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# Antibacterial and cytotoxic prenylated dihydrochalcones from *Eriosema montanum*

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ABSTRACT

Two new prenylated dihydrochalcones (**1**,**2**) and eighteen known secondary metabolites (**3**–**20**) were isolated from the $CH_2Cl_2$-MeOH (1:1) extracts of the roots, the stem bark and the leaves of *Eriosema montanum* Baker f. (Leguminosae). The structures of the isolated compounds were characterized by NMR, IR, and UV spectroscopic and mass spectrometric analyses. The structures of compounds **5**, **10**, **11** and **13** were confirmed by single crystal X-ray diffraction. The antibacterial activity of the crude extracts and the isolated constituents were established against Gram-positive and Gram-negative bacteria. Among the tested compounds, **1**–**4** and **10** showed strong activity against the Gram-positive bacterium *Bacillus subtilis* with minimum inhibitory concentration (MIC) ranging from 3.1 to 8.9 μM, as did the leaf crude extract with an MIC of 3.0 μg/mL. None of the crude extracts nor the isolated compounds were active against *Escherichia coli.* Compounds **1**, **3** and **4** showed higher cytotoxicity, evaluated against the human breast cancer cell line MCF-7, with $EC_{50}$ of 7.0, 18.0 and 18.0 μM, respectively. These findings contribute to the phytochemical understanding of the genus *Eriosema*, and highlight the pharmaceutical potential of prenylated dihydrochalcones.

## 1. Introduction

*Eriosema montanum* Baker f. (Leguminosae), locally known in Rwanda as "Umupfunyantoki", is a perennial shrub that can reach up to 2 meters height [1]. It has yellow flowers, 3-foliate ovate leaves, has ferruginous hairs and is branched [2]. It belongs to the genus *Eriosema*, which is composed of more than 140 species that are distributed in tropical areas [3,4]. Most of these species are used in traditional medicine [5]. In Venezuela, the root decoction of *E. rufum* is used against sterility in women and to accelerate delivery during childbirth. The root decoction of *E. tuberosum* helps in detoxification and to treat diarrhea, orchitis, and hydrophobia, in Yunan Province, China [3]. *E. kraussianum* is used by the Zulu community in South Africa for the treatment of erectile dysfunction and male impotence [6]. In Cameroon, *E. laurentii* decoctions are employed for treatment of infertility and various gynecological problems [7]. *E. robustum* preparations are used to treat cough and skin diseases [8].

In Rwanda, the leaf extract of *E. montanum* is used against conjunctivitis, cough and snake bite [9]. Studies of its ethanolic leaf extract showed anti-HIV and anti-inflammatory activities [10]. Recently, its root decoction was reported to have anti-asthmatic activity [11]. In spite of its importance in Rwandese traditional medicine, there is so far no report on phytochemical investigation of *E. montanum*. Herein, we report the isolation and characterization of two new prenylated dihydrochalcones (**1**, **2**) along with eighteen known compounds (**3**–**20**) from the root, the stem bark, and the leaf extracts of *E. montanum*. Fig. 1 presents the structures of isolated compounds **1**–**20**. The antibacterial activity and the cytotoxicity of the crude extracts and of the isolated constituents have also been evaluated.

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## 2. Results and discussion

The $CH_2Cl_2$-MeOH (1:1) extracts of the roots, stem bark and leaves of *E. montanum*, were subjected to repeated column chromatography over silica gel and Sephadex LH-20, followed by preparative reverse-phase HPLC. This afforded two new prenylated dihydrochalcones (**1, 2**, Fig. 1) and eighteen known compounds (**3**–**20**, Fig. 1).

