Research Article

Nonvolatile Chemical Constituents from the Leaves of *Ligusticopsis wallichiana* (DC.) Pimenov & Kljuykov and Their Free Radical-Scavenging Activity

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Different plant parts of *Ligusticopsis wallichiana* (family: Apiaceae) are widely used as traditional medicines. Although many volatile constituents are already identified from the leaves of *L. wallichiana*, there is no detailed report on the nonvolatile constituents. In the present study, we aimed to isolate and identify the major chemical constituents from the leaves. Bhutkesoside A (**1**), falcarindiol (**2**), ferulic acid (**3**), cnidioside A (**4**), quercetin 3-*O*-β-D-glucopyranoside (**5**), rutin (**6**), 4′-*O*-methylquercetin 3-*O*-β-D-glucopyranoside (**7**), scopoletin (**8**), umbelliferone (**9**), eugenol 4-*O*-β-D-glucopyranoside (**10**) and pumilaside A (**11**) were isolated from the 70% MeOH extract. The structures of isolated compounds were elucidated on the basis of ^{1}H- and ^{13}C-NMR spectroscopic data. Compounds **4–11** are reported for the first time from *L. wallichiana*. Compounds **5** and **6** showed potent free radical-scavenging activity.

1. Introduction

Ligusticopsis wallichiana (DC.) Pimenov & Kljuykov (Syns. *Selinum wallichianum* (DC.) Raizada & H. O. Saxena, *Selinum tenuifolium* Wall. ex C. B. Clarke) is a perennial aromatic herb belonging to family Apiaceae. It is widely distributed in the Himalayan region of Nepal, India, Pakistan, Bhutan, and China between 2700 and 4800 m [1, 2]. In Nepal, it is locally known as "*Bhutkesh*" and the root decoction is used to treat body pain, fever, cough, and cold [1]. Flowers and leaves in the form of infusions are used to treat stomachache, and they are also applied locally for healing cuts and wounds [3]. In India, the root decoction is used for the treatment of diarrhea, stomachache, and vomiting. The flowers and stems are used for stimulant and carminative properties [2]. Previous studies on *L. wallichiana* were mainly focused on the volatile constituents of the different plant parts [2–6], but there is no detailed report on the nonvolatile constituents from the leaves. Recently, we reported two novel compounds, bhutkesoside A (**1**) and bhutkesoside B and ten known compounds from the roots of the same plant [7]. On continuation, in this paper, we report the detailed isolation and spectroscopic identification of major chemical constituents from the leaves of *L. wallichiana* and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity of isolated compounds.

2. Experiment

2.1. General Experimental Procedures. ^{1}H-, ^{13}C-, and 2D-NMR spectra were measured on a JEOL α − 500 (^{1}H-NMR: 500 MHz and ^{13}C-NMR: 125 MHz). Chemical shifts are given in ppm with reference to tetramethyl silane (TMS). Mass spectra were recorded on JEOL JMS-700 MStation. Absorbance was recorded on Immuno-MiniNJ-2300 Microtiter Plate Reader, Biotech Pvt., Ltd. (Tokyo, Japan). Column chromatography was carried out with MCI gel CHP20P (75~150 μm, Mitsubishi Chemical Industries Co., Ltd., Tokyo, Japan), Sephadex LH-20 (Amersham

Pharmacia Biotech, Tokyo, Japan), Chromatorex ODS (30~50 μm, Fuji Silysia Chemical Co., Ltd., Aichi, Japan), and silica gel 60 (0.040–0.063 mm, Merck KGaA, Darmstadt, Germany). TLC was performed on a precoated silica gel 60 F_{254} (aluminum sheet, Merck KGaA, Darmstadt, Germany).

2.2. Chemicals. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and Trolox were purchased from Wako Pure Chemicals, Osaka, Japan, and MES buffer was purchased from Dojindo Chemical Research, Kumamoto, Japan.

2.3. Plant Material. The fresh leaves of *L. wallichiana* were collected from Kurikharkha, Dolkha, Nepal in August 2013. The plant specimen was identified by Mr. Kuber Jung Malla, Senior Scientific Officer, Department of Plant Resources, Nepal. The voucher specimen (Voucher Number: KUNP20130809-015) was deposited at the Museum of Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

