Natural Product Sciences
21(2) : 76-81 (2015)

Phenolic Compounds and Triterpenes from the Barks of *Diospyros burmanica*

Janggyoo Choi[1], Jae Youl Cho[2], Young-Dong Kim[3], Khin Myo Htwe[4], Woo-Shin Lee[5], Jun Chul Lee[1], Jinwoong Kim[1], and Kee Dong Yoon[6,*]

[1]*College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul, Korea*
[2]*Department of Genetic Engineering, Sungkyunkwan University, Suwon 440-746, Korea*
[3]*Department of Life Science, Hallym University, Chuncheon 200-702, Korea*
[4]*Popa Mountain Park, Forest Department, Kyaukpadaung Township, Mandalay Division, Myanmar*
[5]*Department of Forest Sciences, Seoul National University, Seoul 151-921, Korea*
[6]*College of Pharmacy, The Catholic University of Korea, Bucheon 420-743, Korea*

Abstract – *Diospyros burmanica* Kurz. is an evergreen deciduous tree distributed in Mandalay of Myanmar, which belongs to the family of Ebenaceae. In Myanmar, it has been used to treat diarrhea, diabetes, diabetes and also as lumbers. In this study, seven flavonoids (**1** - **7**), a phenolic compound (**8**), and five triterpenes (**9** - **13**) were isolated from the barks of *D. burmanica* and their chemical structures were elucidated. Isolates were identified to be (+)-catechin (**1**), (+)-catechin 3-*O*-α-L-rhamnopyranoside (**2**), (+)-catechin 3-*O*-gallate (**3**), (−)-epicatechin (**4**), (−)-epicatechin 3-*O*-gallate (**5**), (+)-afzelechin 3-*O*-α-L-rhamnopyranoside (**6**), (+)-2,3-*trans*-dihydrokaempferol 3-*O*-α-L-rhamnopyranoside (**7**), methyl gallate (**8**), lupeol (**9**), methyl lup-20(29)-en-3-on-28-oate (**10**), β-amyrin (**11**), α-amyrin (**12**), 3β-hydroxy-D:B-friedo-olean-5-ene (**13**) through MS, ^{1}H NMR and ^{13}C NMR spectroscopic evidences.
Keywords – *Diospyros burmanica,* Flavonoids, Triterpenes

Introduction

Diospyros burmanica Kurz (Ebenaceae) is an evergreen deciduous tree distributed in the Mandalay region of Myanmar, and local traditional practitioners have used this plant as a medicinal plant to treat diabetes, diarrhea and dysentery. Over 350 species of genus *Diospyros* have been known worldwide and many have been used as traditional medicines in the India, Africa and China,[1] and especially *D. kaki* has well been investigated for its phytochemicals and biological activities.[2-6] As for the study of *D. burmanica*, only a paper has been reported revealing bisnaphtoquinones and naphthol derivatives and their leishmanicidal inhibitory activities.[7] This study focused on the further phytochemical investigation of *D. burmanica* and led to the isolation of seven flavonoids, a phenolic compound, and five triterpenes.

Experimental

General experimental procedure – ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AscendTM 500 spectrometer (Bruker, Germany). Mass spectra were recorded by using an Agilent 6530 ESI-QTOF MS (Agilent Technologies, USA) and JEOL JMS-700 spectrometer (JEOL, Japan). A Gilson preparative HPLC system (Gilson, USA) was used to isolate compounds and equipped with a GX-271 liquid handler, binary pumps, and an UV/VIS-155 detector. An MPLC system composed of an IOTA S 300 pump (ECOM, Czech Republic) and a Saphire 600 UV-VIS variable wavelength detector (ECOM, Czech Republic) were used. The preparative HPCCC (Dynamic Extractions, UK) possessed two sets of two bobbins. One bobbin was equipped with an analytical coil (11 mL, 0.8 mm ID), and the other with a preparative coil (492 ml, 4 mm ID). Deionized water was prepared by Millipore Milli-Q water purification system (Millipore, USA), and organic solvents for column chromatography were purchased from Daejung-Chemical and Metals Co. Ltd. (Kyunggi-Do, Korea). Silica gel and reversed-phase silica gel for column chromatography were Kieselgel 60 (230 - 400 mesh, Merck, Germany) and YMC RP-18 resin (YMC, Japan), respectively. HPLC column was YMC-Pack ODS-A (250 × 20 mm, 5 μm, YMC, Japan) and YMC-Pack Ph (250 × 20 mm, 5 μm, YMC, Japan).