The structures of the isolated secondary metabolites were elucidated based on their NMR, IR, UV spectroscopic and mass spectrometric data (Fig. S1-S51, Supplementary Material). The known compounds were identified as 2′,4′,5,6′-tetrahydroxy-4-methoxy-3,3′-diprenyldihydrochalcone (**3**) [12], 2′,4′,4,6′-tetrahydroxy-3,3′-diprenyldihydrochalcone (**4**) [13], genistein (**5**) [14,15], genistin (**6**) [16], quercetin (**7**) [17,18], luteolin (**8**) [19,20], isorhamnetin (**9**) [21,22], lupinalbin A (**10**) [23,24], cedrol (**11**) [25–28], D-pinitol (**12**) [29–31], stigmasterol (**13**) [32–34], stigmasterol glucoside (**14**) [32], betulinic acid (**15**) [35,36], salicylic acid (**16**) [37,38], 4-hydroxy-5,5-dimethyldihydrofuran-2-one (**17**) [39–41], *p*-coumaric acid (**18**) [42,43], 3-(4-methoxyphenyl) propanoic acid (**19**) [44], and dodecyl-3-(3,4-dihydroxyphenyl) propenoate (**20**) [45,46] by comparison of their spectroscopic data to those previously reported in the literature (Fig. S1-S51, Supplementary Material). Compounds **5**, **10**, **11** and **13** were also identified by single crystal X-ray diffraction. Genistein (**5**) crystallizes in the orthorhombic space group Pbca forming a 2-D hydrogen bonded network parallel to the (100) crystallographic plane involving the three phenolic substituents and the ketone. The structure is similar to previously recorded data at 20 K and room temperature [47,48], Lupinalbin A (**10**) crystallizes in the triclinic space group P-1 as a water and methanol solvate (**10**·MeOH·$H_2O$). The solvent molecules engage with the hydroxy-substituents in a 3-D hydrogen bonded network. Furthermore, slipped π-π interactions (ca. 3.6 Å) contribute to the solid-state packing. Two solid-state structures of Cedrol (**11** and **11**·MeOH) have been obtained. Both have a 1D-hydrogen bonded network, but only one has a methanol solvate. While the structure of **11** shows rather long donor-acceptor distances (>3.2 Å), incorporation of MeOH into the 1D-chain significantly strengthens the donor-acceptor interaction (O(Cedrol)-O (MeOH) ca. 2.7 Å). Stigmasterol was also obtained as a water/methanol solvate in the solid-state (monoclinic space group $P2_1$). This structure shows a very pronounced layered structure parallel to the crystallographic (001) plane. Hydrophobic layers alternate with hydrophilic layers consisting of a 2D hydrogen bonded network from stigmasterol's hydroxy group, one methanol and one water molecule, similar to the previously reported monohydrate [49]. For further crystallographic details, see the Supplementary material, page 45–53.

Compound **1** was isolated as a pale yellowish paste. It was assigned the molecular formula $C_{26}H_{32}O_5$ based on HRESIMS ($[M + H]^+$ *m/z* 425.3159, calcd 425.3165) and NMR data analyses (Table 1 and Fig. S1-S8, Supplementary Material). The IR absorptions at 3363 and 1625 $cm^{-1}$ indicated it to possess hydroxy and conjugated carbonyl functionalities. The $^1H$ NMR spectrum with characteristic signals at $\delta_H$ 5.26, $\delta_H$ 3.32 and $\delta_H$ 1.71 were in agreement with the presence of a prenyl group, and at $\delta_H$ 3.72 suggested it to contain a methoxy group. The UV absorbances at $\lambda_{max}$ 286 and 320 nm, along with the NMR signals at $\delta_H$ 3.35 ($CH_2$-8′), 2.92 ($CH_2$-9′) and at $\delta_C$ 205.1 (C-7′), 45.8 ($CH_2$-8′), and 30.3 ($CH_2$-9′) revealed it to be a dihydrochalcone [12,50–54]. This skeleton was further established by the COSY correlation of $\delta_H$ 3.35 (H-8′) with $\delta_H$ 2.92 (H-9′), and the HMBC cross-peaks of $\delta_H$ 3.35 (H-8′) to C-1 ($\delta_C$ 137.4) and C-1′ ($\delta_C$ 105.0), and of $\delta_H$ 2.92 (H-9′) to C-7′ ($\delta_C$ 205.1), C-2 ($\delta_C$ 128.0) and C-6 ($\delta_C$ 128.0) of the B-ring. The NMR data (Table 1 and Figs. S1-S8, Supplementary Material) showed two sets of aromatic singlet peaks at $\delta_H$ 5.92 (H-3′/5′) and $\delta_H$ 6.88 (H-2/6), with their corresponding carbon peaks at $\delta_C$ 95.9 (C-3′/5′) and 128.0 (C-2/6). This is in agreement with the presence of two substituted aromatic rings, which are present in the dihydrochalcone skeleton.

