2018

Dioic acid glycosides, tannins and methylated ellagic acid glycosides from Morella salicifolia bark

Makule, Edna

Elsevier Ltd.

https://doi.org/10.1016/j.phytol.2018.09.013

Dioic acid glycosides, tannins and methylated ellagic acid glycosides from T Morella salicifolia bark

Article · September 2018

CITATIONS
0

READS
51

1 author:

Edna Edward Makule
The Nelson Mandela African Institute of Science and Technology
8 PUBLICATIONS **63** CITATIONS

SEE PROFILE

Phytochemistry Letters 28 (2018) 76–83

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Dioic acid glycosides, tannins and methylated ellagic acid glycosides from *Morella salicifolia* bark

Edna E. Makule[a,b], Birgit Kraus[a], Guido Jürgenliemk[a], Jörg Heilmann[a], Stefan Wiesneth[a,*]

[a] Department of Pharmaceutical Biology, Faculty of Chemistry and Pharmacy, University of Regensburg, Universitätsstraße 31, D-93053 Regensburg, Germany
[b] Department of Food Biotechnology and Nutritional Sciences, Nelson Mandela African Institution of Science and Technology, P. O. Box 447, Arusha, Tanzania

ARTICLE INFO

Keywords:
Morella salicifolia
Myricaceae
Dioic acid glycosides
Proanthocyanidins
Ellagic acid derivatives
Hydrolysable tannins

ABSTRACT

The bark of *Morella salicifolia* (HOCHST. EX A. RICH.) VERDC. & POLHILL (Myricaceae) is a common herbal drug and especially utilized by the Maasai community of northeastern Tanzania. It is traditionally used to treat swellings on the cheek and face, cough, toothache, stomach problems, headaches, and skin diseases. Unless, there is still a lack of information considering the specialized metabolites that may be responsible for the traditional use of this medicinal plant. An isolation study on a methanolic extract revealed three methylated ellagic acids, castalagin, two monomeric flavan-3-ols, three prodelphinidins and three dioic acid glycosides. Among these metabolites, myriside (3,4-dimethyl ellagic acid 4′-*O*-*β*-D-xylopyranoside, **1**), epigallocatechin-3-*O*-gallate-(2*β*→*O*→7, 4*β*→8)-gallocatechin (**9**), 2,7-dimethyl-8-*O*-(6′-*O*-galloyl)-*β*-D-glucopyranoside-deca-2*E*,4*E*-dienedioic acid (**10**), 2,7-dimethyl-8-*O*-*β*-D-glucopyranoside-deca-2*E*,4*E*-dienedioic acid (**11**) as well as 4,9-dimethyl-10-*O*-*β*-D-glucopyranoside-dodeca-2*E*,4*E*,6*E*-trienedioic acid (**12**) were isolated for the first time. To the best of our knowledge, the dioic acid glycosides represent a generally novel structure type. To provide comprehensive information on the condensed tannins, the polymeric proanthocyanidins were enriched and characterized via ^{13}C-NMR. Results confirm chemo-taxonomic relationships within the genera *Morella* and *Myrica* in the Myricaceae.

1. Introduction

Morella salicifolia (HOCHST. EX A. RICH.) VERDC. & POLHILL belongs to the family Myricaceae in the order Fagales. It had formerly been named *Myrica salicifolia* HOCHST. EX A. RICH. until the genus *Myrica* was divided into the two genera *Myrica* and *Morella* (Mbuya et al., 1994). The plant is also known as Olkitalaswa (Maasai) and in Tanzania it is traditionally used to treat swellings on the cheek and face, cough, toothache, stomach problems, headaches, and skin diseases (Getahun, 1976; Kokwaro, 2009). Especially, *M. salicifolia* bark is a common medicinal herbal drug for treatment of many diseases in Maasai community of northeastern Tanzania (Hedberg et al., 1982). Despite the many traditional uses of *M. salicifolia*, information about its phytochemical composition and pharmacological activities is scarce. Very recently, the diarylheptanoid glycoside spectrum of *M. salicifolia* has been characterized (Makule et al., 2017) confirming the close taxonomic relation of the genera *Morella* and *Myrica* from a chemotaxonomic point of view. Aim of the present study, was the in-depth phytochemical investigation of the polar metabolites of a MeOH-extract leading to the isolation of proanthocyanidins, ellagic acid derivatives, and dioic acid glycosides from *M. salicifolia*. To the best of our knowledge dioic acid glycosides were never reported before and represent a new class of specialized metabolites.

2. Results and discussion

Fig. 1 shows isolated ellagic acid derivatives (1–4), flavan-3-ols (5–9) as well as three dioic acid derivatives from *M. salicifolia* (10–12). All compounds are reported the first time for this *Morella* species. To the best of our knowledge, compounds **1** as well as **9–12** are hitherto unknown metabolites.

The spectroscopic and spectrometric data of compound **1** revealed a new ellagic acid derivative substituted with methoxy-groups at C-3 and C-4 and a *β*-D-xylopyranosyl moiety at C-4′. The identity of the sugar moiety including its absolute stereochemistry was verified by hydrolysis and derivatization with (*S*)-(+)-2-methylbutyric anhydride and comparison via ^{1}H-NMR to the reference sugars (York et al., 1997). Here, a congruent ^{1}H NMR spectrum was gained from the per-O-(*S*)-2-methylbutyrate derivative of the *β*-D-xylopyranose. Especially, the chemical shift of the anomeric proton at 5.85 ppm (d, J = 7.2) clearly verified this moiety including its pyranose form and absolute stereochemistry (York et al., 1997). The position of all three substituents were

* Corresponding author.
E-mail address: stefan.wiesneth@ur.de (S. Wiesneth).

https://doi.org/10.1016/j.phytol.2018.09.013
Received 6 July 2018; Received in revised form 4 September 2018; Accepted 11 September 2018