*Author for correspondence
Kee Dong Yoon, College of Pharmacy, The Catholic University of Korea, Bucheon 420-743, Korea
Tel: +82-2-2164-4091; E-mail: kdyoon@catholic.ac.kr

Plant material – Barks and leaves of *D. burmanica* were collected from AKNP of Myanmar in February 2012, and identified by Professor Young-Dong Kim (Hallym University, Chuncheon, Korea). A voucher specimen (CU-2014-2-12) was deposited at the Herbarium of College of Pharmacy, The Catholic University of Korea.

Extraction and isolation – Extraction and isolation - Dried barks (1.3 kg) of *D. burmanica* were extracted with 100 % MeOH in an ultrasonic bath for (5 L × 2 h × 3 times). After evaporating solvent in vacuo, the methanolic extract (221.5 g) was suspended in water and partitioned sequentially with CH_2Cl_2 (8.1 g), EtOAc (68.9 g), and *n*-BuOH (93.9 g). The EtOAc soluble fraction was subjected to silica gel column chromatography (CC) ($CHCl_3$: MeOH, 10:1 → 1:1, v/v) to yield five subfractions (E1~E5). E1 (7.5 g) was subjected to countercurrent chromatography (CCC) with solvent composition of *n*-hexane-EtOAC-MeOH-water (2:8:2:8, v/v) to yield another five fractions (E1-1~E1-5). Compound **2** (243.7 mg) was purified from E1-1 by silica gel column chromatography ($CHCl_3$-MeOH-water, 12:5:1, v/v). Fraction E1-2 was subjected to silica gel CC ($CHCl_3$-MeOH-water, 20:5:1, v/v) to give compound **1** (282.9 mg). Fraction E1-3 was chromatographed on silica gel CC ($CHCl_3$-MeOH, 5:1, v/v) to give four subfraction (E1-3-1~E1-3-4), and E1-3-2 was purified by RP-HPLC (YMC-Pack Ph, MeOH-water, 25:75, v/v) to give compound **8** (56.5 mg) and **7** (27.3 mg). Compound **3** and **5** were obtained from E1-3-4 through silica gel CC ($CHCl_3$-water, 5:1, v/v) and RP-HPLC (YMC-Pack ODS-A, MeOH-water, 30:70, v/v). Compound **4** (1.7 mg) and **6** (7.6 mg) were obtained from E2 through silica gel CC ($CHCl_3$-MeOH-water, 15:5:1, v/v) and RP-HPLC (YMC-Pack Ph, MeOH-water, 33:67, v/v). The CH_2Cl_2 fraction was subjected to silica gel CC ($CHCl_3$: MeOH, 50:1 → 5:1; v/v) to provide seven subfractions (M1~M7). M1 was subjected to silica gel CC (*n*-hexane-EtOAc, 10:1, v/v) to yield two subfractions (M1-1, M1-2). Compound **10** (12.0 mg) was obtained from M1-1 through RP-HPLC (YMC-Pack ODS-A, MeOH-Water, 95:5, v/v). Compound **11** (3.8 mg), **12** (10.2 mg) and **13** (2.2 mg) was obtained from M1-2 through RP-HPLC (ODS-A, MeOH). Compound **9** (21.5 mg) was obtained from M2 through silica gel CC (*n*-hexane-EtOAc, 20:1, v/v) and RP-HPLC (YMC-Pack Ph).

(+)-Catechin (1) – α_D^{25} : +21 (*c* 0.2, MeOH); UV (MeOH) λ_{max} 277 nm; ESI-QTOF MS: *m/z* 291.0866 $[M+H]^+$; ^{1}H NMR (CD_3OD, 500 MHz): δ 4.56 (1H, d, *J* = 7.5 Hz, H-2), 3.96 (1H, m, H-3), 2.85 (1H, dd, *J* = 16.3, 5.3 Hz, H-4eq), 2.50 (1H, d, *J* = 16.2, 8.0 Hz, H-4ax), 5.85 (1H, d, *J* = 2.2 Hz, H-6), 5.92 (1H, d, *J* = 2.2 Hz, H-8), 6.84 (1H, d, *J* = 2.0 Hz, H-2'), 6.76 (1H, d, *J* = 8.0 Hz, H-5'), 6.72 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'); ^{13}C NMR (CD_3OD, 125 MHz): δ 83.0 (C-2), 69.0 (C-3), 28.7 (C-4), 157.7 (C-5), 96.4 (C-6), 158.0 (C-7), 95.6 (C-8), 157.1 (C-9), 100.9 (C-10), 132.4 (C-1'), 115.4 (C-2'), 146.4 (C-3'), 146.4 (C-4'), 116.2 (C-5'), 120.2 (C-6')

