Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Two new diacetylene glycosides: bhutkesoside A and B from the roots of Ligusticopsis wallichiana

Bibek Adhikari, Hari Prasad Devkota, Khem Raj Joshi, Takashi Watanabe & Shoji Yahara

To cite this article: Bibek Adhikari, Hari Prasad Devkota, Khem Raj Joshi, Takashi Watanabe & Shoji Yahara (2015): Two new diacetylene glycosides: bhutkesoside A and B from the roots of Ligusticopsis wallichiana, Natural Product Research, DOI: 10.1080/14786419.2015.1118635

To link to this article: http://dx.doi.org/10.1080/14786419.2015.1118635

NATURAL PRODUCT RESEARCH, 2015
http://dx.doi.org/10.1080/14786419.2015.1118635

Taylor & Francis
Taylor & Francis Group

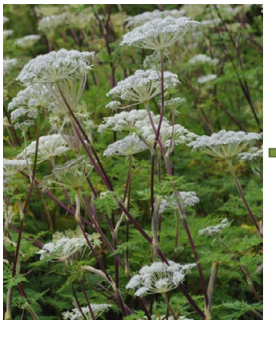
Two new diacetylene glycosides: bhutkesoside A and B from the roots of *Ligusticopsis wallichiana*

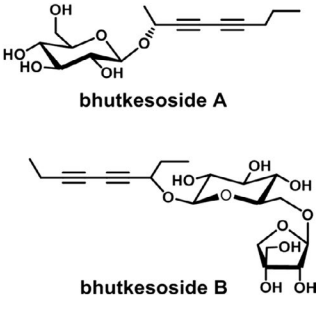
Bibek Adhikari[a,b], Hari Prasad Devkota[a,b], Khem Raj Joshi[a], Takashi Watanabe[a] and Shoji Yahara[a]

[a]Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan; [b]Program for Leading Graduate Schools, HIGO Program, Kumamoto University, Kumamoto, Japan

ABSTRACT

Two new diacetylene glycosides: bhutkesoside A (**1**) and B (**2**), along with 10 known compounds, i.e. falcarindiol (**3**), chlorogenic acid (**4**), 5-*O*-*p*-coumaroyl-quinic acid (**5**), 3,5-di-*O*-caffeoyl-quinic acid (**6**), 4-hydroxy-7-methoxy-phenylethanol (**7**), ferulic acid (**8**), dehydrodiconiferyl alcohol-4-*O*-*β*-D-glucopyranoside (**9**), 5,7-dihydroxy-2-methylchromone-7-*O*-rutinoside (**10**), schumanniofioside B (**11**) and marmesinin (**12**) were isolated from the roots of *Ligusticopsis wallichiana* (DC) Pimenov & Kljuykov (Apiaceae), commonly known as 'Bhutkesh' in Nepal. The structures were determined on the basis of spectroscopic data. Compounds **4** and **6** showed potent antioxidant activity on DPPH free radical scavenging assay.





ARTICLE HISTORY
Received 3 September 2015
Accepted 10 October 2015

KEYWORDS
Ligusticopsis wallichiana; Apiaceae; Bhutkesh; bhutkesoside A; bhutkesoside B

1. Introduction

Ligusticopsis wallichiana (DC) Pimenov & Kljuykov (Syns. *Selinum tenuifolium* Wall. ex C. B. Clarke, *Selinum wallichianum* (DC) Raizada & H.O. Saxena) (Family: Apiaceae), commonly known as 'Bhutkesh' in Nepal, is a perennial aromatic herb, widely distributed in the Himalayan region of Nepal, India, Pakistan, Bhutan and China between 2700 and 4800 m. The roots are

CONTACT Hari Prasad Devkota devkotah@kumamoto-u.ac.jp; Shoji Yahara yaharas1@gpo.kumamoto-u.ac.jp
Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/14786419.2015.1118635.

used to treat body pain, fever, cough and cold (Watanabe et al. 2013) and the flowers and leaves are used in stomach ache, cuts and wounds (Gewali 2008). The root decoction is used for the treatment of diarrhoea, stomach ache and vomiting. The flowers and stem are used for stimulant and carminative properties (Padalia et al. 2012). Previous studies on this plant mainly focused on the volatile constituents of the different plant parts of *L. wallichiana* (Sood et al. 1978; Dev et al. 1984; Chauhan et al. 2012; Padalia et al. 2012) but detailed characterisation of non-volatile constituents is not available. Thus, in the present study, we report the isolation and structure elucidation of two new (**1**, **2**) and 10 known compounds (**3**–**12**) from the roots of *L. wallichiana* and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the isolated compounds.