2.4. Extraction and Isolation. The shade dried leaves (540.0 g) were macerated three times (48 hours for each time) with 70% MeOH (8 L) at room temperature with frequent stirring. The extracts were then combined and evaporated under reduced pressure to give 131.0 g of semisolid extract. A part of the extract (119.0 g) was subjected to MCI gel CHP20P CC and eluted successively with water, 40%~100% MeOH to afford fifteen fractions (1~15). Fraction 6 (2.0 g, 40% MeOH eluate) was subjected successively to Sephadex LH-20 CC (50% MeOH), ODS CC (10% MeOH), and silica gel column (CHCl$_3$: MeOH : H$_2$O = 8 : 2 : 0.1) to obtain compound **4** (7.6 mg) and **11** (21.4 mg). Fraction 7 (2.6 g, 40% MeOH eluate) was applied over Sephadex LH-20 CC (50% MeOH) and ODS CC (20~25% MeOH) to give compounds **5** (34.4 mg) and **6** (127.0 mg). Fraction 9 (2.0 g, 70% MeOH eluate) was applied over Sephadex LH-20 CC (50~100% MeOH) to give ten subfractions (9–1~10). Subfraction 9–2 (927.0 mg) was applied over Sephadex LH-20 CC (40% MeOH) silica gel column (CHCl$_3$: MeOH = 10 : 1), and ODS CC (20% MeOH) to give compounds **1** (13.2 mg) and **10** (20.1 mg). Subfraction 9–4 (321.6 mg) was subjected on silica gel column (hexane : EtOAc = 3 : 2) to obtain compounds **8** (29.2 mg) and **9** (15.6 mg). Subfraction 9–5 was obtained as compound **3** (77.3 mg). Fraction 11 (875 mg, 70% MeOH eluate) was applied over Sephadex LH 20 and ODS CC (40–42% MeOH) to give compound **7** (6.0 mg). Fraction 13 (1.89 g, MeOH eluate) was applied over silica gel column (hexane : EtOAc = 3 : 1) to give compound **2** (375 mg).

2.5. Measurement of Free Radical-Scavenging Activity. The DPPH radical-scavenging activity of the isolated compounds was measured by the method as described by Li and Seeram [8] with slight modifications. Briefly, 50 μL of 200 mM MES (2-(N-morpholino) ethanesulphonic acid) buffer (pH 6.0), 100 μL of samples at different concentrations (in DMSO : ethanol = 1 : 1) and 50 μL of 800 mM DPPH in ethanol solution were mixed in a 96-well plate and kept in dark at

room temperature for 20 minutes. The radical-scavenging activity was measured at 510 nm with UV spectrophotometer using the following formula: radical-scavenging activity (%) = $100 \times (A - B)/A$. where A is the control absorbance of DPPH radicals without samples and B is the absorbance after reacting with samples. Trolox was used as the positive control. From these data, curve was plotted and effective concentration (EC$_{50}$) value was calculated which is defined as the concentration (μM) of the compound required for 50% reduction of the DPPH radical absorbance.

3. Results and Discussion

The shade dried leaves of *L. wallichiana* were extracted with 70% MeOH, and the extract was then subjected to repeated column chromatography (CC) on MCI gel CHP20P, Sephadex LH20, ODS, and silica gel column to obtain 11 compounds (**1–11**). Structures of these compounds were determined on the basis of ^{1}H- and ^{13}C-NMR spectroscopic data and comparison with reference values (Figure 1).

Compound **1**, a pale yellow oil, $[\alpha]_D^{27}$ −117° ($c = 0.35$, MeOH), was identified as 2(R)-hydroxy-3,5-nonadiyn-2-O-β-D-glucopyranoside named as bhutkesoside A, which was a new diacetylene glucoside isolated from the roots of *L. wallichiana* in our previous study [7]. The detailed ^{1}H- and ^{13}C-NMR data for compound **1** are given in Table 1.

Compound **2** was obtained as yellowish orange oil, $[\alpha]_D^{20}$ +99.9° ($c = 0.88$, MeOH). The ^{1}H-NMR spectrum (Table 2) showed three proton signals at δ_H 5.94 (1H, ddd, $J = 5.2$, 10.4, 17.2 Hz), 5.47 (1H, dt, $J = 1.5$, 17.2 Hz), and 5.25 (1H, dt, $J = 1.5$, 10.4 Hz) assignable to terminal vinyl protons. A set of olefenic protons at δ_H 5.51 (1H, dd, $J = 8.5$, 10.6 Hz) and 5.61 (1H, ddd, $J = 1.2$, 7.3, 10.6 Hz) were also present and their coupling constant of 10.6 Hz suggested the *cis* configuration. Two protons attached to oxygen bearing carbon were present at δ_H 5.20 (1H, brd, $J = 8.5$ Hz) and δ_H 4.93 (1H, d, $J = 5.2$ Hz). Two methylene proton at δ_H 2.10 to 1.27 ppm and a methyl signal at δ_H 0.88 (3H, t, $J = 7.0$ Hz) were also observed. The ^{13}C-NMR spectra (Table 2) showed signals equivalent to total seventeen carbons. The natures of these carbons were determined by DEPT spectra. Among these carbon signals, four quaternary carbon signals at δ_C 78.2 (C), 70.3 (C), 79.8 (C), and 68.7 (C) were assignable to a disubstituted acetylene moiety. Two oxygen-bearing carbons at δ_C 63.4 (CH) and 58.5 (CH) and a methyl group at δ_C 14.0 ppm were also observed. On the basis of these data and comparison with literature values, compound **2** was identified as falcarindiol [9].