In support of the above suggestion, $\delta_H$ 5.92 (H-3′/5′) showed HMBC cross-peaks to C-1′ ($\delta_C$ 105.0), C-2′/6′ ($\delta_C$ 162.5) and C-4′ ($\delta_C$ 162.5), and to C-3′/5′ ($\delta_C$ 95.9), which indicated that the A-ring is a *para*-substituted symmetrical aromatic ring. This is further corroborated by the HMBC cross-peaks of $\delta_H$ 14.37 (OH-2′/6′) to C-1′ ($\delta_C$ 105.0) and C-2′/C-6′ ($\delta_C$ 162.5). Furthermore, H-3′/5′ ($\delta_H$ 5.92) showed a long range, $^4J_{CH}$,

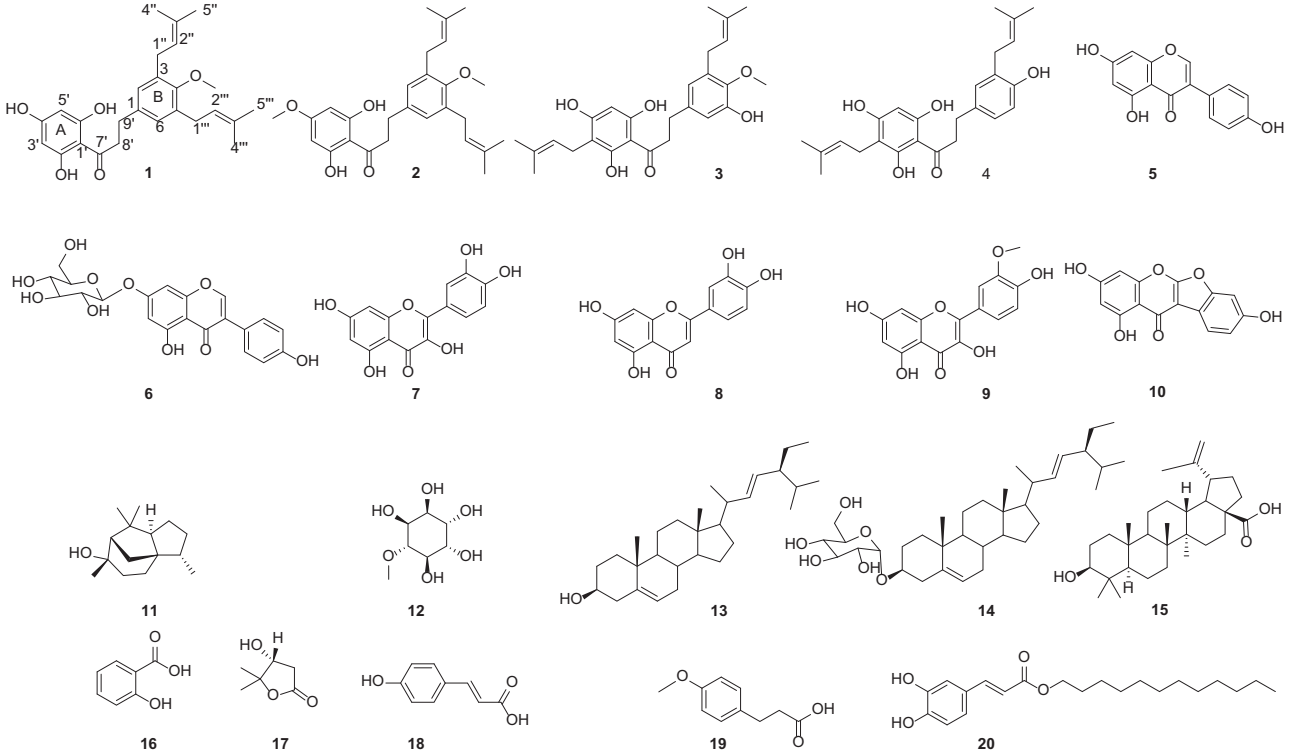


**Fig. 1.** The structures of isolated compounds from *E. montanum*.

**Table 1**
$^{1}H$ and $^{13}C$ NMR spectroscopic data for compounds **1** and **2**.

| **1**[a] | | | **2**[b] | |
|---|---|---|---|---|
| Position | $\delta_C$, type | $\delta_H$ (*J* in Hz) | $\delta_C$, type | $\delta_H$ (*J* in Hz) |
| 1 | 137.4, C | | 137.1, C | |
| 2 | 128.0, CH | 6.88, s | 127.7, CH | 6.88, s |
| 3 | 134.6, C | | 134.4, C | |
| 4 | 154.2, C | | 154.3, C | |
| 5 | 134.6, C | | 134.4, C | |
| 6 | 128.0, CH | 6.88, s | 127.7, CH | 6.88, s |
| 1′ | 105.0, C | | 104.8, C | |
| 2′ | 162.5, C | | 165.5, C | |
| 3′ | 95.9, CH | 5.92, s | 94.4, CH | 5.92, s |
| 4′ | 162.5, C | | 165.5, C | |
| 5′ | 95.9, CH | 5.92, s | 94.4, CH | 5.92, s |
| 6′ | 162.5, C | | 165.5, C | |
| 7′ | 205.1, CO | | 204.7, CO | |
| 8′ | 45.8, $CH_2$ | 3.35, t (7.3) | 45.7, $CH_2$ | 3.35, t (7.3) |
| 9′ | 30.3, $CH_2$ | 2.92, t (8.0) | 30.0, $CH_2$ | 2.92, t (8.2) |
| 1″ | 28.5, $CH_2$ | 3.32, d (7.3) | 28.4, $CH_2$ | 3.32, d (7.3) |
| 2″ | 123.2,CH | 5.26, t (7.0) | 123.1,CH | 5.28, t (7.0) |
| 3″ | 132.5, C | | 132.2, C | |
| 4″ | 18.0, $CH_3$ | 1.71, s | 17.8, $CH_3$ | 1.73, s |
| 5″ | 25.9, $CH_3$ | 1.71, s | 25.7, $CH_3$ | 1.73, s |
| 1‴ | 28.5, $CH_2$ | 3.32, d (7.5) | 28.6, $CH_2$ | 3.32, d (7.3) |
| 2‴ | 123.3, CH | 5.28, t (7.0) | 123.1,CH | 5.28, t (7.0) |
| 3‴ | 132.5, C | | 134.6, C | |
| 4‴ | 18.0, $CH_3$ | 1.71, s | 17.8, $CH_3$ | 1.73, s |
| 5‴ | 25.9, $CH_3$ | 1.71, s | 25.7, $CH_3$ | 1.73, s |
| OH-2′ | | 14.37, s | OH-2’ | 14.00, s |
| $OCH_3$-4 | 61.1, $OCH_3$ | 3.72, s | 55.4, $OCH_3$ | 3.79, s |
| $OCH_3$-4′ | | | 60.9, $OCH_3$ | 3.71, s |

[a] Recorded at 500 MHz ($^{1}H$) and 125 MHz ($^{13}C$) in $CDCl_3$.