2. Results and discussion

The shade dried roots (370 g) were extracted with 70% MeOH at 60 °C and evaporated under reduced pressure to give 99.2 g extract. The extract was then separated into water-soluble and insoluble fractions. The water-soluble fraction was applied on repeated column chromatography (CC) on MCI gel CHP20P, Sephadex LH-20, ODS and silica gel to isolate two new compounds, bhutkesoside A (**1**) and B (**2**), along with 10 known compounds (Figure 1). From the detailed spectral analysis and comparison with literature data, the known compounds were identified as falcarindiol (**3**) (Tamura et al. 2010), chlorogenic acid (**4**), 5-*O*-*p*-coumarylquinic acid (**5**), 3,5-di-*O*-Caffeoyl-quinic acid (**6**) (Kwon et al. 2000), 4-hydroxy-7-methoxy-phenylethanol (**7**) (Kim et al. 2006), ferulic acid (**8**) (Rollinger et al. 2003), dehydrodiconiferyl alcohol 4-*O*-*β*-glucopyranoside (**9**) (Lundgern et al. 1981), 5,7-dihydroxy-2-methylchromone-7-*O*-rutinoside (**10**) (Yoshimatsu et al. 2007), schumanniofioside

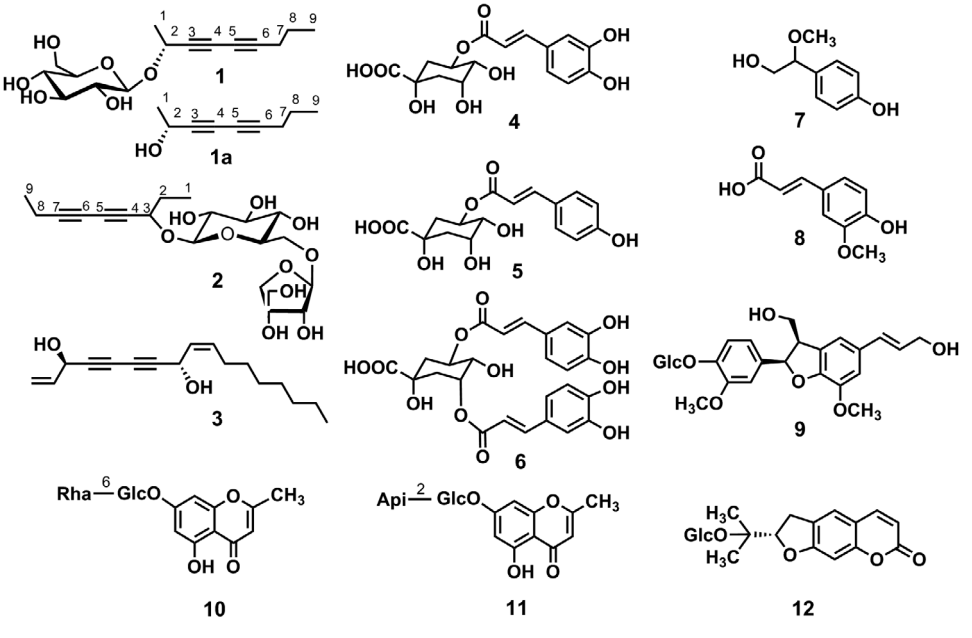


Figure 1. Structures of compounds **1**–**12**.

B (**11**) (Tane et al. 1990) and marmesinin (**12**) (Kitajima et al. 1998). All of these compounds were isolated for the first time from *L. wallichiana*.