(+)-Catechin 3-*O*-α-L-rhamnopyranoside (2) – α_D^{25} : −18.2 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 279 nm; ESI-QTOF MS: *m/z* 437.1454 $[M+H]^+$; ^{1}H NMR (CD_3OD, 500 MHz): δ 4.62 (1H, d, *J* = 7.7 Hz, H-2), 3.93 (1H, m, H-3), 2.88 (1H, dd, *J* = 16.2, 8.3 Hz, H-4eq), 2.64 (1H, d, *J* = 16.1, 8.3 Hz, H-4ax), 5.85 (1H, d, *J* = 2.2 Hz, H-6), 5.93 (1H, d, *J* = 2.4 Hz, H-8), 6.84 (1H, d, *J* = 1.9 Hz, H-2'), 6.76 (1H, d, *J* = 8.0 Hz, H-5'), 6.72 (1H, dd, *J* = 8.2, 1.9 Hz, H-6'), 4.29 (1H, d, *J* = 1.2 Hz, H-1''), 1.25 (3H, d, *J* = 6.1 Hz, H-6''); ^{13}C NMR (CD_3OD, 125 MHz): δ 81.3 (C-2), 76.1 (C-3), 28.1 (C-4), 157.7 (C-5), 95.7 (C-6), 158.1 (C-7), 96.6 (C-8), 157.0 (C-9), 102.3 (C-10), 132.1 (C-1'), 115.2 (C-2'), 146.4 (C-3'), 146.5 (C-4'), 116.3 (C-5'), 120.0 (C-6'), 100.8 (C-1''), 72.2 (C-2''), 72.4 (C-3''), 74.1 (C-4''), 70.5 (C-5''), 18.1 (C-6'')

(+)-Catechin 3-*O*-gallate (3) – α_D^{25} : +8.9 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 277 nm; ESI-QTOF MS: *m/z* 443.2216 $[M+H]^+$; ^{1}H NMR (CD_3OD, 500 MHz): δ 5.06 (1H, d, *J* = 6.1 Hz, H-2), 5.37 (1H, m, H-3), 2.82 (1H, dd, *J* = 16.5, 5.1 Hz, H-4eq), 2.71 (1H, d, *J* = 16.5, 6.0 Hz, H-4ax), 5.94 (1H, d, *J* = 2.2 Hz, H-6), 5.96 (1H, d, *J* = 2.4 Hz, H-8), 6.84 (1H, s, H-2'), 6.72 (2H, s, H-5', 6'), 6.96 (2H, s, H-2'', 6''); ^{13}C NMR (CD_3OD, 125 MHz): δ 75.9 (C-2), 71.3 (C-3), 24.5 (C-4), 156.6 (C-5), 96.6 (C-6), 157.7 (C-7), 95.8 (C-8), 158.2 (C-9), 99.8 (C-10), 131.6 (C-1'), 114.6 (C-2'), 146.3 (C-3'), 146.4 (C-4'), 116.4 (C-5'), 119.4 (C-6'), 121.5 (C-1''), 110.3 (C-2'', 6''), 146.5 (C-3'', 5''), 140.0 (C-4''), 167.7 (-COO-)

(−)-Epicatechin (4) – α_D^{25} : −38.5 (*c* 0.9, MeOH); UV (MeOH) λ_{max} 280 nm; ESI-QTOF MS: *m/z* 291.1953 $[M+H]^+$; ^{1}H NMR (CD_3OD, 500 MHz): δ 4.82 (1H, s, H-2), 4.17 (1H, m, H-3), 2.73 (1H, dd, *J* = 16.5, 2.8 Hz, H-4eq), 2.86 (1H, dd, *J* = 16.5, 4.9 Hz, H-4ax), 5.91 (1H, d, *J* = 2.2 Hz, H-6), 5.94 (1H, d, *J* = 2.2 Hz, H-8), 6.97 (1H, d, *J* = 1.8 Hz, H-2'), 6.75 (1H, d, *J* = 8.2 Hz, H-5'), 6.79 (1H, dd, *J* = 8.4, 1.7 Hz, H-6'); ^{13}C NMR (CD_3OD, 125 MHz): δ 80.0 (C-2), 67.7 (C-3), 29.4 (C-4), 157.9 (C-5), 96.0 (C-6), 158.2 (C-7), 96.5 (C-8), 157.5 (C-9), 100.2 (C-10), 132.5 (C-1'), 115.5 (C-2'), 146.1 (C-3'), 146.0 (C-4'), 116.0 (C-5'), 119.5 (C-6')