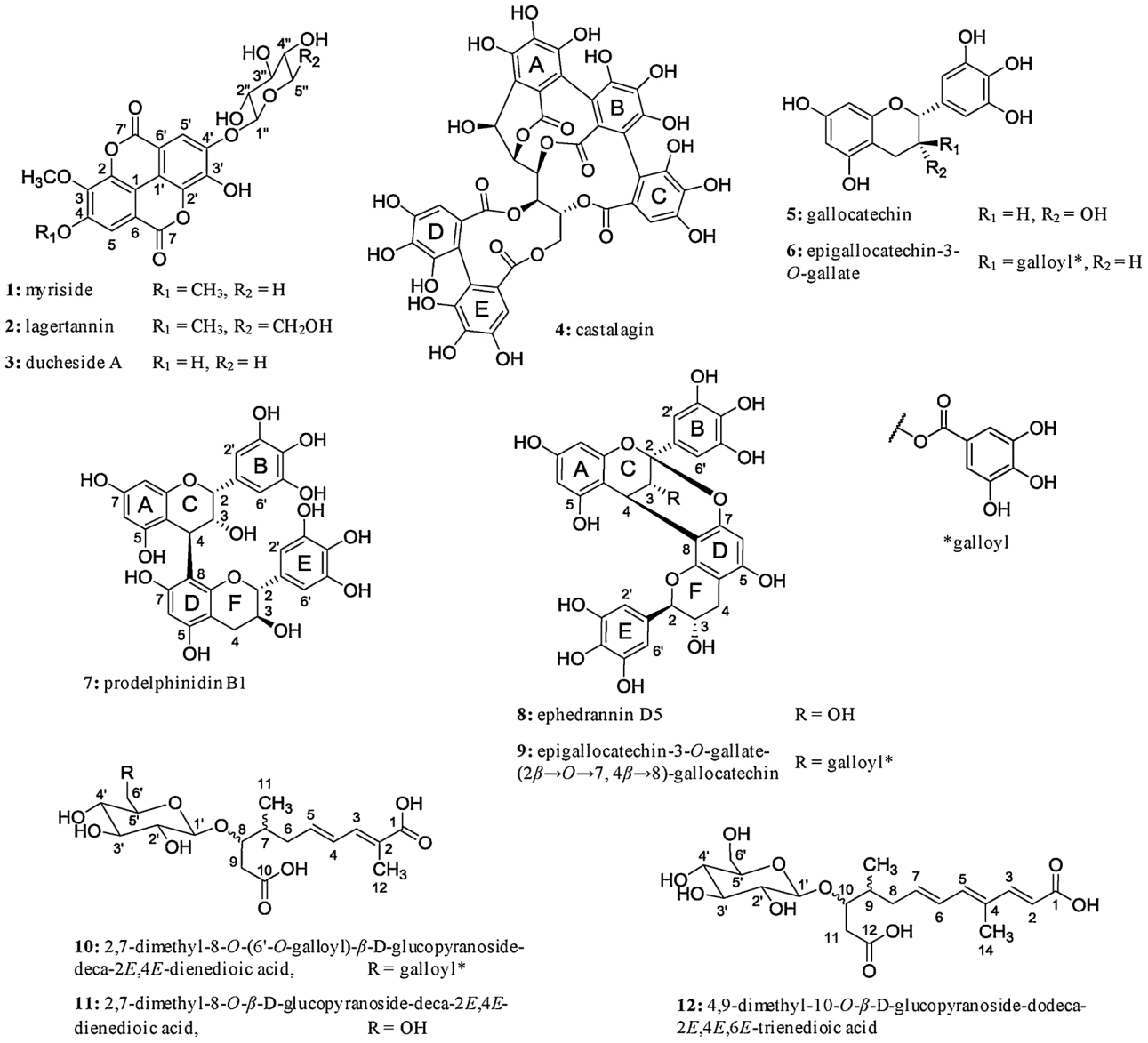

Fig. 1. Isolated ellagic acid derivatives (**1–3**), the hydrolysable tannin castalagin (**4**), monomeric flavan-3-ols (**5**, **6**), condensed tannins (**7–9**) as well as dioic acid glycosides (**10–12**) from the bark of *M. salicifolia*.

evident by their respective HMBC cross-signals. In addition, a NOESY cross-signal between H-5′ of ellagic acid to H-1″ of the sugar was further evidence for a direct neighborhood. The complete NMR-data are presented in Table 1. The MS-fragment with *m/z* 461.0735 $[M-H]^-$ ($C_{21}H_{17}O_{12}$, *calcd.* $[M-H]^-$ *m/z* 461.0720) was detected in a ESI-HRMS experiment and revealed a chemical formula of $C_{21}H_{18}O_{12}$ for **1**. To enable an unambiguous identification of **1** polarimetric and CD-data are also provided. Comparing the NMR-data of compound **1** and **2** some differences in chemical shifts of the aglycone parts appeared, most evident for the carbons C-1′ and C-2′ as well as the proton at C-5′ (Table 1). This phenomenon must be caused by the missing hydroxy-group at C-5″ of the β-D-xylopyranose in case of **1** in combination with a differing solvent (**1**: CD_3OD, **2**: pyridine-d_5). Compound **2** and **3** were also ellagic acid derivatives. Unless they are the first time isolated from *M. salicifolia*, they are already known structures from other sources. The spectroscopic data of **2** matched those from lagertannin. This compound was isolated and elucidated before from *Lagerstoemia speciosa* (L.) PERS. by Takahashi et al. (1976), but without presentation of NMR data. Thus, data were presented in Table 1. Further, the specific rotation angle differed from the reported value (Takahashi et al., 1976). Nevertheless, absolute stereochemistry and identity of the β-D-glucopyranosyl moiety was evident by comparison of the ^{1}H NMR signal of the anomeric proton of the (*S*)-2-methylbutyrate (SMD) derivative of the β-D-glucopyranose at 5.95 ppm (d, $J = 8.3$) (York et al., 1997). Thus, the difference of polarimetric data may be caused by impurities. Compound **3** was identified as ducheside A via matching NMR and ESI-HRMS data. This compound was reported before as a specialized metabolite from *Duchesnea indica* FOCKE (Ye and Yang, 1996) as well as from *Anisophyllea dichostyla* R. BR. (Khallouki et al., 2007). None of these studies provided the CD-data. Therefore, they were included here to complete its stereo-spectroscopic dataset. Compound **4** was unambiguously identified as castalagin. Its NMR-data (Gallo et al., 2006) as well as CD-data (Matsuo et al., 2015) were matching literature data. Qualitatively differences in polarimetry to Mayer et al. (1969) may be caused by impurities. However, **4** is an already known compound from *Myrica esculenta* BUCH.HAM. EX D. DON (Sun et al., 1988).

Compounds **5** and **6** were verified as monomeric flavan-3-ols. Both structures were identified by comparison to literature (**5**: Davis et al., 1996; Lee et al., 1992; Roux and Maihs, 1960; **6**: Davis et al., 1996; Khallouki et al., 2007; Kumar and Rajapaksha, 2005; Lee et al., 1992).

Table 1
NMR-data for myriside, 3,4-dimethyl ellagic acid 4′-*O*-*β*-D-xylopyranoside (**1**) in CD_3OD (1.7 mg/ml) and lagertannin, 3,4-dimethyl ellagic acid 4′-*O*-*β*-D-glucopyranoside (**2**) in pyridine-d_5 (16.7 mg/ml). ^{1}H NMR at 600.25 MHz, ^{13}C NMR at 150.93 MHz, temperature 298 K (*δ* in ppm, *J* in Hz), pt = pseudo triplet.

			1		**2**	
	Pos.		^{13}C	^{1}H	^{13}C	^{1}H
3,4-dimethyl ellagic acid	1		115.2		114.1[a]	
	2		143.4		142.4	
	3		142.6		141.9	
	4		155.6		154.9	
	5		108.0	7.69 (1H, s)	107.9	7.80 (1H, s)
	6		116.1		113.8[a]	
	7		162.2		159.8	
	1′		98.7		107.9	
	2′		139.6		137.8	
	3′		157.6		144.5	
	4′		152.9		149.5	
	5′		113.4	7.69 (1H, s)	113.7	8.48 (1H, s)
	6′		116.5		114.8	
	7′		162.6		159.3	
	3-*O*-CH_3		62.0	4.10 (3H, s)	61.5	4.13 (3H, s)
	4-*O*-CH_3		57.1	4.02 (3H, s)	56.5	3.83 (3H, s)
sugar substituent	1″		104.7	4.77 (1H, d, *J* = 7.6)	103.6	5.95 (1H, d, *J* = 7.7)
	2″		74.7	3.55 (1H, dd, *J* = 7.5, 9.2)	74.8	4.29 (1H, pt, *J* = 8.2)
	3″		77.3	3.45 (1H, pt, *J* = 8.7)	78.4	4.39 (1H, pt, *J* = 8.6)
	4″		71.1	3.59 (1H, ddd, *J* = 5.9, 8.7, 10.3)	71.0	4.36 (1H, pt, *J* = 9.0)
	5″	*α*	67.2	3.43 (1H, dd, *J* = 10.3, 11.4)	79.1	4.14 (1H, m)
		β		3.99 (1H, dd, *J* = 5.9, 11.4)		
	6″				62.1	4.42 (1H, dd, *J* = 4.7, 12.0) 4.55 (1H, dd, *J* = 2.2, 12.1)

[a] Signals interchangeable.

Both have been found in *Myrica gale* L. (Santos and Waterman, 2000) and *Myrica rubra* SIEBOLD. & ZUCC. (Nonaka et al., 1983). Further, **6** was isolated from *Myrica esculenta* BUCH.-HAM. EX D. DON (Sun et al., 1988). This is interesting, due to the close taxonomic relation between the genera *Myrica* and *Morella*. Compound **7** was identified as prodelphinidin B1. Its spectroscopic and spectrometric data were congruent to literature data (Abdel Sattar et al., 1999; Fujii et al., 2013). The positive cotton effects in the CD-spectrum between 213–243 nm are evidence for the *β*-configuration at position 4 of the C-ring (Barrett et al., 1979; Botha et al., 1981, 1978). The difference for the specific rotational angle to the one published by Fujii et al. (2013) again may be caused by impurities. Compound **8** showed sound spectroscopic and spectrometric data by comparison to ephedrannin D5 being an already known specialized metabolite from *Ephedra sinica* STAPF. (Zang et al., 2013). Compound **9** was identified as epigallocatechin-3-*O*-gallate-(2*β*→*O*→7, 4*β*→8)-gallocatechin. Again, the position of galloyl moiety was proven by a ^{1}H-downfield shift of the proton at C-3 (C-ring) to *δ* 5.38 ppm (278 K) in comparison to *δ* 4.04 ppm for **9**. Position of the substituent was further clarified by the respective HMBC cross-signals between H-3 (C-ring) and C-7″ of the galloyl moiety. The 4*β*- and 2*β*-configuration of the C-ring are in accordance with strong positive cotton effects detected between 227–255 nm (Barrett et al., 1979; Botha et al., 1981, 1978). Since negative cotton effects around 270 nm, like detected for **8**, were also present in the CD-spectrum of **9**, absolute stereochemistry of the F-rings of both compounds needs to be identical due to the findings of Hikino et al. (1982) connecting the cotton effects at this wavelength to configuration of C-2 of flavan-3-ol units. As **8** and **9** showed values of molar ellipticity in the same range, both C-2 carbons (C- and F-ring) of **9** need to be *β*-configurated. To the best of our knowledge, this A-type prodelphinidin is reported for the first time. Adenodimerin C (Ting et al., 2014) isolated from *Myrica adenophora* HANCE is very similar to **9**. Hence, CD-, NMR- and MS-data are all sound to this compound except for a cross-signal between H-3 (C-ring) and H-6 (D-ring) being detected in a selective ROESY experiment (Supplement Fig. S3). This result indicates an *α*-configuration at C-3 (C-ring, Kolodziej et al., 1991).