Bhutkesoside A (**1**) was obtained as a pale yellow oil, $[\alpha]_D^{20}$ −127.8° (*c* = 0.28, MeOH). Its molecular formula was determined to be $C_{15}H_{22}O_6$ on the basis of a HR-FAB-MS. The proton nuclear magnetic resonance (^{1}H NMR) spectrum of compound **1** showed signals attached to oxygen bearing carbon from δ_H 3.17 to 4.80 ppm, two methylene proton signals at δ_H 2.27 (2H, t, *J* = 7.3 Hz), 1.55 (2H, dq, *J* = 7.0, 7.3 Hz) and two methyl signals at δ_H 1.42 (3H, d, *J* = 6.7 Hz) and 1.00 (3H, t, *J* = 7.3 Hz). The carbon nuclear magnetic resonance spectra (^{13}C NMR) showed total 15 carbons signals. Among these carbon signals, four quaternary carbon signals at δ_C 82.0 (C), 75.7 (C), 71.3 (C) and 65.4 (C) were assigned to a disubstituted diacetylene moiety (Chauhan et al. 2012). Six carbon signals at δ_C 101.3 (CH), 78.1 (CH), 78.0 (CH), 74.9 (CH), 71.7 (CH) and 62.8 (CH_2) were assigned to a β-glucopyranosyl moiety. The coupling constant of anomeric proton (*J* = 7.6 Hz at δ_H 4.55) also suggested β-configuration of the glucopyranosyl moiety (Roslund et al. 2008). The ^{1}H^{1}H correlation spectroscopy (COSY) showed correlation between proton at δ_H 4.80 (1H, overlapped, D_2O) and methyl protons at δ_H 1.42 (3H, d, *J* = 6.7). Similarly, the protons at δ_H 1.55 (2H, dq, *J* = 7.3) had correlations with methylene protons at δ_H 2.27 (2H, t, *J* = 7.3) and methyl protons at δ_H 1.00 (3H, t, *J* = 7.3). This suggests a substituted ethyl and a propyl moiety present is in the compound. Heteronuclear multiple bond correlation (HMBC) confirmed the correlation of anomeric proton at δ_H 4.55 (1H, d, *J* = 7.6) with oxygenated carbon at δ_C 64.2 (C-2). Methyl proton at δ_H 1.42 showed correlation with acetylenic carbon at δ_C 75.7 (C-3) and oxygenated carbon at δ_C 64.2 (C-2). Similarly, methylene protons δ_H 2.27 of the propyl moiety showed correlation with acetylenic carbon at δ_C 82.0 (C-6) and 65.4 (C-5) suggesting the propyl side chain carbon attached to next side of the acetylenic chain. Acid hydrolysis of compound **1** gave D-glucose $[\alpha]_D^{20}$ +66 (*c* = 0.35 H_2O) and aglycone 3,5-nonadiyn-2-ol (**1a**), confirmed on the basis of ^{1}H and ^{13}C NMR data (Chauhan et al. 2012). The relative configuration of compound **1** was confirmed using β-D-glycosylation shift (Seo et al. 1978). In ^{13}C NMR, the carbon attached with the β-D-glucopyranosyl moiety (C-2, α-carbon) was shifted downfield 5.4 ppm and the adjacent carbons C-1 (β-carbon) and C-3 (β-carbon) were shifted upfield by 2.1 and 3.0 ppm, respectively, which suggested the *R* configuration at position C-2. Thus, the structure of **1** was elucidated as 2(*R*)-hydroxy-3,5-nonadiyn-2-*O*-β-D-glucopyranoside.