(−)-Epicatechin 3-*O*-gallate (5) – α_D^{25} : −12.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 277 nm; ESI-QTOF MS: *m/z* 443.2216 $[M+H]^+$; ^{1}H NMR (CD_3OD, 500 MHz): δ 5.03

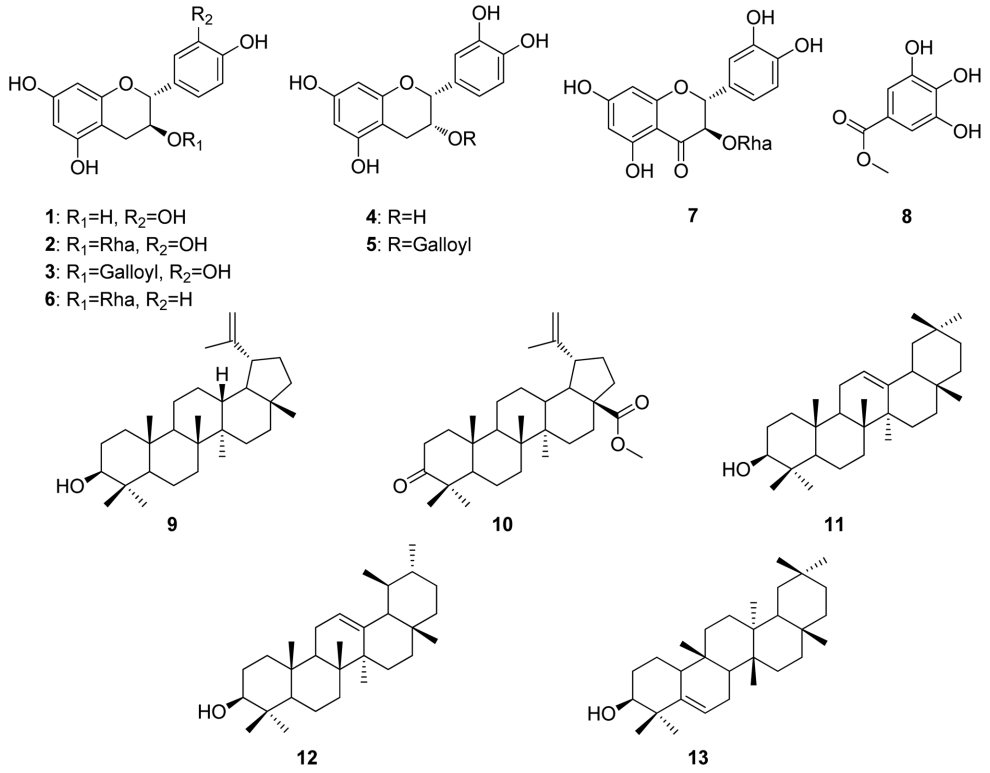

Fig. 1. Chemical structures of compounds **1 - 13** from *D. burmanica* Kurz.

(1H, s, H-2), 5.52 (1H, m, H-3), 2.99 (1H, dd, *J* = 17.3, 4.7 Hz, H-4eq), 2.85 (1H, d, *J* = 17.3, 2.1 Hz, H-4ax), 5.95 (1H, d, *J* = 2.4 Hz, H-6), 5.96 (1H, d, *J* = 2.4 Hz, H-8), 6.93 (1H, d, *J* = 1.9 Hz, H-2'), 6.69 (1H, d, *J* = 8.3 Hz, H-5'), 6.81 (1H, dd, *J* = 8.2, 1.7 Hz, H-6'), 6.95 (2H, s, H-2", 6"); ^{13}C NMR (CD_3OD, 125 MHz): δ 78.8 (C-2), 70.1 (C-3), 27.0 (C-4), 157.4 (C-5), 96.7 (C-6), 158.0 (C-7), 96.0 (C-8), 158.0 (C-9), 99.6 (C-10), 131.6 (C-1'), 115.3 (C-2'), 146.1 (C-3'), 146.1 (C-4'), 116.2 (C-5'), 119.5 (C-6'), 121.6 (C-1"), 110.4 (C-2", 6"), 146.5 (C-3", 5"), 139.9 (C-4"), 167.8 (-COO)