Compounds **10–12** are a new class of specialized metabolites and up to now, there is no report on structurally similar compounds. All three metabolites revealed a dioic acid linked to position 1′ of a *β*-D-glucopyranose. In case of **10**, structure elucidation resulted in a 2,7-dimethyl deca-2*E*,4*E*-diendioic acid with the sugar linked to position 8. Further, HMBC cross-signal between H-6′ and C-7″ detected for **10** indicated the galloyl moiety is attached to the glucose C-6′. The *E*-configuration at positions 2 and 4 in **10** and **11** was elucidated by NOEs between H-12 and H-4 as well as H-3 and H-5, respectively. For **11**, the spectroscopic dataset was nearly identical, except lacking signals of the galloyl moiety. Compound **12** showed NOEs between H-14 and H-2/H-6 as well as H-5 and H-3/H-7, giving evidence for *E*-configuration in all three double bonds. Position of the linkage of the dioic acid to the sugar was verified by HMBC cross-signals between H-1′ and C-8 for **10** and **11** as well as C-10 for **12**. The relative stereochemistry of the *β*-glucoses for all three compounds was elucidated by the typical multiplicity and coupling constants > 7.5 Hz indicating axial positions of all protons (Table 3). Absolute stereochemistry of the glucopyranose of **10–12** was investigated by comparison of the ^{1}H-NMR signal of the anomeric proton of the SMD derivatives to reference sugars and revealed the structure of *β*-D-glucopyranose due to the ^{1}H-NMR signal at *δ*5.95 ppm (d, *J* = 8.3) (York et al., 1997). Unfortunately, absolute stereochemistry considering the stereocenters of the dioic acid parts (**10, 11**: C-7, C-8; **12**: C-9, C-10) couldn't be elucidated due to the many rotational options of the respective sp^3-hybridized carbons. Thus, no valid information could be gained from coupling constants in the 1D-^{1}H NMR experiments nor from the NOESY experiment.

Oligomeric and polymeric proanthocyanidins with more than four monomeric units are very difficult to isolate and to elucidate. Thus, a polymeric prodelphinidin fraction S7 yielded after Sephadex® LH-20 chromatography was characterized via ^{13}C NMR spectroscopy (Czochanska et al., 1980; Eberhardt and Young, 1994; Newman et al., 1987; Porter et al., 1982). Fig. 2 provides the ^{13}C NMR spectrum with the assignments of the respective signals considering the literature. A signal at *δ* 106.7 ppm clearly identified flavan-3-ols with trihydroxylated B-rings (e.g., gallocatechin) as the predominant monomeric units in the polymeric fraction of *M. salicifolia* bark. As there were no typical B-ring signals from dihydroxylated units (e.g., catechin) between *δ* 116–117 ppm (C-2′, C-5′), this type of monomer seems not to be relevant here. The relative amount of 2,3-*cis* to 2,3-*trans* isomers was found to be 36:1 by comparing the integrals of the signals at *δ* 76.8 and 82.3 ppm, respectively. Occurrence of the signals at *δ* 110.6, 121.8, 139.7, 146.5 ppm and especially the carbonyl carbon at *δ* 166.3 ppm indicates the presence of galloyl moieties (Sun et al., 1987). This was further evident by the presence of upfield shifts of C-4 of some extender units (*δ* 35.1 ppm) in addition to the respective units without this substituent (*δ* 37.4 ppm) (Sun et al., 1987). By integration, galloylated units can be estimated ~34% in this polymeric fraction. These findings on 2,3-*cis* configurated monomeric units and degree of galloylation are in accordance to investigations on *M. esculenta* (Sun et al., 1988). The estimated average degree of polymerization was obtained to be 16 obtained from the ratio of 15:1 following integration of the C-3 resonances of the extender (*δ* 73.3 ppm) and terminal units (*δ*68.3 ppm). Likewise, the same ratio of 15:1 was obtained by integrating C-4 signals of the extending (*δ* 35.2–37.4 ppm) and of the terminal units (*δ* 29.6 ppm). In *M. esculenta* vapor pressure osmometry measurements revealed an average degree of polymerization of 14 (Sun et al., 1988),

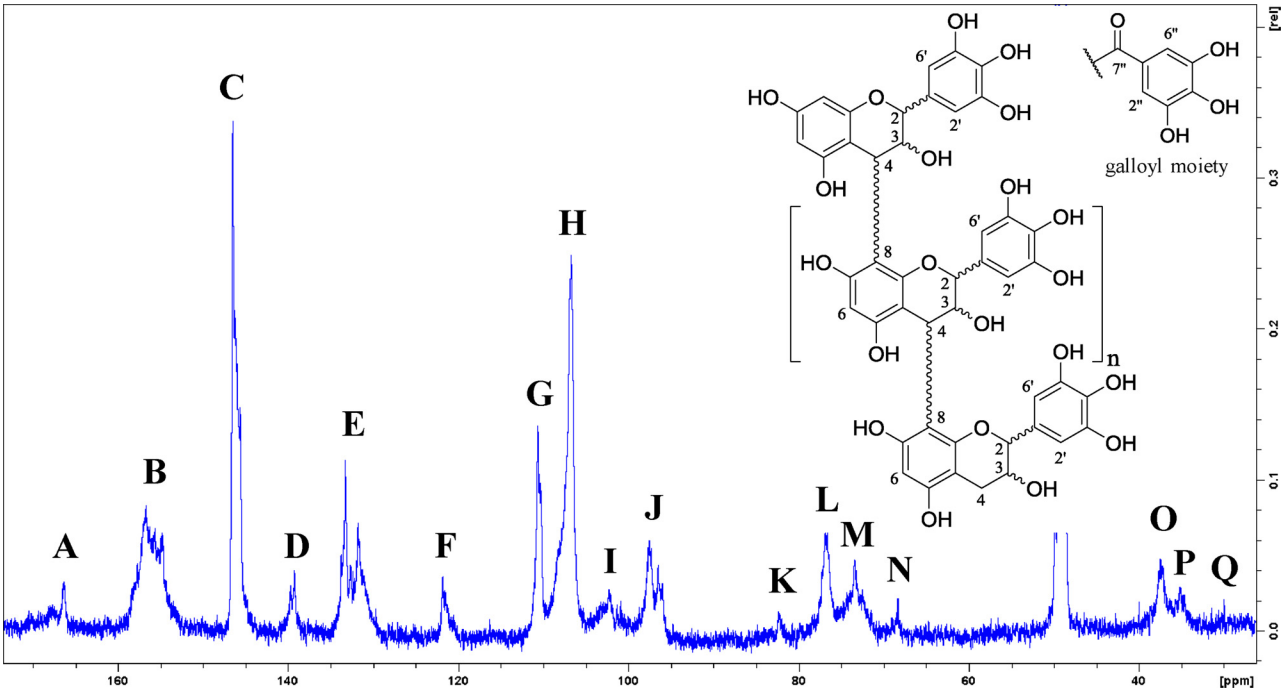

Fig. 2. ^{13}C-NMR of *M. salicifolia* fraction S7 enriched with polymeric proanthocyanidins measured at 150.93 MHz, 298 K (2048 scans); sample dissolved in CD_3OD (214 mg/ml). A (δ 166.3 ppm): C-7″; B (δ 154.8–156.6 ppm): C-5, C-7, C-8a; C (δ 145.6–146.5 ppm): C-3 and C-5; D (δ 139.2–139.7 ppm): C-4″; E (δ 131.7–133.7 ppm): C-1, C-4; F (δ 121.8 ppm): C1″; G (δ 110.6 ppm): C-2″, C-6″; H (δ 106.7 ppm): C-2′, C-6′; I (δ 100.9 ppm): C-2 of A-type units; *J* (δ 96.5–97.5 ppm): unsubstituted C-6 and C-8; K (δ 82.3 ppm): 2,3-*trans* configurated C-2; L (δ 76.8 ppm): 2,3-*cis* configurated C-2; M (δ 73.4 ppm): C-3 of all extender units; N (δ 68.3 ppm): C-3 (terminal units); O (δ 37.4 ppm): C-4 (non-galloylated extending units); P (δ 35.1 ppm): C-4 (galloylated extending units); Q (δ 29.6 ppm): C-4 (terminal units).

being in a comparable range.