Bhutkesoside B (**2**) was obtained as pale yellow oil, $[\alpha]_D^{20}$ −14.5° (*c* = 0.81, MeOH). The molecular formula was determined as $C_{20}H_{30}O_{10}$ on the basis of a HR-FAB-MS. The ^{1}H NMR spectrum of **2** showed protons attached to oxygen-bearing carbon from δ_H 3.18 to 4.99 ppm, two methylene signals δ_H 2.32 (2H, q, *J* = 7.3 Hz), 1.79 (2H, dq, *J* = 7.0, 7.3 Hz) and two methyl signals δ_H 1.15 (3H, t, *J* = 7.3 Hz), 1.01 (3H, t, *J* = 7.3 Hz. The ^{13}C NMR spectra of **2** showed total 20 carbon signals. Among them, four quaternary carbon signals at δ_C 83.2 (C), 75.6 (C), 71.4 (C) and 64.9 (C) were assigned to a disubstituted diacetylene moiety as in **1**. Six oxygenated carbon signals at δ_C 103.2 (CH), 78.1 (CH), 76.8 (CH), 74.9 (CH), 71.5 (CH) and 68.2 (CH_2) were assigned to a C-6-substituted β-glucopyranosyl moiety. In comparison to **1**, six extra carbon signals at δ_C 110.9 (CH), 80.6 (C), 78.0 (CH), 75.1 (CH_2) and 65.8 (CH_2) were observed in **2**, which were assigned to a β-apiofuranosyl moiety (Park et al. 2002). The glucose C-6 carbon was shifted to downfield by 6, which confirmed the apiofuranosyl 1→6 glucopyranoside linkage of the sugars. In the ^{1}H^{1}H COSY spectrum of **2**, protons at δ_H 1.79 were correlated to methyl protons δ_H 1.01 and oxymethine protons at δ_H 4.43, which suggested the presence of a propyloxy moiety. A next correlation was seen between methylene protons at δ_H 2.32

and methyl protons at δ_H 1.15 suggested the presence of ethyl moiety. In the HMBC spectrum, the anomeric proton of glucopyranosyl moiety δ_H 4.39 had correlation with carbon at δ_C 72.3 (C-3). The anomeric proton of apiofuranosyl moiety at δ_H 4.99 was correlated to the carbon at δ_C 68.2 (Glc-C_6). In addition, this carbon δ_C 68.2 was shifted downfield by +6 ppm in comparison to chemical shift in compound **1** (Glc-6, 62.8, CH_2). Further, the proton at δ_H 2.32 was correlated to acetylenic carbon at δ_C 83.2 (C-3), while the protons at δ_H 4.43 and 1.79 were correlated to acetylenic carbon at δ_C 75.6 (C-6) confirming two side chains at either side of the diacetylenic carbons. Glucose and apiose were confirmed on the basis of co-TLC of acid hydrolysate of **2** with authentic samples. Thus, the structure of compound **2** was elucidated as 3-hydroxy-4,6-nonadiyn-3-*O*-β-apiofuranosyl-(1→6)-β-glucopyranoside.

All of these isolated compounds were evaluated for their *in vitro* antioxidant activity by DPPH free radical scavenging assay. Only compounds **6**, **4**, **8** and **5** showed potent activity with EC_{50} values being 39, 89, 127 and 189 µM, respectively, while Trolox used as a positive control had EC_{50} value of 96 µM.

3. Experimental

3.1. Instruments and chemicals

CC was carried out with silica gel 60 (0.040–0.063 mm, Merck (Germany), MCI gel CHP20P (75–150 µm, Mitsubishi Chemical Industries Co., Ltd (Japan), Sephadex LH-20, Amersham Pharmacia Biotech and Silica NH and Chromatorex ODS (30–50 µm, Fuji Silysia Chemical Co., Ltd, (Japan). TLC was performed on precoated silica gel 60 F_{254} plates, 0.2 mm, aluminium sheet, Merck (Germany). NMR spectra were measured on a JEOL α-500 spectrometer (^{1}H: 500 MHz and ^{13}C: 125 MHz) and BRUKER, AVANCE 600 (^{1}H: 600 MHz and ^{13}C: 150 MHz). Chemical shifts in the ^{1}H and ^{13}C NMR spectra are given in ppm with reference to TMS at 0 ppm. Mass spectra were recorded on JEOL JMS 700 MStation, FAB mass spectrometer. Optical rotations were measured with a JASCO DIP-1000KUY polarimeter. Melting points were measured on Yanaco Micromelting point apparatus (MP-J3, MP-S3) and were uncorrected. DPPH was obtained from Wako Pure Chemicals (Osaka,Japan). Trolox from Calbiochem (Denmark) and 2-(N-morpholino)-ethanesulphonic (MES) acid buffer from Dojindo Chemical Research (Kumamoto, Japan). Absorbance was recorded on Immuno-MiniNJ-2300 Microtiter Plate Reader, Biotech Pvt, Ltd, (Tokyo, Japan).