(+)-Afzelechin 3-*O*-α-L-rhamnopyranoside (6) – α_D^{25} : −83.4 (*c* 0.3, MeOH); UV (MeOH) λ_{max} 225, 279 nm; ESI-QTOF MS: *m/z* 421.2333 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 4.66 (1H, d, *J* = 7.9 Hz, H-2), 3.94 (1H, m, H-3), 2.65 (1H, dd, *J* = 16.3, 8.9 Hz, H-4eq), 2.91 (1H, d, *J* = 15.9, 5.7 Hz, H-4ax), 5.85 (1H, d, *J* = 2.2 Hz, H-6), 5.94 (1H, d, *J* = 2.4 Hz, H-8), 7.23 (2H, d, *J* = 8.5 Hz, H-2', 6'), 6.79 (2H, d, *J* = 8.5 Hz, H-3', 5'), 4.25 (1H, br s, H-1"), 1.25 (3H, d, *J* = 6.3 Hz, H-6"); ^{13}C NMR (CD_3OD, 125 MHz): δ 81.3 (C-2), 76.4 (C-3), 28.4 (C-4), 157.1 (C-5), 95.6 (C-6), 157.7 (C-7), 96.6 (C-8), 158.1 (C-9), 100.8 (C-10), 131.4 (C-1'), 129.5 (C-2', 6'), 116.2 (C-3', 5'), 158.7 (C-4'), 102.4 (C-1"), 72.1 (C-2"), 72.4 (C-3"), 74.1 (C-4"), 70.5 (C-5"), 18.1 (C-6")

(+)-2,3-*trans*-Dihydrokaempferol 3-*O*-α-L-rhamnopyranoside (7) – α_D^{25} : −15.3 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 290, 332 nm; ESI-QTOF MS: *m/z* 435.2365 $[M+H]^+$; ^{1}H NMR (CD_3OD, 500 MHz): δ 5.14 (1H, d, *J* = 11.2 Hz, H-2), 4.62 (1H, d, *J* = 11.2 Hz, H-3), 5.92 (1H, d, *J* = 2.2 Hz, H-8), 5.89 (1H, d, *J* = 2.2 Hz, H-6), 7.36 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.84 (1H, d, *J* = 8.8 Hz, H-3', 5'), 4.00 (1H, d, *J* = 1.4 Hz, H-1"), 1.18 (3H, d, *J* = 6.2 Hz, H-6"); ^{13}C NMR (CD_3OD, 125 MHz): δ 84.0 (C-2), 78.8 (C-3), 196.2 (C-4), 165.7 (C-5), 97.6 (C-6), 164.3 (C-7), 96.4 (C-8), 168.9 (C-9), 102.4 (C-10), 128.8 (C-1'), 130.2 (C-2', 6'), 116.6 (C-3', 5'), 159.6 (C-4'), 102.6 (C-1"), 71.9 (C-2"), 72.3 (C-3"), 73.9 (C-4"), 70.7 (C-5"), 18.0 (C-6")

Methyl gallate (8) – α_D^{25} : + 19.3 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} 277 nm; ESI-Q-TOF MS: *m/z* 185.0443 $[M+H]^+$; ^{1}H NMR (CD_3OD, 500 MHz): δ 7.04 (2H, s, H-2, 6), 3.81 (3H, s, -OMe); ^{13}C NMR (CD_3OD, 125 MHz): δ 121.6 (C-1), 110.2 (C-2, 6), 146.6 (C-3, 5), 139.9 (C-4), 169.2 (C=O), 52.4 (-OMe)

Lupeol (9) – α_D^{25} : +31.5 (*c* 0.5, $CHCl_3$); ESI-Q-TOF MS: *m/z* 426.3862 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.16 (1H, dd, *J* = 11.5, 5.2 Hz, H-3), 2.35 (1H, m, H-19), 1.90 (1H, m, H-21), 0.77 (3H, s, H-23), 0.81 (3H, s, H-24), 0.92 (3H, s, H-25), 0.94 (3H, s, H-26), 1.01 (3H, s, H-27), 0.74 (3H, s, H-28), 4.54 (1H, m, H-29a), 4.66 (1H,

d, J = 2.4 Hz, H-29b), 1.66 (3H, s, H-30); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 39.0 (C-1), 27.6 (C-2), 79.2 (C-3), 39.1 (C-4), 55.5 (C-5), 18.6 (C-6), 34.5 (C-7), 41.1 (C-8), 50.7 (C-9), 37.4 (C-10), 21.2 (C-11), 25.4 (C-12), 38.3 (C-13), 43.1 (C-14), 27.7 (C-15), 35.8 (C-16), 43.2 (C-17), 48.6 (C-18), 48.2 (C-19), 151.2 (C-20), 30.1 (C-21), 40.2 (C-22), 28.2 (C-23), 15.6 (C-24), 16.3 (C-25), 16.2 (C-26), 14.8 (C-27), 18.2 (C-28), 109.5 (C-29), 19.5 (C-30)