Compound **3** was obtained as white needles. The ^{1}H-NMR spectrum (Table 3) showed three proton signals in the aromatic region at δ_H 7.27 (1H, $J = d$, 2.1 Hz), 7.08 (1H, dd, $J = 2.1$, 8.2 Hz), and 6.80 (1H, $J = d$, 8.2 Hz) assignable to a 1,3,4-trisubstituted aromatic ring. Two signals at δ_H 7.51 (1H, d, $J = 15.9$ Hz) and 6.36 (1H, d, $J = 15.9$ Hz) suggested presence of *trans* olefenic protons. A methoxy signal at δ_H 3.82 (3H, s) attached to aromatic ring was also observed. The ^{13}C-NMR (Table 3) showed six carbon signals at δ_C 149.1 (C), 147.9 (C), 125.8 (C), 122.8 (CH), 115.6 (CH), and 111.2 (CH), which confirmed a 1,3,4-trisubstituted aromatic ring

FIGURE 1: Structures of isolated compounds.

and signals at δ_C 144.6 (CH) and 115.5 (CH) confirmed a *trans* olefinic moiety. Further quaternary carbon at δ_C 168.1 (C) for a carbonyl carbon and methoxy signal at δ_C 55.7 (OCH$_3$) were also observed. On the basis of these data and comparison with literature values, compound **3** was identified as ferulic acid [10].

Compound **4** was obtained as white amorphous powder, $[\alpha]_D^{27}$ −33.4° (c = 0.45, pyridine). The ^{1}H-NMR spectrum (Table 4) of compound **4** showed four aromatic or olefinic protons at δ_H 7.82 (1H, d, J = 2.2 Hz), 7.37 (1H, s), 7.32 (1H, s), and 6.81 (1H, d, 2.2 Hz). Seven proton signals attached to oxygen bearing carbons assignable to a sugar moiety were present at δ_H 4.82 (1H, d, J = 7.3 Hz), 3.72 (1H, dd, J = 5.01, 11.8 Hz), 3.26–3.30 (2H, br, m), 3.16 (2H, t, J = 9.1 Hz), and 3.75 (1H, dd, J = 1.8, 11.8 Hz). The proton signals at δ_H 4.82 (1H, d, J = 7.3 Hz) was assignable to the anomeric proton for a sugar moiety. Further two methylene protons signals coupled each other at δ_H 2.87 (2H, t, J = 7.6 Hz) and 2.40 (2H, t, J = 7.6 Hz) were observed. The ^{13}C-NMR spectra (Table 4) showed signals equivalent to total eighteen carbons and among them eight aromatic carbons at δ_C 153.5 (C), 153.4 (C), 144.9 (CH), 126.6 (C), 120.8 (C), 120.6 (CH), 106.2 (CH), and 98.3 (CH) revealed a benzofuran moiety. Six carbon signals at δ_C 101.5 (CH), 73.3 (CH), 77.0 (CH), 69.9 (CH), 76.6 (CH), and 60.8 (CH$_2$) were assignable to a β-glucopyranosyl moiety which was also supported by the coupling constant of anomeric proton (J = 7.3 Hz). Three

TABLE 1: ^{1}H- and ^{13}C-NMR spectroscopic data of compound **1** in CD$_3$OD.

Carbon number	δ_H, mult. (J in Hz)	δ_C
1	1.42, d (6.7)	22.3, CH$_3$
2	4.80, brs	64.2, CH
3	—	75.7, C
4	—	71.3, C
5	—	65.4, C
6	—	82.0, C
7	2.27, t (7.3)	21.7, CH$_2$
8	1.55, dq (7.3)	22.8, CH$_2$
9	1.00, t (7.3)	13.7, CH$_3$
Glc-1	4.55, d (7.6)	101.3, CH
Glc-2	3.17, dd (7.6, 9.2)	74.9, CH
Glc-3	3.38, t (9.2)	78.0, CH
Glc-4	3.27, m	71.7, CH
Glc-5	3.27, m	78.1, CH
Glc-6	3.80, dd (11.5, 1.5) 3.64, dd (11.5, 5.4)	62.8, CH$_2$

carbon signals δ_C 174.8 (C), 35.3 (CH$_2$), and 26.1 (CH$_2$) revealed a propanoic acid derivative. On the basis of these data and comparison with literature values, compound **4** was identified as cnidioside A [11].

TABLE 2: ^{1}H- and ^{13}C-NMR spectroscopic data of compound **2** in CDCl$_3$.