In conclusion, this study revealed 12 compounds being reported the first time for *M. salicifolia*. Amongst these, five previously unknown compounds were isolated and described. The dioic acid derivatives (**10–12**) might be potential characteristic substances for this species, since they were never reported for any natural source before. Compound **4** was already isolated from *M. esculenta* (Sun et al., 1988) and **5** as well as **6** were isolated before from members of the genus *Myrica* being closely related to *Morella* (Nonaka et al., 1983; Santos and Waterman, 2000; Sun et al., 1988). Further, Adenodimerin C an A-type prodelphinidin was reported for *M. adenophora* (Ting et al., 2014) being very similar to **9** except the configuration at C-3 (C-ring). These three examples could be a further confirmation for the near chemotaxonomic relationship within the two genera. The characterization of the fraction enriched with polymeric proanthocyanidins showed the same characteristics as described for the isolated monomeric and dimeric flavan-3-ols. Thus, this species seems to prefer 2,3-*cis* configurated (C-ring), trihydroxylated (B-ring) flavan-3-ols and shows the tendency to link galloyl moieties to the respective structures. Again, these results and the average degree of polymerization are showing great similarity to a fraction containing polymeric proanthocyanidins from *M. esculenta* (Sun et al., 1988). Thus, the polymeric proanthocyanidins also confirm close chemotaxonomic relationship within the genera *Myrica* and *Morella*. These findings are also in accordance to the results considering the diarylheptanoids (Makule et al., 2017) and even a phylogenetic study revealing the family tree for Myricaceae (*Canacomyrica* (*Comptonia* (*Myrica*, *Morella*))), indicating a close neighborhood of the two genera *Myrica* and *Morella* (Herbert, 2005).

3. Experimental

3.1. Material

Methanol (MeOH), chloroform ($CHCl_3$), isopropyl alcohol, acetic acid (HOAc), formic acid (HCO_2H), hydrochloric acid 37%, sulfuric acid as well as D-xylose (Art.Nr. 8689) were purchased from Merck Chemicals GmbH (Darmstadt, Germany) in highest available quality. methanol-d_4 (CD_3OD, Art.Nr. 151947-10G-GL, 99.8 atom % D), *p*-anisaldehyde, vanillin (Fluka), trifluoroacetic acid (TFA), (*S*)-(+)-2-methylbutyric anhydride 95% (Art.Nr. 348562-1 G), D glucose ≥ 99.5% (Art.Nr. G7528-250 G), acetone (Me_2CO) for analysis, ethanol (EtOH) absolute and sodium carbonate (Na_2CO_3) were from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany). Acetone-d_6 ($(D_3C)_2CO$, Art.Nr. 00105-10 ml, 99.8 atom % D) and pyridine-d_5 (Art.Nr. 03403-10 ml, 99.5 atom % D) were from Deutero GmbH (Kastellaun, Germany). Ethyl acetate (EtOAc) for analysis and methylene chloride for analysis (CH_2Cl_2) were purchased from Acros Organics (Geel, Belgium). L-Glucose ≥ 98% (Art.Nr. 7509.2) was from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Toluene was from VWR International GmbH (Darmstadt, Germany). Water (H_2O) for analysis was generated by an Astacus Reagent bench (MembraPure GmbH, Henningsdorf/Berlin, Germany).

3.2. Instruments and methods

Optical rotations were measured in MeOH for spectroscopy with a Unipol L 1000 polarimeter (Schmidt + Haensch GmbH & Co., Berlin, Germany), CD spectra were recorded in MeOH for spectroscopy on a J-710 spectropolarimeter (JASCO Deutschland GmbH, Gross-Umstadt, Germany), at wavelength range of 195–350 nm. 1D-^{1}H, 1D-^{13}C, [^{1}H-^{13}C]-HSQC, [^{1}H-^{13}C]-HMBC, [^{1}H-^{1}H]-COSY, [^{1}H-^{1}H]-ROESY, [^{1}H-^{1}H]-NOESY and selective 1D-[^{1}H-^{1}H]-ROESY NMR spectra were measured at a Bruker AVANCE 600 spectrometer equipped with a 5 mm TCI CryoProbe (Bruker Corporation, Billerica, MA, USA; 600.25 MHz for ^{1}H and 150.93 MHz for ^{13}C) or AVANCE III HD spectrometer (Bruker; 400.13 MHz for ^{1}H and 100.63 MHz for ^{13}C). 1D-^{1}H NMR-spectra of SMB-derivatives were acquired with an AVANCE 300 (Bruker Corporation; 300.13 MHz for ^{1}H) or AVANCE 600 spectrometer.

Characterization of the enriched polymeric prodelphinidin fraction via ^{13}C NMR was also achieved with an AVANCE 600 (150.93 MHz for ^{13}C, number of scans = 2048, fraction S7 214 mg/ml). For NMR experiments, samples were dissolved in either CD_3OD, $(D_3C)_2CO$ or pyridine-d_5. The recorded NMR spectra were recorded at ambient temperature (298 K) or low temperature (278 K) and referenced against residual nondeuterated solvent. ESI-LRMS was measured on a TSQ 7000 (Thermo Quest, Finnigan) and ESI-HRMS on a Q-TOF 6540 UHD mass spectrometer (Agilent Technologies Sales & Services GmbH & Co. KG, Waldbronn, Germany).

Centrifugal partition chromatography (CPC) was performed using a SPOT centrifugal partition chromatography with 250 ml rotor (Armen Instrument, Saint-Avé, France; saturated EtOAc/H_2O system). First, system was run in ascending mode with upper phase (EtOAc) and second, after no further spots were detected at TLC-control, in descending mode with lower phase (H_2O) as mobile phase. Rotation = 800 rpm, flow rate 5 ml/min.

MPLC-chromatography was achieved via a Flash Spot On machine (Armen Instrument) using the following methods: MPLC system A: RP-18 pre-packed SVP D40-RP18 cartridge (Merck Chemicals GmbH), eluent A = H_2O, eluent B = MeOH, flow = 40 ml/min, gradient: 20% → 40% B (0–10 min), 40% → 100% B (10–30 min), 100% B isocratic (30–60 min). MPLC system B: pre-packed SVP D40-RP18 cartridge (Merck Chemicals GmbH), eluent A: H_2O, eluent B: acetonitrile, flow = 5 ml/min, gradient: 20% → 100% B (0–60 min), 100% B isocratic (60–75 min). MPLC system C: 170 g MCI-Gel® CHP20 P (Mitsubishi Chemical Europe GmbH, Düsseldorf, Germany), column: 600 × 25 mm (BESTA-Technik für Chromatographie GmbH, Wilhelmsfeld, Germany), eluent A = H_2O, eluent B = MeOH, flow = 5 ml/min, gradient: 20–60% B stepwise in 10% intervals (40 min each), final washing step 100% B for 60 min. MPLC system D: pre-packed Reveleris® 20 g silica gel 20 µm cartridge (Büchi Labortechnik AG, Flawil, Switzerland), eluent A = $CHCl_3$, eluent B = MeOH, flow = 15 ml/min, gradient: 5% → 12% B (0–30 min), 12–60% B (30–31 min), 60–100% B (31–40 min), 100% B isocratic (40–50 min).