Methyl lup-20(29)-en-3-on-28-oate (10) – α_D^{25} : +14.3 (c 0.35, $CHCl_3$); LRFAB MS: m/z 469 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.98 (1H, m, H-3), 2.47 (1H, m, H-19), 0.90 (3H, s, H-23), 0.93 (3H, s, H-24), 0.95 (3H, s, H-25), 0.99 (3H, s, H-26), 1.04 (3H, s, H-27), 4.58 (1H, m, H-29a), 4.71 (1H, m, H-29b), 1.66 (3H, s, H-30), 3.65 (3H, s, 28-OC<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 38.4 (C-1), 26.6 (C-2), 218.2 (C-3), 39.7 (C-4), 56.6 (C-5), 19.7 (C-6), 33.7 (C-7), 42.5 (C-8), 55.0 (C-9), 36.9 (C-10), 21.4 (C-11), 25.6 (C-12), 37.0 (C-13), 47.0 (C-14), 29.7 (C-15), 34.2 (C-16), 47.4 (C-17), 49.9 (C-18), 49.4 (C-19), 150.5 (C-20), 32.1 (C-21), 40.6 (C-22), 30.6 (C-23), 15.8 (C-24), 19.4 (C-25), 16.0 (C-26), 14.7 (C-27), 176.7 (C-28), 109.7 (C-29), 21.1 (C-30), 51.3 (28-O<u>C</u>H$_3$)

β-Amyrin (11) – α_D^{25} : +89.2 (c 0.3, $CHCl_3$); HRFAB MS: m/z 426.3855 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.20 (1H, dd, J = 10.2, 4.5 Hz, H-3), 5.16 (1H, t, J = 3.6 Hz, H-12), 0.98 (3H, s, H-23), 0.77 (3H, s, H-24), 0.92 (3H, s, H-25), 0.95 (3H, s, H-26), 1.11 (3H, s, H-27), 0.81 (3H, s, H-28), 0.85 (6H, s, H-29, H-30); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 38.8 (C-1), 27.2 (C-2), 79.3 (C-3), 39.0 (C-4), 55.4 (C-5), 18.6 (C-6), 32.9 (C-7), 41.9 (C-8), 47.9 (C-9), 37.2 (C-10), 23.9 (C-11), 121.9 (C-12), 145.4 (C-13), 40.0 (C-14), 28.3 (C-15), 26.4 (C-16), 32.9 (C-17), 47.5 (C-18), 47.1 (C-19), 32.7 (C-20), 35.0 (C-21), 37.4 (C-22), 28.6 (C-23), 15.7 (C-24), 15.8 (C-25), 17.0 (C-26), 26.2 (C-27), 27.5 (C-28), 33.6 (C-29), 23.8 (C-30)

α-Amyrin (12) – α_D^{25} : +51.6 (c 0.7, $CHCl_3$); HRFAB MS: m/z 426.3856 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.21 (1H, dd, J = 10.4, 5.1 Hz, H-3), 5.10 (1H, t, J = 3.6 Hz, H-12), 0.98 (3H, s, H-23), 0.76 (3H, s, H-24), 0.93 (3H, s, H-25), 0.99 (3H, s, H-26), 1.05 (3H, s, H-27), 0.78 (3H, s, H-28), 0.78 (3H, s, H-29), 0.89 (3H, s, H-30); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 39.0 (C-1), 27.5 (C-2), 79.3 (C-3), 39.0 (C-4), 55.4 (C-5), 18.6 (C-6), 33.2 (C-7), 40.2 (C-8), 47.9 (C-9), 37.1 (C-10), 23.6 (C-11), 124.6 (C-12), 139.8 (C-13), 42.3 (C-14), 26.8 (C-15), 28.4 (C-16), 34.0 (C-17), 59.3 (C-18), 39.9 (C-19), 39.8 (C-20), 31.5 (C-21), 41.7 (C-22), 28.3 (C-23), 15.9 (C-24), 15.8 (C-25), 17.1 (C-26), 23.5 (C-27), 29.0 (C-28), 17.7 (C-29), 21.6 (C-30)