3.2. Plant material

Fresh roots of *L. wallichiana* were collected from forest of Kurikharkha, Dolkha on August 2013 at altitude 2696 m. The roots were shade dried for a month. The plant specimen was identified by Mr Kuber Jung Malla, Senior Scientific Officer, Department of Plant Resources, Nepal. The voucher specimen (Voucher No.: KUNP20130809-015) was deposited on Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

3.3. Extraction and isolation

The shade dried roots (370 g) were extracted with 70% MeOH at 60 °C and the extract was evaporated under reduced pressure to give 99.2 g extract. The extract was then suspended in water to separate water soluble (94.2 g) and water insoluble fraction (5.0 g). The water-soluble

fraction was run over MCI gel CHP20P CC and eluted successively with water, 40%, 60%, 80%, 100% MeOH and $CHCl_3$ to give ten fractions. Fraction 2 (497.0 mg) was subjected to Sephadex LH-20 CC (H_2O) and ODS CC (H_2O, 10% MeOH) to give compound **4** (35.4 mg). Fraction 3 (4.22 g, 40% MeOH eluate) was applied on Sephadex LH-20 CC (50% MeOH) and ODS CC (10% MeOH) to give compound **5** (68.0 mg) and subfraction 3-3-9 (34.5 mg) was subjected to silica gel CC ($CHCl_3$: MeOH: H_2O = 9: 1: 0.1) to afford compound **7** (20.7 mg). Fraction 4 (1.15 g 40% MeOH eluate) was applied on Sephadex LH-20 CC (50% MeOH), ODS CC (25% MeOH), Sephadex LH-20 CC (100% MeOH) and silica gel CC ($CHCl_3$: MeOH: H_2O = 8: 2: 0.1) to afford compound **10** (20.0 mg) and compound **11** (9.1 mg). Fraction 6 (207.7 mg, 70% MeOH eluate) was applied over Sephadex LH-20 to give compound **6** (3.3 mg). Subfraction 6-2 (196.0 mg) was applied over ODS column (25–45% MeOH), silica gel column to compound **2** (2.9 mg). Fraction 7 (2.86 g, 70% MeOH eluate) was subjected to Sephadex LH-20 CC (MeOH) and ODS (40% MeOH) to give fraction 7-3-4 which was recrystallized in 20% MeOH to give compound **8** (84.2 mg). Similarly, subfraction 7-2 (1563.0 mg) was subjected to ODS (40–70% MeOH) and silica gel column to afford compound **9** (18.4 mg). Subfraction 7-2-4 (93.0 mg) was applied over silica gel column and recrystallized in 50% MeOH to get compound **12** (3.0 mg). Subfraction 7-2-9 (214.0 mg) was applied over silica gel column ($CHCl_3$: MeOH: H_2O = 9: 2: 0.1) and ($CHCl_3$: MeOH = 10:1) to give of compound **1** (23.0 mg). Subfraction 7-2-6, (323.0 mg) was applied over silica gel CC and ODS CC (40% MeOH) to give compound **2**. (9.1 mg). similarly, subfraction 7-4 (93.4 mg) was applied over ODS CC 20% MeOH to give compound 6 (14.4 mg). Fraction 8 (1.63 g, 100% MeOH eluate) was subjected to silica gel CC ($CHCl_3$: MeOH = 20:1) and (Hexane:EtOAc = 9:1) to give compound **3** (41.6 mg). The water-insoluble fraction was partitioned into hexane and methanol fractions. The methanol fraction was applied over Sephadex LH20 and silica gel (Hexane: EtOAc = 10:1) to give compound **1** (14.5 mg).