[b] Recorded at 400 MHz ($^{1}H$) and 100 MHz ($^{13}C$) in $CDCl_3$.

HMBC cross-peak to the carbonyl carbon (C-7′, $\delta_C$ 205.1), Fig. S6, Supplementary Material, as has been reported for structurally related compounds [55–57].

The aromatic singlet at $\delta_H$ 6.88 (H-2/6) showed HMBC cross-peaks to C-4 ($\delta_C$ 154.2), C-9′ ($\delta_C$ 30.3) and C-1‴($\delta_C$ 28.5). Further, it showed an HMBC cross-peak to C-2/6 ($\delta_C$ 128.0), which revealed that the B-ring has a substitution pattern similar to that of the A-ring. The placement of the prenyl groups at C-3 ($\delta_C$ 134.6) and C-5 ($\delta_C$ 134.6) on the B-ring was identified by the HMBC cross-peaks of H-2/6 ($\delta_H$ 6.88) to C-1″ ($\delta_C$ 28.5) and C-1‴($\delta_C$ 28.5), respectively. Furthermore, the placement of the methoxy group at C-4 ($\delta_C$ 154.2) was supported by the HMBC cross-peaks of $OCH_3$-4 ($\delta_H$ 3.72) and of H-2/6 ($\delta_H$ 6.88) to C-4 ($\delta_C$ 154.2). In addition, the NOESY correlations of H-1″/1‴($\delta_H$ 3.32) with H-2/6 ($\delta_H$ 6.88) and with $OCH_3$ protons ($\delta_H$ 3.72) further supported the placement of the methoxy group at C-4 ($\delta_C$ 154.2), and of the prenyl groups at C-3 ($\delta_C$ 134.6) and C-5 ($\delta_C$ 134.6) in the B-ring (Fig. 2, Fig. S6, Supplementary Material). Based on the above spectroscopic data, the new compound montachalcone A (**1**) was characterized as 2′,4′,6′-trihydroxy-4-methoxy-3,5-diprenyldihydrochalcone.

Compound **2** was isolated as a pale yellowish gum, and was assigned the molecular formula $C_{27}H_{34}O_5$ based on HRESIMS ($[M + H]^+$ *m/z* 439.3236, calcd 439.3241) and NMR data analyses (Table 1 and Fig. S10-S15, Supplementary Material). Compound **2** showed similar spectroscopic features to that of **1**, with the only difference being the presence of an additional methoxy group. The placement of this methoxy group on C-4′ ($\delta_C$ 165.5) was confirmed by the HMBC cross-peaks between $OCH_3$-4′ ($\delta_H$ 3.71) to C-4′ ($\delta_C$ 165.5) and H-3′/5′ ($\delta_H$ 5.92) to C-4′ ($\delta_C$ 165.5). Therefore, this new compound, montachalcone B (**2**), was characterized as 2′,6′-dihydroxy-4,4′-dimethoxy-3,5-diprenyldihydrochalcone.