Carbon number	δ_H, mult. (J in Hz)	δ_C
1	5.47, dt (17.2, 1.5) 5.25, dt (10.4, 1.5)	117.3, CH$_2$
2	5.94, ddd (5.2, 10.4, 17.2)	135.7, CH
3	4.93, brd (5.2)	63.4, CH
4	—	78.2, C
5	—	70.3, C
6	—	68.7, C
7	—	79.8, C
8	5.20, brd (8.5)	58.5, CH
8		
9	5.51, brdd (8.5, 10.6)	127.6, CH
10	5.61, ddd (1.2, 7.3, 10.6)	134.6, CH
11	2.10, dq (1.2, 7.3)	31.7, CH$_2$
12	1.38, t like (7.3)	29.2, CH$_2$
13	1.27–1.29, m	29.2, CH$_2$
14	1.27–1.29, m	29.2, CH$_2$
15	1.27–1.29, m	27.2, CH$_2$
16	1.27–1.29, m	22.1, CH$_2$
17	0.88, t (7.0)	14.0, CH$_3$

TABLE 3: ^{1}H- and ^{13}C-NMR spectroscopic data of compound **3** in DMSO-d_6.

Carbon number	δ_H, mult. (J in Hz)	δ_C
1	—	125.8, C
2	7.27, d (2.1)	111.2, CH
3	—	149.1, C
4	—	147.9, C
5	6.80, d (8.5)	115.6, CH[a]
6	7.08, dd (2.1, 8.2)	122.8, CH
7	7.51, d (15.9)	144.6, CH
8	6.36, d (15.9)	115.5, CH[a]
9	—	168.1, C
OCH$_3$	3.82, s	55.7, OCH$_3$

[a]Assignments with the same superscript may be interchanged in the same column.

TABLE 4: ^{1}H- and ^{13}C-NMR spectroscopic data of compound **4** in CD$_3$OD.

Carbon number	δ_H, mult. (J in Hz)	δ_C
1	—	126.6, C[a]
2	—	153.5, C[b]
3	7.37, s	98.3, CH
3a	—	153.4, C[b]
4	7.82, d (2.2)	144.9, CH
5	6.81, d (2.2)	106.2, CH
5a	—	120.8, C[a]
6	7.32, s	120.6, CH
7	2.87, t (7.6)	26.1, CH$_2$
8	2.40, t (7.6)	35.3, CH$_2$
9	—	174.8, C
Glc-1	4.82, d (7.3)	101.5, CH
Glc-2	3.26–3.30, m	73.3, CH
Glc-3	3.16, t (9.1)	77.0, CH[c]
Glc-4	3.16, t (9.1)	69.9, CH
Glc-5	3.26–3.30, m	76.6, CH[c]
Glc-6	3.72, dd (5.0, 11.8) 3.75, dd (1.8, 11.8)	60.8, CH$_2$

[a,b,c]Assignments with the same superscripts may be interchanged in the same column.

156.2 (C), 144.8 (C), 148.5 (C), 133.4 (C), 121.6 121.2 (C), 115.2 (CH), 116.2 (CH), 104.0 (C), and 98.7 (CH), were assignable to a 3-O-substituted quercetin. The remaining six signals at δ_C 100.9 (CH), 77.5 (CH), 76.5 (CH), 74.1 (CH), 69.9 (CH), and 61.0 (CH$_2$) for a monosaccharide revealed the β-glucopyranosyl moiety which was supported by the coupling constant ($J = 7.0$ Hz) of anomeric proton. On the basis of these data and comparison with literature values, compound **5** was identified as quercetin 3-O-β-D-glucopyranoside [12].

Compound **6** was obtained as pale yellow crystalline powder, $[\alpha]_D^{23}$ −35.5° ($c = 0.37$, pyridine). The ^{1}H-NMR spectrum (Table 5) of compound **6** was similar to that of compound **5** except some additional signals of sugar moiety. Proton signal for anomeric proton at 5.29 (1H, brs) and a methyl group at δ_H 1.00 (3H, d, $J = 6.1$ Hz) suggested the presence of rhamnopyranosyl moiety. The ^{13}C-NMR spectra (Table 5) showed signals equivalent to total twenty-seven carbons. Similar to compound **5**, fifteen carbon signals were assignable to a 3-O-substituted quercetin moiety. Among the remaining 12 signals, six signals at δ_C 101.2 (CH), 76.4 (CH), 75.9 (CH), 74.1 (CH), 70.6 (CH), and 68.2 (CH$_2$) were assignable to a β-glucopyranosyl moiety and other six carbons signals at δ_C 100.7 (CH), 70.4 (CH), 70.0 (CH), 71.8 (CH), 67.0 (CH), and 17.7 (CH$_3$) were assignable to a α-rhamnopyranosyl moiety. The downfield shift of C-6 of glucopyranosyl moiety at 68.2 ppm suggested Rha-1→Glc-6 linkage. On the basis of these data and comparison with literature values, compound **6** was identified as rutin [12].