Final purification of isolated compounds was achieved on a ProStar HPLC (Varian Deutschand GmbH, Darmstadt, Germany) coupled with an Eclipse XDB-C18, 250 × 9.4 mm, 5 µm (Agilent, column A) or a Purospher STAR RP-18e column 250 × 4 mm, 5 µm (Merck Chemicals, column B) using the following methods: HPLC system A: column A, eluent A = H_2O, eluent B = MeOH, flow = 2 ml/min, gradient: 50% → 60% B (0–20 min), 60% → 100% B (20–21 min), 100% B isocratic (21–25 min). HPLC system B: column A, eluent A = H_2O (+0.02% TFA), eluent B = MeOH (+0.02% TFA), flow 2 ml/min, gradient: 25% → 35% B (0–30 min), 35% → 65% B (30–31 min), 65% B isocratic (31–35 min). HPLC system C: column A, eluent A = H_2O (+0.05% TFA), eluent B = acetonitrile (+0.05% TFA), flow = 2 ml/min, gradient: 25% → 40% B (0–30 min), 40% → 100% B (30–35 min), 100% B isocratic (35–38 min). HPLC system D: column A, eluent A = H_2O (+0.02% TFA), eluent B = MeOH (+0.02% TFA), flow 2 ml/min, gradient: 20% → 30% B (0–15 min), 30% B isocratic (15–16 min), 30% → 70% B (16–18 min), 70% B isocratic (18–24 min). HPLC system E: column B, eluent A = H_2O (+0.02% TFA), eluent B = MeOH (+0.02% TFA), flow = 1 ml/min, gradient: 15% → 19% B (0–5 min), 19% → 23% B (5–20 min), 23% → 60% B (20–22 min), 60% B isocratic (22–25 min). HPLC system F: HPLC system A: column A, eluent A = H_2O, eluent B = MeOH, flow = 2 ml/min, gradient: 60% B isocratic (0–10 min), 60% → 100% B (10–11 min), 100% B isocratic (11–15 min).

3.3. Plant material

The bark of *Morella salicifolia* (HOCHST EX A. RICH) VERDC. & POLHILL was collected in February 2013 at Monduli mountain ranges in the Arusha region, Tanzania. Identification of the plant was achieved by senior botanist Daniel Sitoni from Tanzania National Herbarium (TNH, Arusha, Tanzania) as well as Canisius J. Kayombo from the Olmotonyi Forestry Institute (Arusha, Tanzania) and a voucher specimen (Nr. CK 7792) was stored at TNH. The collected bark material was spread on a clean cotton cloth under direct sunlight with temperature between 30–35 °C until complete dryness.

3.4. Extraction

Dried and pulverized *M. salicifolia* bark (390.1 g) was mixed with 400 g of sea sand, packed in a column and macerated overnight with 1 l of CH_2Cl_2. After maceration, subsequent bulk extraction of *M. salicifolia* bark was performed using CH_2Cl_2, EtOAc, MeOH and MeOH-H_2O (50 + 50 [V/V], approximately 4 l each), resulting in extracts of 7.7, 1.1, 162.2 and 25.9 g, respectively.

3.5. Isolation

MeOH extract (119 g) was subjected in portions of 12 g to Sephadex® LH-20 column chromatography (GE Healthcare GmbH, München, Germany; 265 g, column: 95 × 3 cm, flow = 0.5 ml/min) with two eluents. First EtOH-H_2O (70 + 30 [V/V]) resulting in fractions S1–S6 and second Me_2CO (70 + 30 [V/V]) yielding fraction S7 (96.5 g, enriched polymeric prodelphinidins). Fraction control was achieved via TLC on silica gel 60 F_{254} (Merck Chemicals GmbH), using EtOAc-H_2O-HOAc-HCO_2H (100 + 26 + 11 + 11 [V/V/V/V]) and derivatization with vanillin/hydrochloric acid or anisaldehyde/sulfuric acid as detection reagents. Fraction S2 (450–560 ml, 15.7 g) was fractionated by MPLC system A. Fraction S2.F4 (760 mg) was fractionated by CPC (all methods in chapter 2.2). S2.F4.C2 (49.8 mg) yielded to **3** (R_t = 12.2 min), **10** (R_t = 9.6 min) and **12** (R_t = 10.4 min) via HPLC system A. Further, **11** (R_t = 16.0 min) and **3** (R_t = 12.2 min) were gained from S2.F4.C3 (53.9 mg) and S2.F4.C6 (288.0 mg) yielded **2** (R_t = 15.8 min) using HPLC system A. Fraction S2.F5 (1.44 g, 851–920 ml) was also fractionated by CPC. S2.F5.C4 (22.7 mg) yielded **1** (R_t = 16.0 min) via HPLC system B. Further, **1** was re-isolated from S2.F6 (0.14 g) by a subsequent combination of MPLC system D and HPLC system F (R_t = 5.9 min). S4 (1251–1430 ml, 0.4 g) was fractionated by MPLC system B and revealed fraction S4.2 (121–771 ml) with the already pure compound **5**. S5 (1851–2070 ml, 0.32 g) was fractionated by MPLC system C. The fraction S5.M4 (9.0 mg) lead to **7** (R_t = 5.0 min), S5.M6 (9.4 mg) to **4** (R_t = 6.5 min) and S5.M9 (17.0 mg) to **8** (R_t = 7.0 min) via HPLC system C. S6 (2271–2820 ml, 420.5 mg) was also subjected to MPLC system C. The fraction S6.M6 (32.0 mg) yielded **9** (R_t = 10.7 min) using HPLC system D. Compound **6** was gained from fraction S6.M13 (16.0 mg) after a combination of HPLC system D (R_t = 10.0 min) followed by HPLC system E (R_t = 8.0 min).

3.6. Determination of absolute configuration of glycosides

Determination of the glycosides and their absolute configuration was achieved by recording the ^1H NMR spectra of the per-*O*-(*S*)-2-methylbutyrate (SMB) derivatives and compared to ^1H NMR data of the SMB derivatives of reference sugars (York et al., 1997), according to Makule et al. (2017). In brief, sugars were hydrolyzed with 2 M TFA followed by a derivatization with (*S*)-(+)-2-methylbutyric anhydride. The ^1H-NMR spectra of the gained SMB-derivatives were compared to reference sugars.

3.7. Isolated compounds

Myriside, 3,4-dimethyl ellagic acid 4′-O-β-D-xylopyranoside (**1**, 1.5 mg). Yellowish amorphous powder. $[\alpha]_D^{25}$ −38.3 (c 0.1, MeOH). CD $[\Theta]_{204}$ −35,458.1, $[\Theta]_{213}$ −18,411.8, $[\Theta]_{227}$ +10,529.8, $[\Theta]_{248}$ +20,077.3, $[\Theta]_{273}$ −9009.1 (MeOH, 0.006). UV (MeOH, log ε) λ_{max} 255.1 nm (3.7), 300.1 nm (3.6). NMR data in Table 1 and 2D-NMR

Table 2
NMR-data for epigallocatechin-3-*O*-gallate-(2*β*→*O*→7, 4*β*→8)-gallocatechin (**9**). 1H NMR at 600.25 MHz, ^{13}C NMR at 150.93 MHz, temperature 278 K and 298 K, in CD_3OD (5 mg/ml) (δ in ppm, *J* in Hz).