*Eriosema* species have been reported to be a rich source of biologically active compounds including terpenoids, isoflavones and chalcones [5,6,8]. We have evaluated the *in vitro* antibacterial activity of the crude extracts and of the isolated constituents of *E. montanum* against the

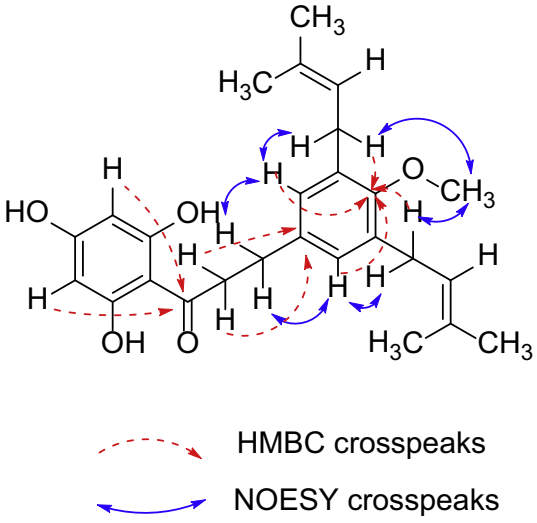


**Fig. 2.** Key HMBC and NOESY correlations of montachalcone A (**1**).

Gram-negative bacterium *E. coli* and the Gram-positive bacterium *B. subtilis*.

The leaf crude extract and compounds **1**–**4** and **10** were active against *B. subtilis* with MIC ranging from 3.1 to 8.9 μM. They also inhibited MCF-7 cells with montachalcone A (**1**) being the most potent of the samples (Table 2). Within the concentration range used in this study (<2000 μM), none of the samples were active against *E. coli*. The biological activity of compounds **1**–**4** and **10** corroborated with previously reported activities of chalconoids of *Eriosema* species [3,58]. Compounds **1**, **3** and **4** showed highest cytotoxicity, evaluated using the human MCF-7 cell line, with $EC_{50}$ 7.0, 18.0 and 18.0 μM, respectively.

## 3. Materials and methods

### 3.1. General experimental procedures

The dried plant material was finely ground using Retsch SM 100 and Retsch ZM 200 grinding machines. UV spectra were measured using a SHIMADZU UV-1650 PC UV–vis spectrophotometer. IR spectra were recorded on a Perkin Elmer Spectrum One instrument with universal ATR sampling accessory. NMR spectra were recorded with Agilent MR400-DD2 ($^{1}H$ at 400 MHz, $^{13}C$ at 100 MHz) equipped with a OneNMR probe, Bruker Avance Neo ($^{1}H$ at 500 MHz, $^{13}C$ at 125 MHz) equipped with a TXO (CRPHe TR-$^{13}C$/$^{15}N$/$^{1}H$ 5 mm-Z) probe or Bruker Avance Neo ($^{1}H$ at 600 MHz, $^{13}C$ at 150 MHz) equipped with TCI (CRPHe TR-$^{1}H$ &$^{19}F$/$^{13}C$/$^{15}N$ 5 mm-EZ) probe spectrometers. The data were processed using the MestReNova x64-14.1.0 software, and chemical shifts were referenced to the residual solvent signals ($CDCl_3$: $\delta_H$ 7.26, $\delta_C$ 77.16;

**Table 2**
Antibacterial (MIC, μM) and cytotoxic activities ($EC_{50}$, μM) of the crude extracts and isolated compounds from *E. montanum*.

| Sample | MIC (μM) | $EC_{50}$ (μM) |
|---|---|---|
| **1** | 3.1 | 7.0 |
| **2** | 7.7 | 27.0 |
| **3** | 8.9 | 18.0 |
| **4** | 5.5 | 18.0 |
| **10** | 27 | n.d |
| **17** | 1650 | 45 |
| Crude extract of leaves | 3.0 μg/mL | 18.0 μg/mL |
| Crude extract of root | 21.0 μg/mL | 40.0 μg/mL |
| Crude extract of stem bark | 270.0 μg/mL | 6.4 μg/mL |

n.d = no detectable activity. Ampicillin has been used as positive control (Fig. S52, Supplementary Material).