3.4. Bhutkesoside A (1)

A pale yellow oil, $[\alpha]_D^{20}$ −127.8° (*c* = 0.28, MeOH). HR-FAB-MS *m/z* 321.1314 $[M + Na]^+$ (Calcd for $C_{15}H_{22}O_6Na$, 321.1314). ^1H NMR (500 MHz, CD_3OD): δ 4.80 (1H, overlapped, D_2O, C_2-H), 4.55 (1H, d, *J* = 7.6, Glc-C_1-H), 3.80 (1H, dd, *J* = 11.5, 1.5, Glc-C_{6a}-H), 3.64 (1H, dd, *J* = 11.5, 5.4, Glc-C_{6b}-H), 3.38 (1H, t, *J* = 9.2, Glc-C_3-H), 3.27 (1H, m, Glc-C_4-H), 3.27 (1H, m, Glc-C_5-H), 3.17 (1H, dd, *J* = 7.6, 9.2 Glc-C_2-H), 2.27 (2H, t, *J* = 7.3, C_7-H), 1.55 (2H, dq, *J* = 7.3, C_8-H), 1.42 (3H, d, *J* = 6.7, C_1-H), 1.00 (3H, t, *J* = 7.3, C_9-H). ^{13}C NMR (125 MHz, DMSO-d_6): 101.3 (C-Glc-1), 82.0 (C-6), 78.1 (C-Glc-5), 78.0 (C-Glc-3), 75.7 (C-3), 74.9 (C-Glc-2), 71.7 (C-Glc-4), 71.3 (C-4), 65.4 (C-5), 64.2 (C-2), 62.8 (C-Glc-6), 22.8 (C-8), 22.3 (C-1), 21.7 (C-7), 13.7 (C-9).

3.5. Acid hydrolysis of bhutkesoside A (1)

Compound **1** (20.0 mg) was hydrolysed with 2.0 mL of 2 M HCl in a water bath at 60 °C for 3 h in a sealed tube. The reaction mixture was extracted with diethyl ether and separated in to diethyl ether and aqueous layers. The diethyl ether layer was purified in silica gel chromatography with ($CHCl_3$: MeOH: H_2O = 9:1:0.1 v/v/v) to isolate the aglycone (**1a**, 4.0 mg). The aqueous layer was passed over Chromatorex Silica NH to ensure neutralisation and evaporated to give D-glucose (3.5 mg), which confirmed on the basis of co-TLC with authentic sample and optical rotation, $[\alpha]_D^{20}$ +66.0° (*c*, 0.35, H_2O).

3.6. 2(R)-hydroxy-3,5-nonadiyne (1a)

A colourless oil, $[\alpha]_D^{20}$ −6.5° (*c* 0.40, MeOH), ^{1}H NMR, (600 MHz, CD_3OD): δ_H 4.50 (1H, q, *J* = 6.7 Hz, C_2-H), 2.25 (2H, t, *J* = 7.3 Hz, C_7-H), 1.53 (2H, dq, *J* = 7.3 Hz, C_8-H), 1.39 (3H, d, *J* = 6.7 Hz, C_1-H), 0.90 (3H, t, *J* = 7.3 Hz, C_9-H). ^{13}C NMR (CD_3OD, 150 MHz): δ_C 81.6 (C-6), 78.7 (C-3), 69.1 (C-4), 65.4 (C-5), 58.8 (CH-2), 24.4 (C-1), 22.8 (C-7), 21.7 (C-8) and 13.7 (C-9).

3.7. Bhutkesoside B (2)

A yellowish oil, $[\alpha]_D^{20}$ −14.5° (*c* 0.81, MeOH). HR-FAB-MS *m/z* 453.1737 $[M + Na]^+$ (Calcd for $C_{20}H_{30}O_{10}Na$, 453.1752). ^{1}H NMR (500 MHz, CD_3OD): δ_H 4.99 (1H, d, *J* = 2.1 Hz, Api-C_1-H), 4.43 (1H, t like, *J* = 7.3 Hz, C_3-H), 4.39 (1H, d, *J* = 7.6 Hz, Glc-C_1-H), 3.98 (1H,d, *J* = 9.5 Hz, Api-C_{4a}-H), 3.97 (1H,dd, *J* = 11.5, 2.1 Hz, Glc-C_{6a}-H), 3.91 (1H, d, *J* = 2.1 Hz, Api-C_2-H), 3.77 (1H, d, *J* = 9.5 Hz, Api-C_{4b}-H), 3.61 (1H, dd, *J* = 11.5, 5.4, Glc-C_{6b}-H), 3.60 (2H, s, Api-C_5-H), 3.37 (1H, m, Glc-C_5-H), 3.33 (1H, m, Glc-C_4-H), 3.31 (1H, m, Glc-C_3-H), 3.18 (1H, dd, *J* = 7.9, 9.5 Hz, Glc-C_2-H), 2.32 (2H, quartet, *J* = 7.3 Hz, C_8-H), 1.79 (2H, dq, *J* = 7.0,7.3 Hz, C_2-H), 1.15 (3H, t, *J* = 7.3 Hz, C_9-H), 1.01 (3H, t, *J* = 7.3 Hz, C_1-H). ^{13}C NMR (CD_3OD): δ_C 110.9 (CH, C-Api-1), 103.2 (CH, C-Glc-1), 83.2 (C-7), 80.6 (C-Api-3), 78.1 (C-Glc-3), 78.0 (C-Api-2), 76.8 (C-Glc-5), 75.6 (C-4), 75.1 (C-Api-4), 74.9 (C-Glc-2), 72.3 (C-3), 71.5 (C-Glc-4), 71.4 (C-5), 68.2 (C-Glc-6), 65.8 (C-Api-5), 64.9 (C-6), 29.7 (C-2), 13.7 (C-9), 13.5 (C-8), 9.7 (C-1).

