, 1,3-bis(1,1-dimethylethyl)-	Benzene, 1,3-bis(1,1-dimethylethyl)-	2.70±0.07	1262
4	10.99	Glycerol, tris(trimethylsilyl) ether	Glycerine	4.51±0.06	1285
5	11.51	Butanedioic acid, bis(trimethylsilyl) ester	Butanedioic acid	1.44±0.06	1320
6	14.75	Trimethyl(2,6 ditert.-butylphenoxy)silane	2,6-ditert-butylphenol	9.70±0.29	1556
7	18.26	Tetradecanoic acid, trimethylsilyl ester	Tetradecanoic acid	2.58±0.1	1852.3
8	19.60	Benzoic acid, 3,4,5-tris(trimethylsiloxy)-, trimethylsilyl ester	3,4,5-trihydroxy benzoic acid or gallic acid	12.06±0.9	1978
9	20.33	Hexadecanoic acid, trimethylsilyl ester	Hexadecanoic acid	31.6±1.003	-
10	22.23	Octadecanoic acid, trimethylsilyl ester	Octadecanoic acid	25.21±0.57	-
11	23.75	cis-11,14-Eicosadienoic acid, trimethylsilyl ester	cis-11,14-eicosadienoic acid	1.69±0.06	-
12	23.96	Eicosanoic acid, trimethylsilyl ester	Eicosanoic acid	6.79±0.16	-

RT = Retention time, SD= Standard deviation, n= Number of experiment replication, RI= Retention index

Table S3. ^{1}H NMR (CD_3OD) data for the five synthetic gallate derivatives.

Compound name	Chemical shifts					
	H-2, 6	H-8	H-9	H-10	H-11	H-12
Propan-2-yl 3,4,5-trihydroxybenzoate (**1**)	7.06, s	5.13, m	1.34, d, *J* = 6.4 Hz	1.34, d, *J* = 6.4 Hz		
2-Methylpropyl 3, 4, 5-trihydroxybenzoate (**2**)	7.08, s	4.02, d, *J* = 6.6 Hz	2.04, m	1.03, d, *J* = 6.6 Hz	1.03, d, *J* = 6.6 Hz	
2-Methylbutyl 3, 4, 5-trihydroxybenzoate (**3**)	7.07, s	4.10, m	1.84, m	1.47, m; 1.30, m	0.95, t, *J* = 7.5 Hz	1.02, d, *J* = 6.8 Hz
3-Methylbut-2-en-1-yl 3, 4, 5-trihydroxybenzoate (**4**)	7.05, s	4.74, d, *J* = 7.2 Hz	5.45, m		1.79, s	1.82, s
3-Methylbutyl 3, 4, 5-trihydroxybenzoate (**5**)	7.06, s	4.27, t, *J* = 6.6 Hz	1.64, m	1.25, m	0.99, d, *J* = 6.6 Hz	0.99, d, *J* = 6.6 Hz

Table S4. ^{13}C NMR (CD_3OD) data for the five synthetic gallate derivatives.

Compound name	Chemical shifts									
	C-1	C-2, 6	C-3, 5	C-4	C-7	C-8	C-9	C-10	C-11	C-12
Propan-2-yl 3,4,5-trihydroxybenzoate (**1**)	120.8	108.6	145.0	138.2	166.7	67.8	20.8			
2-Methylpropyl 3, 4, 5-trihydroxybenzoate (**2**)	120.3	108.6	145.1	138.3	167.2	70.3	27.8	18.1	18.1	
2-Methylbutyl 3, 4, 5-trihydroxybenzoate (**3**)	120.3	108.6	145.1	138.3	167.2	68.8	34.3	25.8	10.3	15.5
3-Methylbut-2-en-1-yl 3, 4, 5-trihydroxybenzoate (**4**)	120.4	108.6	145.1	138.3	167.1	61.0	118.7	138.6	16.7	24.5
3-Methylbutyl 3, 4, 5-trihydroxybenzoate (**5**)	120.3	108.6	145.1	138.3	167.2	62.8	37.3	25.0	21.5	21.5

References

Li, W.W., Barz, W., 2006. Structure and accumulation of phenolics in elicited Echinacea purpurea cell cultures. Planta Medica 72, 248-254.

Tene, M., Chabert, P., Note, O., Kenla, T.J.N., Tane, P., Lobstein, A., 2011. Triterpenoid saponins from Cylicodiscus gabunensis. Phytochem Lett 4, 89-92.