Compound **7** was obtained as yellow powder, $[\alpha]_D^{27}$ −14.7° ($c = 0.60$, pyridine). The ^{1}H-NMR spectrum (Table 5) showed

Compound **5** was obtained as pale yellow crystalline powder, $[\alpha]_D^{23}$ −31.9° ($c = 0.99$, pyridine). The ^{1}H-NMR spectrum (Table 5) showed five proton signals at δ_H 7.59 (1H, d, $J = 2.2$ Hz), 7.58 (1H, brd, $J = 8.4$ Hz), 6.85 (1H, d, $J = 8.4$ Hz), 6.41 (1H, d, $J = 1.8$ Hz), and 6.21 (1H, d, $J = 1.8$ Hz) assignable to proton signals of quercetin. Seven proton signals attached to oxygen bearing carbon were present at δ_H 5.47 (1H, d, $J = 7.0$ Hz), 3.10 (1H, m), 3.25 (2 H, m), 3.10 (1H, m), 3.59 (1H, d, $J = 11.3$ Hz), and 3.33 (1H, d $J = 11.3$ Hz) were present. Among them proton at δ_H 5.47 (1H, d, $J = 7.0$ Hz) was assignable to anomeric proton of the sugar moiety. The ^{13}C-NMR spectra of (Table 5) showed signals equivalent to total twenty-one carbons, in which 15 carbon signals at δ_C 177.5 (C), 164.3 (C), 161.2 (C), 156.3 (C),

TABLE 5: ^{1}H- and ^{13}C-NMR spectroscopic data of compounds **5**, **6**, and **7** in DMSO-d_6.

Carbon number	5		6		7	
	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C
2	—	156.3, C[a]	—	156.4, C[b]	—	156.2, C[a]
3	—	133.4, C	—	133.3, C	—	133.5, C
4	—	177.5, C	—	177.4, C	—	177.4, C
5	—	161.2, C[c]	—	161.2, C[d]	—	161.2, C[c]
6	6.21, d (1.8)	98.7, CH	6.19, d (2.2)	100.7, CH[e]	6.20, d (1.8)	98.7, CH
7	—	164.3, C[c]	—	164.0, C[d]	—	164.1, C[c]
8	6.41, d (1.8)	93.5, CH	6.38, d (2.2)	93.6, CH	6.41, d (1.8)	93.6, CH
9	—	156.2, C[a]	—	156.6, C[b]	—	155.7, C[a]
10	—	104.0, C	—	104.0, C	—	103.9, C
1′	—	121.2, C	—	121.2, C	—	122.6, C
2′	7.59, d (2.2)	115.2, CH[f]	7.52, d (2.2)	115.2, CH[f]	7.56, d (2.2)	115.7, CH[f]
3′	—	144.8, C	—	144.7, C	—	145.8, C
4′	—	148.5, C	—	148.4, C	—	149.9, C
5′	6.85, d (8.4)	116.2, CH[f]	6.81, d (8.4)	116.3, CH[f]	7.04, d (8.4)	114.8, CH[f]
6′	7.58, brd (8.4)	121.6, CH	7.54, brd (8.4)	121.6, CH	7.70, brd (8.4)	121.4, CH
OCH$_3$	—	—	—	—	3.85, s	55.6, OCH$_3$
Glc-1	5.47, d (7.0)	100.9, CH	5.34, d (7.3)	101.2, CH[e]	5.48, d (7.0)	100.8, CH
Glc-2	3.10, m	74.1, CH	3.22–3.42, m	74.1, CH	3.09, m	74.0, CH
Glc-3	3.24–3.25, m	77.5, CH[e]	3.22–3.42, m	76.4, CH[c]	3.17–3.24, m	77.5, CH[e]
Glc-4	3.10, m	69.9, CH	3.07, dd (9.5, 9.2)	70.6, CH[a]	3.09, m	69.8, CH
Glc-5	3.24–3.25, m	76.5, CH[e]	3.22–3.42, m	75.9, CH[c]	3.17–3.24, m	76.4, CH[e]
Glc-6	3.59, brd (11.3)	61.0, CH$_2$	3.67, brd (10.2)	67.0, CH$_2$	3.57, brd (11.3)	60.9, CH$_2$
	3.33, brd (11.3)		3.70, brd (10.9)		3.60, m	
Rha-1	—	—	5.29, brs	100.7, CH[e]	—	—
Rha-2	—	—	3.22–3.42, m	70.4, CH[a]	—	—
Rha-3	—	—	3.22–3.42, m	70.0, CH[a]	—	—
Rha-4	—	—	3.22–3.42, m	71.8, CH[a]	—	—
Rha-5	—	—	3.22–3.42, m	68.2, CH	—	—
Rha-6	—	—	1.00, d (6.1)	17.7, CH$_3$	—	—

[a,b,c,d,e,f]Assignment with the same superscript may be interchanged in the same column.