3.8. Acid hydrolysis of bhutkesoside B (2)

Compound **2** (1.0 mg) was hydrolysed with 2.0 mL of 2 M HCl in a water bath at 60 °C for 3 h in a sealed tube. Co-TLC of the reaction mixture with authentic samples confirmed the presence of glucose and apiose.

3.9. Known compounds

Falcarindiol (**3**), a yellowish orange oil, $[\alpha]_D^{25}$ +99.9° (*c* 0.88, MeOH); chlorogenic acid (**4**), a pale yellow powder, $[\alpha]_D^{25}$ −26.3° (*c* 0.99, MeOH + H_2O, 1:1); 5-*O*-*p*-coumarylquinic acid (**5**), a white amorphous powder, $[\alpha]_D^{25}$ −27.6° (*c* 1.0, MeOH + H_2O, 1:1); 3,5-di-*O*-Caffeoyl quinic acid (**6**), a pale yellow powder, $[\alpha]_D^{25}$ −138.8° (*c* 0.95, MeOH + H_2O, 1:1); 4-hydroxy-7-methoxy-phenylethanol (**7**), a crystalline powder (MeOH), mp 92 °C, $[\alpha]_D^{25}$ ±0° (*c* 0.98, MeOH); ferulic acid (**8**), needle-shaped crystals (MeOH), mp 174 °C; dehydrodiconiferyl alcohol 4-*O*-*β*-glucopyranoside (**9**), a pale yellow mass, $[\alpha]_D^{25}$ −31.4° (*c* 0.98, MeOH); 5,7-dihydroxy-2-methylchromone-7-*O*-rutinoside (**10**), a pale yellow mass $[\alpha]_D^{25}$ −91.2° (*c* 0.99, MeOH); schumanniofioside B (**11**), a white crystalline powder (MeOH), mp 250 °C, $[\alpha]_D^{27}$ −58.8° (*c* 0.90, MeOH + H_2O); marmesinin (**12**), a white crystalline powder (MeOH), mp 258 °C, $[\alpha]_D^{20}$ −10° (*c* 0.16, MeOH).

3.10. Antioxidant activity

The DPPH radical scavenging activity of the isolated compounds was measured by the method as described previously (Joshi et al. 2014).

4. Conclusion

Two new diacetylenic glycosides, bhutkesoside A (**1**) and bhutkesoside B (**2**) and 10 known compounds (**3**–**12**) were isolated from the dried roots of *L. wallichiana*. All these compounds were isolated first time from the title plant. Among the phenolic compounds, 3, 5-di-*O*-Caffeoyl-quinic acid (**6**) and chlorogenic acid (**4**) had potent antioxidant activity against DPPH free radical.

Acknowledgements

The authors are grateful to the Program for Leading Graduate Schools 'HIGO Program' (Health life science: Interdisciplinary and Glocal Oriented), MEXT, Japan for scholarship to Mr Bibek Adhikari. We are grateful to Ms Teruo Tanaka and Mr Toshiyuki Iriguchi of Institute of Resource Development and Analysis, Kumamoto University for measurement of NMR and mass spectra, respectively.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Chauhan RS, Nautiyal MC, Tava A, Mella M. 2012. Chemical composition of the volatile oil from the roots of *Selinum tenuifolium* Wall. Helv Chim Acta. 95:780–787.