3β-Hydroxy-D:B-friedo-olean-5-ene (13) – α_D^{25} : +73.1 (c 0.5, $CHCl_3$); HRFAB MS: m/z 426.3853 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.50 (1H, m, H-3), 5.66 (1H, m, H-12), 1.08 (3H, s, H-23), 1.17 (3H, s, H-24), 0.88 (3H, s, H-25), 1.13 (3H, s, H-26), 1.04 (3H, s, H-27), 1.19 (3H, s, H-28), 1.02 (3H, s, H-29), 0.98 (3H, s, H-30); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 18.4 (C-1), 28.0 (C-2), 76.6 (C-3), 41.1 (C-4), 141.8 (C-5), 122.3 (C-6), 23.9 (C-7), 47.7 (C-8), 35.1 (C-9), 49.9 (C-10), 34.8 (C-11), 30.6 (C-12), 38.1 (C-13), 39.5 (C-14), 32.3 (C-15), 36.2 (C-16), 30.3 (C-17), 43.3 (C-18), 35.3 (C-19), 28.5 (C-20), 33.3 (C-21), 39.1 (C-22), 29.2 (C-23), 25.7 (C-24), 16.4 (C-25), 18.6 (C-26), 19.8 (C-27), 32.2 (C-28), 32.6 (C-29), 34.7 (C-30)

Results and Discussion

The methanolic extract of *D. burmanica* was partitioned successively with CH_2Cl_2, EtOAc, and *n*-BuOH. The EtOAc and CH_2Cl_2 soluble fraction were subjected diverse column chromatography to give six flavan 3-ol derivatives, a dihydroflavonol glycoside, a phenolic compound and five triterpenes. Spectroscopic data of isolates were compared with those of literature values to determin (+)-catechin (**1**),[8] (+)-catechin 3-O-α-L-rhamnopyranoside (**2**),[8] (+)-catechin 3-*O*-gallate (**3**),[9] (–)-epicatechin (**4**),[8] (–)-epicatechin 3-*O*-gallate (**5**),[10] (+)-afzelechin 3-*O*-α-L-rhamnopyranoside (**6**),[11] (+)-2,3-*trans*-dihydrokaempferol 3-*O*-α-L-rhamnopyranoside (**7**),[12] methyl gallate (**8**),[13] lupeol (**9**),[14-15] methyl lup-20(29)-en-3-on-28-oate (**10**),[16] β-amyrin (**11**),[17] α-amyrin (**12**),[18] 3β-hydroxy-D:B-friedo-olean-5-ene (**13**).[19] To the best of our knowledge, all isolates were isolated from *D. burmanica* for the first time.

Compound **1** was obtained as brownish amorphous powder and the molecular formula, $C_{15}H_{14}O_6$, was established by the positive ion mode ESI-QTOF MS (m/z 291.0866 $[M+H]^+$). The ^{1}H NMR spectrum showed the signals for 1,3,4-substituted aromatic protons [δ_H 6.84 (1H, d, J = 2.0, H-2'), 6.76 (1H, d, J = 8.0, H-5'), 6.72 (1H, dd, J = 8.0, 2.0, H-6')], two meta coupling protons [δ_H 5.92 (1H, d, J = 2.2, H-8), 5.85 (1H, d, J = 2.2, H-6)], a methene group [δ_H 2.85 (1H, dd, J = 16.3, 5.3, H-4$_{eq}$), 2.50 (1H, dd, J = 16.2, 8.0, H-4ax)] and two methine protons [δ_H 4.56 (1H, d, J = 7.5, H-2), 3.96 (1H, m, H-3)]. The 2,3-*trans* configuration was confirmed from the large J value of H-2 (J = 7.5 Hz). The ^{13}C NMR detected 15 carbon signals including twelve aromatic carbons, one oxygenated aliphatic carbon and two aliphatic carbons. Thus, compound **1** was identified as (+)-catechin based on the spectroscopic evidences and comparison of literature values.

Compound **2** was isolated as dark brownish amorphous powder and the molecular formula was deduced to be $C_{21}H_{24}O_{10}$ from its ESI-QTOF MS ion peak at *m/z* 437.1454 $[M+H]^+$. The ^1H and ^{13}C NMR spectra showed similar patterns with those of compound **1** except for the sugar moiety. The sugar moiety was elucidated to be α-rhamnopyranoside from the anomeric proton signal at δ_H 4.29 (1H, d, J = 1.2, H-1") and six alipathic carbon signals at δ_C 100.8 (C-1"), 72.2 (C-2"), 72.4 (C-3"), 74.1 (C-4"), 70.5 (C-5"), 18.1 (C-6"). On the basis of spectroscopic data with comparison of literature values, the structure of compound **2** was determined to be (+)-catechin 3-*O*-α-L-rhamnopyranoside.