	Pos.	^{13}C	^{1}H	HMBC	COSY	ROESY
upper unit	2	99.0				
	3	69.8	5.38 (1H, d, *J* = 3.6), 278 K 5.40 (1H, d, *J* = 3.6), 298 K	4a,7″	4	4,2′/6′
	4	27.3	4.49 (1H, d, *J* = 3.5), 278 K 4.50 (1H, d, *J* = 3.6), 298 K	3,4a,5,6,8a; lower unit: 7,8,8a	3	3
	4a	103.7				
	5	156.6				
	6	98.5	5.95 (1H, d, *J* = 2.2), 278 K 5.95 (1H, d, *J* = 2.3), 298 K	4a,5,7,8	8	–
	7	158.4				
	8	96.5	6.12 (1H, brs) [a], 278 K 6.14 (1H, d, *J* = 2.3), 298 K	4a,6,7,8a	6	2′/6′
	8a	152.0				
	1′	130.8				
	2′/6′	107.5	6.70 (2H, s), 278 K 6.72 (2H, s), 298 K	2,1',2′/6',3′/5'		3,8
	3′/5′	146.4				
	4′	134.9				
galloyl moiety	1″	120.6				
	2″/6″	110.3	6.72 (2H, s), 278 K 6.72 (2H, s), 298 K	2″/6″,3″/5″,4″,7″		–
	3″/5″	146.3				
	4″	140.2				
	7″	166.8				
lower unit	2	84.7	4.73 (1H, d, *J* = 7.3), 278 K 4.74 (1H, d, *J* = 7.3), 298 K	3,4*α*,4*β*,8a, 1‘,2‘/6‘	3	3,4*α*,4*β*
	3	68.4	4.10 (1H, m), 278 K 4.11 (1H, m), 298 K	–	2,4*α*,4*β*	2,4*α*,4*β*, 2‘/6‘
	4*β*	28.7	2.59 (1H, dd, *J* = 7.8, 16.4), 278 K 2.60 (1H, dd, *J* = 7.8, 16.5), 298 K	2,3,4a,5,8a	4*α*	2,3, 4*α*
	4*α*		2.88 (1H, dd, *J* = 5.4, 16.4), 278 K 2.90 (1H, dd, *J* = 5.3, 16.5), 298 K		4*β*	3, 4*β*
	4a	103.4				
	5	156.8				
	6	96.5	6.12 (1H, s) [a], 278 K 6.13 (1H, s), 298 K	4a,5,7,8		–
	7	154.4				
	8	105.9				
	8a	151.9				
	1′	130.4				
	2′/6′	108.0	6.45 (2H, s), 278 K 6.46 (2H, s), 298 K	2,2′/6',3′/5',4'		2,3
	3′/5′	147.0				
	4′	134.7				

Cross-signals are considering the same unit, exceptions are indicated. a) Signals are overlapping (278 K).

cross-signals in Table S4. Negative ESI-HRMS *m/z* 461.0735 $[M-H]^-$ ($C_{21}H_{17}O_{12}$, *calcd.* $[M-H]^-$ *m/z* 461.0720).

Lagertannin, 3,4-dimethyl ellagic 4′ O-*β*-D-*xylopyranoside* (**2**, 6.0 mg). Yellowish amorphous powder. $[\alpha]_D^{25}$ −24.6 (c 0.1, MeOH). UV (pyridine, log ε) λ_{max} 354.9 nm (3.7). NMR data in Table 1 and 2D-NMR cross-signals in Table S4. Negative ESI-HRMS *m/z* 491.0821 $[M-H]^-$ ($C_{22}H_{19}O_{13}$, *calcd.* $[M-H]^-$ *m/z* 491.0826).

Ducheside A, 3-O-methyl ellagic acid 4′-O-*β*-D-*xylopyranoside* (**3**, 18.6 mg). Yellowish amorphous powder. $[\alpha]_D^{25}$ −60.0 (c 0.1, MeOH). UV (MeOH, log ε) λ_{max} 254.9 nm (4.0), 354.9 nm (3.5). Positive ESI-HRMS *m/z* 449.0716 $[M+H]^+$ ($C_{20}H_{17}O_{12}$, *calcd.* $[M+H]^+$ *m/z* 449.0720).

Castalagin (**4**, 1.6 mg). Off-white amorphous powder. $[\alpha]_D^{25}$ +15.6 (c 0.1, MeOH). CD $[\Theta]_{199}$ −18,317.8, $[\Theta]_{206}$ +2623.0, $[\Theta]_{210}$ −6958.4, $[\Theta]_{218}$ +4458.7, $[\Theta]_{228}$ +17,230.8, $[\Theta]_{235}$ +23,569.7, $[\Theta]_{260}$ −9153.8, $[\Theta]_{274}$ -8786.7, $[\Theta]_{284}$ +3297.1, $[\Theta]_{294}$ +970.5, $[\Theta]_{300}$ +2187.3, $[\Theta]_{310}$ +1244.4, $[\Theta]_{320}$ −2836.2, $[\Theta]_{325}$ +1445.3, $[\Theta]_{333}$ −920.6, $[\Theta]_{341}$ +3667.7 (MeOH, 0.006). UV (MeOH, log ε) λ_{max} 275.0 nm (3.4). Negative ESI-HRMS *m/z* 933.0800 $[M-H]^-$ ($C_{41}H_{25}O_{26}$, *calcd.* $[M\text{-}H]^-$ *m/z* 933.0634).

(+)-*Gallocatechin* (**5**, 0.2 g). Light orange amorphous powder. $[\alpha]_D^{22.4}$ +25.1 (c 0.1, MeOH). UV (MeOH, log ε) λ_{max} 269.9 nm (2.7). Positive ESI-HRMS *m/z* 307.0815 $[M+H]^+$ ($C_{15}H_{15}O_7$, *calcd.* $[M+H]^+$ *m/z* 307.0818).

Epigallocatechin-3-O-*gallate* (**6**, 1.8 mg). Light orange amorphous powder. $[\alpha]_D^{25}$ -96.8 (c 0.1, MeOH). UV (MeOH, log ε) λ_{max} 275.0 nm (3.0). Positive ESI-HRMS *m/z* 459.0922 $[M+H]^+$ ($C_{22}H_{19}O_{11}$, *calcd.* $[M+H]^+$ *m/z* 459.0927).

Prodelphinidin B1, epigallocatechin-(4β→8)-gallocatechin (**7**, 2.3 mg). Amorphous light orange powder. CD $[\Theta]_{207}$ −35,037.4, $[\Theta]_{218}$ +25,726.9, $[\Theta]_{229}$ +11,708.0, $[\Theta]_{238}$ +9130.9, $[\Theta]_{246}$ −7344.56, $[\Theta]_{284}$ −3964.5 (MeOH, 0.006). UV (MeOH, log ε) λ_{max} 269.9 nm (2.8). Positive ESI-HRMS *m/z* 611.1395 $[M+H]^+$ ($C_{30}H_{27}O_{14}$, *calcd.* $[M+H]^+$ *m/z* 611.1401).

Ephedrannin D5, epigallocatechin-(2β→O→7, 4β→8)-gallocatechin (**8**, 6.0 mg). Amorphous light orange powder. CD $[\Theta]_{201}$ −25,227.9, $[\Theta]_{213}$ +3762.2, $[\Theta]_{216}$ +1569.9, $[\Theta]_{225}$ +17,397.4, $[\Theta]_{232}$ +14,525.9, $[\Theta]_{238}$ +11,237.4, $[\Theta]_{271}$ −11,888.9 (MeOH, 0.006). UV (MeOH, log ε) λ_{max} 269.9 nm (2.8). Positive ESI-MS *m/z* 609.1 $[M+H]^+$.

Epigallocatechin-3-O-*gallate-(2β→O→7,4β→8)-gallocatechin* (**9**, 2.6 mg). Amorphous orange powder. $[\alpha]_D^{25}$ +20.5 (c 0.1, MeOH). CD $[\Theta]_{208}$ −4910.4, $[\Theta]_{217}$ −29,532.6, $[\Theta]_{238}$ +13,323.4, $[\Theta]265$ −9666.0, $[\Theta]_{272}$ −13,436.8, $[\Theta]_{296}$ −3480.1, $[\Theta]_{306}$ −3017.3 (MeOH, 0.006). UV (MeOH, log ε) λ_{max} 275.0 nm (3.1). NMR data in Table 2. Negative ESI-HRMS *m/z* 759.1206 $[M-H]^-$ ($C_{37}H_{27}O_{18}$,

Table 3
NMR-data for 2,7-dimethyl-8-*O*-(6′-*O*-galloyl)-*β*-D-glucopyranoside-deca-2*E*,4*E*-dienedioic acid (**10**, 2.3 mg/ml), 2,7-dimethyl-8-*O*-*β*-D-glucopyranoside-deca-2*E*,4*E*-dienedioic acid (**11**, 4.0 mg/ml) and 4,9-dimethyl-10-*O*-*β*-D-glucopyranoside-dodeca-2*E*,4*E*,6*E*-trienedioic acid (**12**, 1 mg/ml). ^{1}H-NMR at 600.25 MHz, ^{13}C-NMR at 150.93 MHz, 298 K, in CD_3OD (δ in ppm, J in Hz), pt = pseudo triplet.