Dev V, Oka M, Mathela CS, Murari ND, Stevens TH. 1984. The volatile constituents of the roots of *Selinum tenuifolium*. J Nat Prod. 47:904–905.

Gewali MB. 2008. Aspects of traditional medicine in Nepal. Toyama: Institute of Natural Medicine, University of Toyama.

Joshi KR, Devkota HP, Watanabe T, Yahara S. 2014. Thotneosides A, B and C: potent antioxidants from nepalese crude drug, leaves of *Aconogonon molle*. Chem Pharm Bull. 62:191–195.

Kim J, Kim JC, Shim SH, Lee EJ, Bae K, Son KH, Kim HP, Kang SS, Chang HW. 2006. Chemical constituents of the roots of *Dystaenia takeshimana* and their anti-inflammatory activity. Arch Pharmacal Res. 29:617–623.

Kitajima J, Okamura C, Ishikawa T, Tanaka Y. 1998. Coumarin glycosides of *Glehnia lifforalis* root and rhizoma. Chem Pharm Bull. 46:1404–1407.

Kwon HC, Jung CM, Shin CG, Lee JK, Cho SU. 2000. A new caffeoyl quinic acid from *Aster scaber* and its inhibitory activity against human immunodeficiency virus-1(HIV-1) integrase. Chem Pharm Bull. 48:1796–1798.

Lundgern LN, Popoff T, Theander O. 1981. Dilignol glycosides from needles of *Picea abies*. Phytochemistry. 20:1967–1969.

Padalia RC, Verma RS, Chauhan A, Chanotiya CS, Yadav A. 2012. Variation in the volatile constituents of different plant parts of *Ligusticopsis wallichiana* from western Himalaya. India Nat Prod Commun. 7:1077–1078.

Park JY, Min BS, Jung HJ, Kim YH, Lee HK, Bae KH. 2002. Polyacetylene glycosides from *Gymnaster koraiensis*. Chem Pharm Bull. 50:685–687.

Rollinger JM, Zidorn C, Dobner MJ, Ellmerer EP, Stuppner HZ. 2003. Lignans, phenylpropanoids and polyacetylenes from *Chaerophyllum aureum* L. (Apiaceae). J Naturforsch. 58:553–557.

Roslund MU, Tahtinen P, Nietmitz M, Sjoholm R. 2008. Complete assignment of ^{1}H and ^{13}C chemical shifts and J^{CHH} coupling constants in NMR spectra of D-glucopyranose and all D-glucopyranosyl-D-glucopyranosides. Carbohydr Res. 343:101–112.

Seo S, Tomita Y, Tori K, Yoshimura Y. 1978. Determination of the absolute configuration of a secondary hydroxy group in a chiral secondary alcohol using glycosidation shifts in carbon-13 nuclear magnetic resonance spectroscopy. J Am Chem Soc. 100:3331–3339.

Sood SS, Chopra MM, Jamwal RK. 1978. Essential oil of *Selenium tenuifolium*. Indian Perfurmer. 22: 127–129.

Tamura S, Ohno T, Hattori Y, Murakami Y. 2010. Establishment of absolute stereostructure of falcarindiol, algicidal principle against Heterocapsa circularisquama from *Notopterygii Rhizoma*. Tetrahedron Lett. 51:1523–1525.

Tane P, Ayafor JA, Sondangam BL, Connolly JD. 1990. Chromone glycosides from *Schumanniophyton magnificum*. Phytochemistry. 29:1004–1007.

Watanabe T, Rajbhandari KR, Malla KJ, Devkota HP, Yahara S. 2013. A handbook of medicinal plants of Nepal supplement I. Kanagawa: Ayurseed Life Environmental Institute.

Yoshimatsu H, Nishida M, Hashimoto F, Tanaka M, Sakata Y, Okawa M, Nohara T. 2007. Chromone and flavonol glycosides from *Delphinium hybridum* cv. "Belladonna Casablanca". J Nat Med. 61:334–338.