$CD_3OD$: $\delta_H$ 3.31, $\delta_C$ 49.0; DMSO-$d_6$: $\delta_H$ 2.50, $\delta_C$ 39.5). Mass spectra were acquired on a Waters Micromass ZQ Multimode Ionization ESCI using LC-MS in ESI mode, connected to an Agilent 1100 series gradient pump system and a $C_{18}$ Atlantis T3 column (3.0 × 50 mm, 5 μm), using Milli-Q water-MeCN (5:95 to 95:5, with 1% $HCO_2H$, flow rate 0.75 mL/min over 6 min). HRESIMS spectra were acquired with a Q-TOF LC/MS spectrometer with a lockmass-ESI source (Stenhagen Analysis Lab AB, Gothenburg, Sweden), using a 2.1 × 30 mm, 1.1 μm RP-$C_{18}$ column and $H_2O$-MeCN gradient (5:95 to 95:5, with 0.2% $HCO_2H$). TLC analysis was performed on Merck pre-coated silica gel 60 $F_{254}$ aluminum plates using UV detection at 254 and 365 nm. Gravitational column chromatography was done using silica gel 60 (230–400 mesh). Gel filtration was done using Sephadex LH-20 (GE Healthcare). Preparative HPLC experiments were carried out on a Waters 600E system using Chromulan v. 0.88 (Pikron Ltd) software and a RP-$C_8$ Kromasil® column (250 mm × 25 mm, 5 μm), or on an Interchim Ultra Performance Flash Purification (PF-430) system using Interchim v 5.1d.02 software and RP-$C_8$ Kromasil® column (250 mm × 25 mm, 5 μm).

### 3.2. Plant and chemical material

#### 3.2.1. Plant material

The roots, the stem bark, and the leaves of *E. montanum* were collected from Uruyange village, Nyarugano cell, Ruramba sector, Nyaruguru District, southern Province, Rwanda in February 2019. The plant was identified by Mr. Alphonse Murerwa, an experienced Rwandan traditional healer, and further authenticated by Mr. Samuel Nshutiyayesu, a senior botanist, Department of Biology, University of Rwanda. A voucher specimen (UMDAN: 001–2019) was deposited in the herbarium of the National Industrial Research and Development Agency (NIRDA), Rwanda.

### 3.3. Extraction and isolation

The shade-dried and finely ground leaves of *E. montanum* (1.6 kg) were extracted with $CH_2Cl_2$-MeOH (1:1) (3 × 2 L) at room temperature for 72 h. The extract was filtered, and the solvent removed under vacuum using a rotary evaporator at 50 °C to obtain 40 g of crude extract. The extract was adsorbed on silica gel and subjected to column chromatography on silica gel (400 g) eluting with isohexane containing increasing percentages of EtOAc to yield 43 fractions. Fractions 1–10 eluted with 0–4% EtOAc in isohexane gave a mixture of fatty acids that was not investigated further. Fractions 11–15 eluted with 12% EtOAc, crystallized shortly after elution from the column and were washed with isohexane to yield betulinic acid (**15**, 12.3 mg), the washing liquid afforded subsequently stigmasterol (**13**, 24.0 mg) and dodecyl-3-(3,4-dihydroxyphenyl)propenoate (**20**, 11.0 mg). Fractions 16–25 eluted with 25% EtOAc were combined and purified over Sephadex LH-20 ($CH_2Cl_2$-MeOH, 1:1) and further purified over PTLC (isohexane-EtOAc 4:6) to afford 2′,6′-dihydroxy-4,4′-dimethoxy-3,5-diprenyldihydrochalcone (**1**, 7.5 mg) and 2′,4′,6′-trihydroxy-4-methoxy-3,5-diprenyldihydro-chalcone (**2**, 11.0 mg). Fractions 26–31 eluted with 30% EtOAc were combined and purified over Sephadex LH-20 ($CH_2Cl_2$-MeOH, 1:1) followed by preparative HPLC (MeCN-MeOH, gradient elution 10% to 90% MeOH) to yield 2′,4′,5,6′-tetrahydroxy-4-methoxy-3,3′-diprenyldihydrochalcone (**3**, 7.3 mg) and 2′,4′,4,6′-tetrahydroxy-3,3′-diprenyldihydrochalcone (**4**, 8.0 mg). Fractions 32–43 eluted with 40% EtOAc were combined and purified by column chromatography over silica gel, eluting with 35–50% EtOAc in isohexane, 27 subfractions were collected. Subfractions 1–11 were combined and purified over Sephadex LH-20 ($CH_2Cl_2$-MeOH, 1:1), to yield colorless needle crystals of lupinalbin A (**10**, 18.0 mg). Sub-fractions 12–27 eluting with 45% EtOAc were combined and purified by PTLC followed by preparative HPLC with isocratic elution of 40% $H_2O$-MeOH to afford 4-hydroxy-5,5-dimethyldihydrofuran-2-one (**17**, 5.1 mg).