		10		**11**		**12**	
	Pos.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
deca-2*E*,4*E*-dienedioic acid/dodeca-2*E*,4*E*,6*E*-trienedioic acid	1	172.8		172.5		172.5	
	2	126.2		126.3		118.9	5.84 (1H, d, J = 15.6)
	3	140.4	7.03 (1H, d, J = 11.0)	140.2	7.16 (1H, d, J = 11.3)	150.0	7.27 (1H, d, J = 15.5)
	4	128.3	6.25 (1H, dd, J = 11.4, 14.9)	128.5	6.45 (1H, dd, J = 11.4, 15.0)	132.6	
	5	143.4	5.97 (1H, m)	143.6	6.14 (1H, m)	139.9	6.38 (1H, d, J = 11.2)
	6a	37.6	1.96 (1H, m)	37.1	2.04 (1H, m)	128.9	6.49 (1H, dd, J = 11.3,
					2.57 (1H, m)	140.8	14.9)
	6b		2.42 (1H, m)				6.02 (1H, m)
	7	38.5	1.86 (1H, m)	38.4	1.93 (1H, m)	37.2	2.02 (1H, m)
	8	81.7	4.11 (1H, m)	80.9	4.12 (1H, m)		2.56 (1H, m)
	9a	39.2	2.45 (1H, dd, J = 5.8, 14.9)	38.5	2.48 (1H, dd, J = 5.5, 15.5)	38.7	1.91 (1H, m)
	9b		2.53 (1H, dd, J = 7.3, 15.2)		2.55 (1H, dd, J = 7.4, 15.6)	81.4	4.11 (1H, m)
	10	178.1		177.3		39.2	2.44 (1H, dd, J = 5.4, 15.2)
	11	14.7	0.84 (3H, d, J = 6.9)	14.9	0.93 (3H, d, J = 6.9)		2.52 (1H, dd, J = 7.5, 15.2)
	12	12.7	1.80 (3H, s)	12.7	1.89 (3H, d, J = 0.8)	178.3	
	13					15.0	0.93 (3H, d, J = 6.8)
	14					12.6	1.88 (3H, s)
β-D-glucopyranoside	1′	103.9	4.44 (1H, d, J = 7.7)	103.8	4.39 (1H, d, J = 7.7)	103.9	4.39 (1H, d, J = 7.7)
	2′	75.5	3.20 (1H, pt, J = 8.4)	75.4	3.16 (1H, dd, J = 7.9, 8.9)	75.4	3.16 (1H, dd, J = 7.9, 8.8)
	3′	78.1	3.40 (1H, pt, J = 8.9)	78.1	3.36 (1H, pt, J = 8.9)	78.1	3.36 (1H, pt, J = 8.9)
	4′	72.0	3.36 (1H, pt, J = 8.9)	71.6	3.31 (1H, pt, J = 9.1)	71.6	3.31 (1H, pt, J = 9.6)
	5′	75.5	3.57 (1H, ddd, J = 1.9, 6.7, 9.3)	77.9	3.26 (1H, ddd, J = 2.4, 5.3, 9.4)	77.9	3.26 (1H, ddd, J = 2.3, 5.3, 9.5)
	6′	65.0	4.38 (1H, dd, J = 6.6, 11.7)	62.8	3.69 (1H, dd, J = 5.3, 11.8)	62.9	3.69 (1H, dd, J = 5.2, 11.7)
			4.51 (1H, dd, J = 2.1, 11.7)		3.85 (1H, dd, J = 2.3, 11.8)		3.85 (1H, dd, J = 2.3, 11.8)
galloyl moiety	1″	121.7					
	2″/6″	110.4	7.10 (2H, s)				
	3″/5″	146.6					
	4″	140.0					
	7″	168.4					

calcd. $[M-H]^-$ *m/z* 759.1197).

2,7-Dimethyl-8-O-*(6′-O-galloyl)-β-D-glucopyranoside-deca-2*E,*4*E-*dienedioic acid* (**10**, 1.4 mg). Off-white amorphous powder. $[\alpha]_D^{25}$ −19.0 (c 0.1, MeOH). UV (MeOH, log ε) λ_{max} 265.0 nm (3.7). NMR data in Table 3 and 2D NMR cross-signals in Table S5. Positive ESI-HRMS *m/z* 557.1862 $[M+H]^+$ ($C_{25}H_{33}O_{14}$, *calcd.* $[M+H]^+$ *m/z* 557.1870).

2,7-Dimethyl-8-O-β-D-*glucopyranoside-deca-2*E,*4*E-*dienedioic acid* (**11**, 2.4 mg). Off-white amorphous powder. $[\alpha]_D^{25}$ −24.0 (c 0.1, MeOH). UV (MeOH, log ε) λ_{max} 265.0 nm (3.7). NMR data in Table 3 and 2D NMR cross-signals in Table S5. Positive ESI-HRMS *m/z* 422.2022 $[M + NH_4]^+$ ($C_{18}H_{28}O_{10}$, *calcd.* $[M + NH_4]^+$ *m/z* 422.2026).

4,9-Dimethyl-10-O-β-D-*glucopyranoside-dodeca-2*E,*4*E,*6*E-*trienedioic acid* (**12**, 0.6 mg). Off-white amorphous powder. $[\alpha]_D^{25}$ −22.0 (c 0.1, MeOH). UV (MeOH, log ε) λ_{max} 295.0 nm (3.6). NMR data in Table 3 and 2D-NMR cross-signals in Table S5. Positive ESI-HRMS *m/z* 448.2175 $[M + NH_4]^+$ ($C_{20}H_{31}O_{10}$, *calcd.* $[M + NH_4]^+$ *m/z* 448.2183).

Authors' contributions

E.M. did the isolation work and performed the structure elucidation and experiments together with S.W. S.W. wrote the manuscript together with J.H. and E.M. G.J. supervised the isolation and structure elucidation strategies. B.K. and J.H. designed the project. All authors read and approved the manuscript.

Acknowledgements

This research was financially supported by MoEVT, DAAD and NM-AIST giving a grant to E.M. Many thanks are due to Philipp Nitschke (Research Group Ruth M. Gschwind, Institute of Organic Chemistry, University of Regensburg) for performing the selective ROESY experiments. Fritz Kastner is kindly acknowledged for performing the standard NMR-experiments, Josef Kiermaier and Wolfgang Söllner for acquiring the MS data (all: Zentrale Analytik, Faculty of Chemistry and Pharmacy, University of Regensburg). Daniel Sitoni (National Herbarium of Tanzania, Arusha, Tanzania) and Canisius J. Kayombo (Olmotonyi Forestry Institute, Arusha, Tanzania) are acknowledged for identifying the plant material.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2018.09.013.

References

Abdel Sattar, E., El Olemy, M.M., Elhag, H., El-Domiaty, M.M., Mossa, J.S., Petereit, F., Nahrstedt, A., 1999. Flavan-3-ols and prodelphinidins from *Catha edulis*. Sci. Pharm. 67, 159–165.

Barrett, M.W., Klyne, W., Scopes, P.M., Fletcher, A.C., Porter, L.J., Haslam, E., 1979. Plant proanthocyanidins. Part 6. Chiroptical studies. Part 95. Circular dichroism of procyanidins. J. Chem. Soc. Perkin Trans. 1, 2375–2377. https://doi.org/10.1039/p19790002375.

Botha, J.J., Ferreira, D., Roux, D.G., 1978. Condensed tannins. Circular dichroism method of assessing the absolute configuration at C-4 of 4-arylflavan-3-ols, and stereochemistry of their formation from flavan-3,4-diols. J. Chem. Soc. Chem. Commun. (16), 698–700. https://doi.org/10.1039/c39780000698.

Botha, J.J., Young, D.A., Ferreira, D., Roux, D.G., 1981. Synthesis of condensed tannins. Part 1. Stereoselective and stereospecific syntheses of optically pure 4-arylflavan-3-ols, and assessment of their absolute stereochemistry at C-4 by means of circular dichroism. J. Chem. Soc. Perkin Trans. 1, 1213–1219. https://doi.org/10.1039/p19810001213.