signals similar to compound **5** except an additional signal for methoxy group at δ_H 3.85 (3H, s). The ^{13}C-NMR spectra of compound **7** (Table 5) showed signals equivalent to total twenty-two carbons, in which 15 carbon signals at δ_C 177.5 (C), 164.3 (C), 161.2 (C), 156.3 (C), 156.2 (C), 144.8 (C), 148.5 (C), 133.4 (C), 121.6 121.2 (C), 115.2 (CH), 116.2 (CH), 104.0 (C), and 98.7 (CH), were assignable to a 3-O-substituted quercetin. The remaining six signals at δ_C 101.5 (CH), 72.7 (CH), 70.8 (CH), 68.2 (CH), and 63.7 (CH$_2$) confirmed the presence of a β-glucopyranosyl which was also supported by the coupling constant ($J = 7.9$ Hz) of the anomeric proton. Signal at δ_C 55.6 was assigned to a methoxy group. In differential NOE experiment, irradiation of the methoxy signal at δ_H 3.85 (3H, s) increased the intensity of proton signal assignable to C-5′ at δ_H 7.04 (d, 8.4 Hz) while no effect was seen in the protons at C-2′ at δ_H 7.56, 1H (d, 2.2 Hz), which suggested that the methoxy group was attached at C-4′ position in B-ring of quercetin. On the basis of these data and comparison with literature values, compound **7** was identified as 4′-O-methylquercetin 3-O-β-D-glucopyranoside [13].

Compound **8** was obtained as white crystals. The TLC spot for compound **8** showed blue colour under UV (365 nm), suggesting a coumarin derivative. The ^{1}H-NMR spectrum (Table 6) showed four proton signals in aromatic or olefinic region at δ_H 7.84 (1H d, $J = 9.5$ Hz), 7.10 (1H, s), 6.76 (1H, s), and 6.20 (1H, d, $J = 9.5$ Hz). Further a proton singlet at δ_H 3.90 (3H, s) suggested a methoxy group. The ^{13}C-NMR spectra (Table 6) showed signals equivalent to total 10 carbons and among them, 9 carbon signals at δ_C 164.1 (C), 152.9 (C), 151.5 (C), 147.1 (C), 146.1 (CH), 112.6 (C), 112.7 (CH), 110.0 (CH), and 104.0 (CH) were assignable to a 6,7-dihydroxycoumarin derivative. Moreover, signal at δ_C 56.8 was assigned to a methoxy group. In differential NOE experiment, irradiation of a proton signal at 7.10 δ_H (1H, s) assignable to proton attached to C-5 position of coumarin increased the intensity of methoxy signal at δ_H 3.90 (3H, s) as well as proton assignable to C-4 position at

TABLE 6: ^{1}H- and ^{13}C-NMR spectroscopic data of compounds **8** and **9** in CD$_3$OD.

Carbon number	8		9	
	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C
2	—	164.1, C	—	163.7, C[a]
3	6.20, d (9.5)	112.7, CH	6.18, d (9.5)	114.5, CH[b]
4	7.84, d (9.5)	146.1, CH	7.84, d (9.5)	146.0, CH
5	7.10, s	110.0, CH	7.45, d (8.5)	130.7, CH
6	—	147.1, C	6.77, dd (2.4,8.5)	112.3, CH[b]
7	—	152.9, C[c]	—	163.1, C[a]
8	6.76, s	104.0, CH	6.70, d (2.4)	103.4, CH
9	—	151.5, C[c]	—	157.2, C[c]
10	—	112.6, C	—	111.6, C
OCH$_3$	3.90, s	56.8, CH$_3$	—	—

[a,b,c]Assignment with the same superscript may be interchanged in the same column.

7.84 (1H d, $J = 9.5$ Hz), which suggested that the methoxy group was attached to C-6 position. On the basis of these data and comparison with literature values, compound **8** was identified as scopoletin [14].

Compound **9** was obtained as white crystals. The TLC spot for compound **9** also showed blue colour under UV (365 nm), suggesting a coumarin derivative. The ^{1}H-NMR spectrum (Table 6) showed five protons in the aromatic region two proton signals coupled each other at δ_H 6.18 (1H, d, $J = 9.5$ Hz) and 7.84 (1H d, $J = 9.5$ Hz) and three proton signals at δ_H 7.45 (1H, d, $J = 8.5$ Hz), 6.77 (1H, dd, $J = 2.4$, 8.5 Hz), and 6.70 (1H, d, $J = 2.4$ Hz), suggesting that the compound **9** was a 7-hydroxycoumarin. The ^{13}C-NMR spectra (Table 6) showed signals equivalent to total 9 carbons at δ_C 163.7 (C), 163.1 (C), 157.2 (C), 146.0 (CH), 130.7 (CH), 114.5 (CH), 111.6 (C), 112.3 (CH), and 103.4 (CH). These carbon signals were superimposable with that of umbelliefone [14].