The shade-dried and finely ground root bark of *E. montanum* (1.8 kg) was extracted with $CH_2Cl_2$-MeOH, 1:1 (3 × 3.6 L) at room temperature for 72 h. Following filtration, the solvent was removed under vacuum using a rotary evaporator at 50 °C, to yield 90 g of crude extract. Prior to fractionation over silica gel chromatography, the crude extract was partitioned between isohexane and methanol. The methanol layer (50 g) was subjected to column chromatography on silica gel, eluting with isohexane-EtOAc mixture containing increasing amount of EtOAc to yield 25 fractions. Based on TLC analysis, similar fractions were combined for further purification. Fractions 1–5 eluted with 12% EtOAc were washed with isohexane and gave stigmasterol (**13**, 21.5 mg). Fractions 5 and 6 eluted with 18% EtOAc gave colorless needle crystals (MeOH) of cedrol (**11**, 12.3 mg). Fractions 7–10 eluted with 20% EtOAc were combined and purified over Sephadex LH-20 ($CH_2Cl_2$-MeOH, 1:1) followed by further purification on a column chromatography over silica gel, eluting with 40% EtOAc in isohexane, and Sephadex LH-20 ($CH_2Cl_2$-MeOH, 1:1) to yield 3-(4-methoxyphenyl)propanoic acid (**19**, 16.0 mg). The fractions 11–16 eluted with 25% EtOAc were combined and subjected to column chromatography over Sephadex LH-20 ($CH_2Cl_2$-MeOH, 1:1) to afford quercetin (**7**, 11.0 mg) and isorhamnetin (**9**, 8.5 mg). Fractions 17 and 18 were combined and purified on preparative HPLC (MeOH-$H_2O$, gradient elution 10% - 90% $H_2O$) to yield genistein (**5**, 13.2 mg) and luteolin (**8**, 9.5 mg). Fractions 19–25 were combined and purified by column chromatography on a silica gel (40 g) eluting with a mixture isohexane-EtOAc (1:4) to give genistin (**6**, 17.8 mg).

The shade-dried and finely ground stem bark of *E. montanum* (1 kg) was extracted with a $CH_2Cl_2$-MeOH, 1:1 (3 × 2 L) at room temperature for 72 h. After filtration, the solvent was removed under vacuum using a rotary evaporator at 50 °C to yield 30 g of crude extract. The crude extract (30 g) was subjected to column chromatography on silica gel (300 g) eluting with mixture isohexane-EtOAc of increasing polarity to yield 20 fractions. Except fractions 17–20, other fractions showed similar TLC profile as compounds isolated from previous fractions of root and leaves. Therefore, fractions 17–20 were combined and purified over Sephadex LH-20 ($CH_2Cl_2$-MeOH, 1:1) to give a colorless precipitate *D*-pinitol (**12**, 14.0 mg), colorless needle crystals (MeOH) of salicylic acid (**16**, 8.0 mg) and *p*-coumaric acid (**18**, 16.0 mg).

### 3.4. Physical and spectrosopic data of compounds 1 and 2

*Montachalcone A (**1**)*: Pale yellowish paste; UV (MeOH) $\lambda_{max}$ (log ε) 286 (3.15), 320 (3.20) nm; IR $\nu_{max}$ 3363, 1625 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) and $^{13}C$ NMR (125 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 425.3159 $[M + H]^+$ (calcd for $C_{26}H_{32}O_5$, 425.3165).

*Montachalcone B (**2**):* Pale yellowish paste; UV (MeOH) $\lambda_{max}$ (log ε) 285 (3.22), 317 (3.17) nm; IR $\nu_{max}$ 3352, 1629 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) and $^{13}C$ NMR (100 MHz, $CDCl_3$) see Table 1; HRESIMS *m/z* 439.3236 $[M + H]^+$ (calcd for $C_{27}H_{34}O_5$, 439.3241).