Czochanska, Z., Foo, L.Y., Newman, R.H., Porter, L.J., 1980. Polymeric proanthocyanidins. Stereochemistry, structural units, and molecular weight. J. Chem. Soc. Perkin

Trans. 1 (2278). https://doi.org/10.1039/p19800002278.
Davis, A.L., Cai, Y., Davies, A.P., Lewis, J.R., 1996. ^{1}H and^{13}C NMR assignments of some green tea polyphenols. Magn. Reson. Chem. 34, 887–890. https://doi.org/10.1002/(SICI)1097-458X(199611)34:11<887:AID-OMR995>3.0.CO;2-U.
Eberhardt, T.L., Young, R.A., 1994. Conifer seed cone proanthocyanidin polymers: characterization by ^{13}C NMR spectroscopy and determination of antifungal activities. J. Agric. Food Chem. 42, 1704–1708. https://doi.org/10.1021/jf00044a023.
Fujii, W., Toda, K., Matsumoto, K., Kawaguchi, K., Kawahara, S.-i., Hattori, Y., Fujii, H., Makabe, H., 2013. Syntheses of prodelphinidin B1, B2, and B4 and their antitumor activities against human PC-3 prostate cancer cell lines. Tetrahedron Lett. 54, 7188–7192. https://doi.org/10.1016/j.tetlet.2013.10.113.
Gallo, M.B.C., Rocha, W.C., da Cunha, U.S., Diogo, F.A., da Silva, F.C., Vieira, P.C., Vendramim, J.D., Fernandes, J.B., da Silva, M.F., das, G.F., Batista-Pereira, L.G., 2006. Bioactivity of extracts and isolated compounds from *Vitex polygama* (Verbenaceae) and *Siphoneugena densiflora* (Myrtaceae) against *Spodoptera frugiperda* (Lepidoptera: noctuidae). Pest Manag. Sci. 62, 1072–1081. https://doi.org/10.1002/ps.1278.
Getahun, A., 1976. Some Common Medicinal and Poisonous Plants Used in Ethiopian Folk Medicine. Amare Getahun.
Hedberg, I., Hedberg, O., Madati, P.J., Mshigeni, K.E., Mshiu, E.N., Samuelsson, G., 1982. Inventory of plants used in traditional medicine in Tanzania. I. Plants of the families acanthaceae-cucurbitaceae. J. Ethnopharmacol. 6, 29–60. https://doi.org/10.1016/0378-8741(82)90070-8.
Herbert, J., 2005. Systematics and Biography of Myricaceae. PhD Thesis. St. Andrews.
Hikino, H., Shimoyama, N., Kasahara, Y., Takahashi, M., Konno, C., 1982. Structures of mahuannin A and B, hypotensive principles of *Ephedra* roots. Heterocycles 19, 1381. https://doi.org/10.3987/R-1982-08-1381.
Khallouki, F., Haubner, R., Hull, W.E., Erben, G., Spiegelhalder, B., Bartsch, H., Owen, R.W., 2007. Isolation, purification and identification of ellagic acid derivatives, catechins, and procyanidins from the root bark of *Anisophyllea dichostyla* R. Br. Food Chem. Toxicol. 45, 472–485. https://doi.org/10.1016/j.fct.2006.09.011.
Kokwaro, J.O., 2009. Medicinal Plants of East Africa, 3rd ed. Univ. of Nairobi Press, Nairobi, Kenya 478.
Kolodziej, H., Sakar, M.K., Burger, J.F.W., Engelshowe, R., Ferreira, D., 1991. A-type proanthocyanidins from *Prunus spinosa*. Phytochemistry 30, 2041–2047. https://doi.org/10.1016/0031-9422(91)85064-7.
Kumar, N.S., Rajapaksha, M., 2005. Separation of catechin constituents from five tea cultivars using high-speed counter-current chromatography. J. Chromatogr. A 1083, 223–228. https://doi.org/10.1016/j.chroma.2005.06.013.
Lee, M.-W., Morimoto, S., Nonaka, G.-I., Nishioka, I., 1992. Flavan-3-ol gallates and proanthocyanidins from P*ithecellobium lobatum*. Phytochemistry 31, 2117–2120. https://doi.org/10.1016/0031-9422(92)80375-O.
Makule, E., Schmidt, T.J., Heilmann, J., Kraus, B., 2017. Diarylheptanoid glycosides of *Morella salicifolia* bark. Molecules 22 (12). https://doi.org/10.3390/molecules22122266.
Matsuo, Y., Wakamatsu, H., Omar, M., Tanaka, T., 2015. Reinvestigation of the stereochemistry of the C-glycosidic ellagitannins, vescalagin and castalagin. Org. Lett. 17, 46–49. https://doi.org/10.1021/ol503212v.
Mayer, W., Seitz, H., Jochims, J.C., 1969. Über die Gerbstoffe aus dem Holz der Edelkastanie und der Eiche, IV[1)] Die Struktur des Castalagins. Justus Liebigs Ann. Chem. 721, 186–193. https://doi.org/10.1002/jlac.19697210123.
Mbuya, L.P., Msanga, H.P., Ruffo, C.K., Birnie, A., Tengnäs, B., 1994. Useful Trees and Shrubs for Tanzania: Identification, Propagation and Management for Agricultural and Pastoral Communities. Regional Soil Conservation Unit SIDA, Nairobi, pp. 542.
Newman, R.H., Porter, L.J., Foo, L.Y., Johns, S.R., Willing, R.I., 1987. High-resolution^{13}C NMR studies of proanthocyanidin polymers (condensed tannins). Magn. Reson. Chem. 25, 118–124. https://doi.org/10.1002/mrc.1260250207.
Nonaka, G.-I., Muta, M., Nishioka, I., 1983. Myricatin, a galloyl flavanonol sulfate and prodelphinidin gallates from *Myrica rubra*. Phytochemistry 22, 237–241. https://doi.org/10.1016/S0031-9422(00)80097-7.
Porter, L.J., Newman, R.H., Foo, L.Y., Wong, H., Hemingway, R.W., 1982. Polymeric proanthocyanidins. ^{13}C N.M.R. studies of procyanidins. J. Chem. Soc. Perkin Trans. 1, 1217–1221. https://doi.org/10.1039/p19820001217.
Roux, D.G., Maihs, E.A., 1960. Condensed tannins. 3. Isolation and estimation of (−)-7:3′:4′:5′-tetrahydroxyflavan-3-ol, (+)-catechin and (+)-gallocatechin from black-wattle-bark extract. Biochem. J. 74, 44–49.
Santos, S.C., Waterman, P.G., 2000. Condensed tannins from *Myrica gale*. Fitoterapia 71, 610–612. https://doi.org/10.1016/S0367-326X(00)00198-2.
Sun, D., Wong, H., Foo, L.Y., 1987. Proanthocyanidin dimers and polymers from *Quercus dentata*. Phytochemistry 26, 1825–1829. https://doi.org/10.1016/S0031-9422(00)82297-9.
Sun, D., Zhao, Z., Wong, H., Foo, L.Y., 1988. Tannins and other phenolics from *Myrica esculenta* bark. Phytochemistry 27, 579–583. https://doi.org/10.1016/0031-9422(88)83145-5.
Takahashi, M., Osawa, K., Ueda, J., Yamamoto, F., Tsai, C.-T., 1976. The components of the plants of *Lagerstroemia* genus. III. On the structure of the new tannin "lagertannin" from the leaves of *Lagerstroemia speciosa* (L.) PERS. Yakugaku Zasshi 96, 984–987. https://doi.org/10.1248/yakushi1947.96.8_984.
Ting, Y.-C., Ko, H.-H., Wang, H.-C., Peng, C.-F., Chang, H.-S., Hsieh, P.-C., Chen, I.-S., 2014. Biological evaluation of secondary metabolites from the roots of *Myrica adenophora*. Phytochemistry 103, 89–98. https://doi.org/10.1016/j.phytochem.2014.04.003.
Ye, L., Yang, J.S., 1996. New ellagic glycosides and known triterpenoids from *Duchesnea indica* Focke. Yaoxue Xuebao 31, 844–848.
York, W.S., Hantus, S., Albersheim, P., Darvill, A.G., 1997. Determination of the absolute configuration of monosaccharides by ^{1}H NMR spectroscopy of their per-*O*-(*S*)-2-methylbutyrate derivatives. Carbohydr. Res. 300, 199–206. https://doi.org/10.1016/S0008-6215(97)00050-5.
Zang, X., Shang, M., Xu, F., Liang, J., Wang, X., Mikage, M., Cai, S., 2013. A-type proanthocyanidins from the stems of *Ephedra sinica* (Ephedraceae) and their antimicrobial activities. Molecules 18, 5172–5189. https://doi.org/10.3390/molecules18055172.