Compound **10** was obtained as white amorphous powder, $[\alpha]_D^{27}$ −35.5° ($c = 0.92$, pyridine). The ^{1}H-NMR spectrum (Table 7) showed three proton signals in the aromatic region at δ_H 6.81 (1H, d, $J = 1.8$ Hz), 7.07 (1H, d, $J = 8.2$ Hz), and 6.71 (1H, dd, $J = 8.2$, 1.8 Hz), which were assignable to a 1,3,4-substituted aromatic ring. Further three proton signals due to terminal alkene at δ_H 5.94 (1H, ddt, $J = 17.0$, 10.2, 6.7 Hz), 5.05 (1H, dd, $J = 17.0$, 1.8 Hz), and 5.01 (1H, dd, $J = 10.2$, 1.8 Hz) coupled with two proton signals at δ_H 3.31 (2H, d, $J = 6.7$ Hz) were observed, which suggested the presence of allyl moiety. A singlet at δ_H 3.83 (3H, s) suggested the presence of a methoxy group. Further seven protons assignable to a sugar moiety were present at δ_H 4.83 (1H, d, $J = 7.3$ Hz), 3.48 (1H, dd, $J = 7.3$, 9.1 Hz), 3.37–3.39 (2H, m), 3.45 (1H, dd, $J = 9.1$, 9.8 Hz), 3.68 (1H, dd, $J = 12.2$, 5.4 Hz), and 3.86 (1H, dd, $J = 12.2$, 1.5 Hz). The ^{13}C-NMR spectra (Table 7) showed signals equivalents to total 16 carbons, in which 9 carbon signals at δ_C 150.8 (C), 146.4 (C), 139.0 (CH), 136.5 (C), 122.1 (CH), 118.3 (CH), 115.1 (CH$_2$), 114.2 (CH), and 40.7 (CH$_2$), were assignable to a 1,3,4-trisubstituted aromatic ring with an allyl moiety. Other six signals δ_C 103.1 (CH), 78.2 (CH), 77.9 (CH), 74.9 (CH), 71.4 (CH), and 62.5 (CH$_2$) were assignable to β-glucopyranosyl

TABLE 7: ^{1}H- and ^{13}C-NMR spectroscopic data of compound **10** in CD$_3$OD.

Carbon number	δ_H, mult. (J in Hz)	δ_C
1	—	136.5, C
2	6.81, d (1.8)	118.3, CH
3	—	146.4, C
4	—	150.8, C
5	7.07, d (8.2)	114.2, CH
6	6.71, dd (8.2, 1.8)	122.1, CH
7	3.31, d (6.7)	40.7, CH$_2$
8	5.94, ddt (10.2, 17.0, 6.7)	139.0, CH
9	5.05, dd (17.0, 1.8) 5.01, dd (10.2, 1.8)	115.1, CH$_2$
OCH$_3$	3.83, s	56.8, CH$_3$
Glc-1	4.83, d (7.3)	103.1, CH
Glc-2	3.48, dd (7.3, 9.1)	74.9, CH
Glc-3	3.37–3.39, m	78.2, CH[a]
Glc-4	3.45, dd (9.5, 9.1)	71.4, CH
Glc-5	3.37–3.39, m	77.9, CH[a]
Glc-6	3.68, dd (12.2, 5.4) 3.86, dd (12.2, 1.5)	62.5, CH$_2$

[a]Assignments with the same superscripts may be may be interchanged in the same column.

moiety as in the case of previous compounds. The carbon signal at δ_C 56.8 was assigned to a methoxy group. In differential NOE experiment, irradiation of the methoxy signal at δ_H 3.83 (3H, s) enhanced the intensity of proton at δ_H 6.79 (1H, d, $J = 1.8$ Hz) assignable to C-2 of the aromatic ring, which suggested that the methoxy group was present at C-3 position, and glucopyranosyl moiety was attached to C-4. On the basis of these data and comparison with literature values, compound **10** was identified as eugenol 4-O-β-D-glucopyranoside [15].

Compound **11** was obtained as colourless gum, $[\alpha]_D^{27}$ −25.9°. Its molecular formula was determined to be C$_{21}$H$_{38}$O$_8$ on the basis of a HR-FAB-MS peak of [M + Na]$^+$ at 441.2482 (calculated for C$_{21}$H$_{38}$O$_8$Na, 441.2464). ^{1}H-NMR

TABLE 8: ^{1}H- and ^{13}C-NMR spectroscopic data of compound **11** in DMSO-d_6.