### 3.5. X-ray diffraction analysis of compounds 5, 10, 11 and 13

Single crystals were obtained by slow solvent evaporation. Single crystals were mounted on a fiber loop and fixated using Fomblin oil. The data were collected at 150(2) K on a Bruker D8 APEX-II equipped with a APEX-II CCD camera using $MoK_\alpha$ radiation (λ = 0.71073 Å). Data reduction was performed with SAINT [59], absorption corrections for the area detector were performed using SADABS [60]. Structures were solved by direct methods and refined by least squares methods on F2 using the SHELX and the OLEX2 software suits, respectively [61,62]. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were constrained in geometrical positions to their parent atoms The absolute structures are based on the known absolute configurations. The X-ray structures (cif) data of **5** (CCDC 2033906), **10** (CCDC 2033905), **11** (CCDC 2033904, and 2033903 for the methanol solvate) and **13** (CCDC 2033902) have been deposited with the Cambridge Crystallographic Data Centre. Further details of the X-ray data acquisition are given in the Supplementary Material.

### 3.6. Antibacterial assays

The antibacterial activity of the isolated compounds was determined against *Escherichia coli* (CGSC #6300) and *Bacillus subtilis* (NBRC #111470). The compounds were first re-dissolved at 10 mg/mL in 100% DMSO, then further diluted 30 times in $H_2O$ and stored at −20 °C. *E. coli* and *B. subtilis* were cultured as previously described by Mueller and Hinton [63] and Doyle [64]. For the *in vitro* assays, both bacterial species were cultured by standard procedures [63,65,66]. In brief, a culture of bacteria was grown in Mueller-Hinton Broth II medium to OD $600_{nm}$ = 0.5 and then diluted 10 times with pre-warmed medium. All compounds to be tested were pre-dissolved in 100% DMSO, obtaining various concentrations based on their solubility and further stored at −20 °C. The compounds were diluted to a starting concentration of 3% *v*/v in diluted bacterial culture (final OD $600_{nm}$ = 0.05) and a 2-fold serial dilution was set up in 384-well microtiter plates and incubated. Bacterial viability was measured through a standard resazurin-based assay (addition of 10% AlamarBlue solution (DAL1025, Invitrogen) followed by 1 to 4 h incubation), as described elsewhere [67]. Fluorescence was measured using a POLARstar Omega microplate reader (BMG Labtech) with the excitation filter set to 544 nm and emission filter to 590 nm. Negative viability control was performed by exposing to 40 μg/mL ampicillin and positive control by exposing bacteria to an equivalent concentration of DMSO. Bleed-through between wells was controlled by assuring <1% fluorescence between adjacent wells. Each compound activity was performed in three independent replicates. Effective concentrations (EC) and minimum inhibitory concentrations (MIC) were calculated using the Quest Graph $EC_{50}$ calculator webtool (AAT Bioquest, Inc).

### 3.7. Cytotoxicity assays

The cytotoxicity was evaluated against human MCF-7 cells (ATCC® HTB-22™) following standard procedures [68]. Briefly, cells grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum were reseeded into 96-well microtiter plates and allowed to settle for 24 h. Stock solution of the compounds were added for final concentration of 0.3% *v*/v of the solvent (DMSO) in the culture medium. According to the manufacturer's instructions, PrestoBlue reagent (ThermoFisher) was used to measure cell viability after 24 h incubation in the presence of the compounds. Measurements were performed based on resorufin fluorescence at 544 nm excitation/590 nm emission, using a POLARstar Omega microplate reader (BMG Labtech). Cell survival was expressed as percentage of solvent-only control and $EC_{50}$ values for each compound were calculated from three independent replicate experiments, with 2-fold dilution intervals, using the Quest Graph EC50 calculator web tool (AAT Bioquest, Inc).

## 4. Conclusions

The phytochemical investigation of *E. montanum* afforded twenty compounds including two new montachalcones A (**1**) and B (**2**) along with eighteen known natural products (**3–20**). Both newly characterized montachalcones showed activity against Gram-positive bacteria, with montachalcone B (**2**) showing lower cytotoxicity and thus, a promising therapeutic window to be further explored in future studies. Compounds **1–4** and **10** were active against *B. subtilis*, whereas compounds **1**, **3** and **4** showed cytotoxicity against the human MCF-7 cell line.

## Author contributions

The authors contributed as follows: Extraction and isolation of compounds was performed by D.U and T.M; spectroscopic characterization of isolates was done by D.U, Y.A, M.E and A.G. The antibacterial and cytotoxic activities were evaluated by A.R, Y.G, C.B and P.S. The X-ray diffraction analysis was conducted by A.K.G and A.O. All authors contributed to the preparation of the manuscript.

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## Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2020.104809.

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