Carbon number	δ_H, mult. (J in Hz)	δ_C
1	3.08, dd (4.2, 9.2)	77.9, CH
2	1.36–1.48, m	27.9, CH$_2$
3	1.36–1.48, m	39.9, CH$_2$
4	—	71.3, C
5	1.74, d (11.6)	49.9, CH
6	4.48, dd (4.6, 11.6)	76.8, CH
7	1.83, m	40.3, CH
8	1.62, d (14.2)	22.4, CH$_2$
	1.36–1.48, m	
9	1.36–1.48, m	35.5, CH$_2$
	1.14, m	
10	—	41.1, C
11	2.11, dq (7.0, 6.7)	24.6, CH
12	0.90, d (6.7)	22.6, CH$_3$
13	1.03, d (6.7)	23.1, CH$_3$
14	0.83, s	13.8, CH$_3$
15	1.23, s	23.6, CH$_3$
Glc-1	4.36, d (7.3)	98.3, CH
Glc-2	2.93, m	74.3, CH
Glc-3	3.10–3.16, m	77.0, CH
Glc-4	3.02–3.04, m	70.4, CH
Glc-5	3.10–3.16, m	76.9, CH
Glc-6	3.42, dd (5.0, 11.2)	61.4, CH$_2$
	3.70, dd (4.3, 11.2)	

spectrum of compound **11** (Table 8) showed several proton signals from 1 to 2 ppm, clear signals for two methyl doublets were present at δ_H 0.90 (3H, d, $J = 6.7$ Hz) and 1.03 (3H, d, $J = 6.7$ Hz), two methyl singlets were present at δ_H 0.83 (3H, s) and 1.23 (3H, s). Remaining ten proton signal equivalents were present from δ_H 2.11 to δ_H 4.48 ppm. The ^{13}C-NMR spectra (Table 8) showed total 21 carbon signals and among them, six signals at δ_C 98.3 (CH), 77.0 (CH), 76.9 (CH), 74.3 (CH), 70.4 (CH), and 61.4 (CH$_2$) were assignable to a glucopyranosyl moiety. The rest fifteen signals at δ_C 77.9 (CH), 76.8 (CH), 71.3 (C), 49.9 (CH), 40.3 (CH), 39.9 (CH$_2$), 35.5 (CH$_2$), 27.9 (CH$_2$), 24.6 (CH), 23.6 (CH$_3$), 23.1 (CH$_3$), 22.6 (CH$_3$), 22.4 (CH$_2$), and 13.8 (CH$_3$) can be assignable to a eudesmane-type sesquiterpenoid moiety. On the basis of these data and comparison with literature values, compound **11** was identified as pumilaside A [16].

Among these eleven compounds isolated from the leaves of *L. wallichina* in this study, a diacetylene glucoside, bhutkesoside A (**1**); a polyacetylene derivative, falcarindiol (**2**); and a phenylpropanoid derivative, ferulic acid (**3**) were also isolated from the roots of same plant, which were reported in a previous paper [7]. All other compounds (**4**–**11**) were isolated for the first time from this plant which included a benzofuran derivative, cnidioside A (**4**); three flavonoid derivatives, quercetin 3-*O*-β-D-glucopyranoside (**5**), rutin (**6**), and 4′-*O*-methylquercetin 3-*O*-β-D-glucopyranoside (**7**); two coumarin derivatives, scopoletin (**8**) and umbelliferone (**9**); a phenylpropene derivative,

eugenol 4-*O*-β-D-glucopyranoside (**10**), and a eudesmane sesquiterpene glucoside, pumilaside A (**11**). It was the first study on the isolation and identification of nonvolatile compounds from the leaves of *L. wallichiana*. Regarding coumarin derivatives, three furocoumarins such as bergapten, heraclenin, and heraclenol were isolated from the roots of *L. wallichiana* [17]. This is the first report on the presence of flavonoids in *L. wallichiana* and presence of coumarin derivatives in the leaves.

All these isolated compounds were evaluated for their DPPH free radical-scavenging activity. Among them, only two flavonoids, rutin (**6**) (EC$_{50}$ 52.4 μM) and quercetin-3-*O*-β-D-glucopyranoside (**5**) (EC$_{50}$ 54.5 μM) showed potent free radical-scavenging activity as compared to positive control, Trolox (EC$_{50}$ 96.1 μM). Flavonoids with unsubstituted hydroxyl groups in C$_{3'}$ and C$_{4'}$ position (**5** and **6**) showed potent activity; however, a compound with C-4′ methoxy substitution, 4′-*O*-methylquercetin 3-*O*-β-D-glucopyranoside (**7**), did not show any activity in the free radical-scavenging assay. These results were similar to previous studies on the free radical-scavenging activities of flavonoids [18, 19].

In conclusion, eleven nonvolatile compounds belonging to different chemical classes were isolated and identified for the first time from the leaves of *L. wallichiana*. Some of the isolated compounds also showed potent free radical-scavenging activity. Further studies should focus on the detailed biological activities of extracts and isolated compounds to provide the scientific evidence for their traditional uses.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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