Compound **3** was obtained as dark purple amorphous powder. Its molecular formula was identified as $C_{22}H_{18}O_{10}$ from the positive ion mode ESI-QTOFMS (*m/z* 443.2216 $[M+H]^+$). The ^1H and ^{13}C NMR spectra was similar to compound **1** (see Experimental) except for the presence of a gallic acid moiety at δ_H 6.96 (2H, s, H-2", 6") and δ_C 121.5 (C-1"), 110.3 (C-2", 6"), 146.5 (C-3", 5"), 140.0 (C-4"), 167.7 (-COO). The HMBC correlation of δ_H 5.37 (H-3) to δ_C 167.7 indicated that gallic acid was linked to C-3 of (+)-cathecin. Thus, compound **3** was confirmed to be (+)-catechin 3-*O*-gallate

Compound **4** was isolated as brown amorphous powder and its molecular formula was determined to be $C_{15}H_{14}O_6$ by the positive ion mode ESI-QTOFMS (*m/z* 291.1953 $[M+H]^+$). The ^1H NMR indicated flavan 3-ol moiety including 1,3,4-substitued aromatic proton signals at δ_H 6.97 (1H, d, J = 1.8, H-2'), 6.75 (1H, d, J = 8.2, H-5'), 6.79 (1H, dd, J = 8.4, 1.7, H-6'), two *meta*-coupled aromatic proton signals at δ_H 5.91 (1H, d, J = 2.2, H-6), 5.94 (1H, d, J = 2.2, H-8) and a methene group at δ_H 2.73 (1H, dd, J = 16.5, 2.8, H-4_{eq}), 2.86 (1H, dd, J = 16.5, 4.9, H-4ax) and two methine protons at δ_H 4.82 (1H, s, H-2), 4.17 (1H, m, H-3). The 2,3-*cis* configuration was confirmed from the singlet signal of H-2. Based on the spectroscopic evidences and comparison with literature values, compound **4** was determined to be (-)-epicatechin.

Compound **5** was obtained as dark purple amorphous powder showing its molecular as $C_{22}H_{18}O_{10}$ from ESI-QTOFMS (*m/z* 443.2216 $[M+H]^+$). The ^1H and ^{13}C NMR spectra detected (−)-epicathecin skeleton and additionally observed the presence of a gallic acid moiety (see Experimental). The HMBC correation of δ_H 5.52 (H-3) to δ_C 167.8 revealed that gallic acid was linked to C-3 of (−)-epicatechin. Thus, compound **5** was elucidated as (−)-epicatechin 3-*O*-gallate.

The molecular formula of compound **6** was determined to be $C_{21}H_{24}O_9$ by at *m/z* 421.2333 $[M+H]^+$ ion peak of ESI-QTOFMS spectrum. The ^1H and ^{13}C NMR spectra displayed similar patterns with those of compound **2** except for the signals of 1,4-substituted aromatic proton signals [δ_H 7.23 (2H, d, J = 8.5, H-2', 6'), 6.79 (2H, d, J = 8.5, H-3', 5'); δ_C 131.4 (C-1'), 129.5 (C-2', 6'), 116.2 (C-3', 5'), 158.7 (C-4')]. From these spectroscopic data and through comparison with literature values, compound **6** was assigned to be (+)-afzelechin 3-*O*-α-L-rhamnopyranoside.

Compound **7** was isolated as yellowish amorphous powder and the molecular formula was determined to be $C_{21}H_{22}O_{10}$ by ESI-QTOFMS ion peak at *m/z* 435.2365 $[M+H]^+$. The ^1H NMR spectrum displayed dihydrokaempferol skeleton including 1,4-substituted aromatic proton signals [δ_H 7.36 (2H, d, J = 8.8, H-2', 6'), 6.84 (2H, d, J = 8.8, H-3', 5'), two *meta*-coupled aromatic protons at δ_H 5.92 (1H, d, J = 2.2, H-8), 5.89 (1H, d, J = 2.2, H-6) and two aliphatic proton signals at δ_H 5.14 (1H, d, J = 11.2, H-2), 4.62 (1H, d, J = 11.2, H-3). The coupling constant (J = 11.2 Hz) between H-2 and H-3 indicated that 2,3-*trans* configuration. Furthermore, α-rhamnopyranoside was detected at δ_H 4.00 (1H, d, J = 1.4, H-1") and δ_C 102.6 (C-1"), 71.9 (C-2"), 72.3 (C-3"), 73.9 (C-4"), 70.7 (C-5"), 18.0 (C-6"). The anomeric proton signal of rhamnose (δ_H 4.00) was correlated to δ_C 78.8 indicating sugar moiety was attached to C-3 position of dihydrokaempferol moiety. Based on the spectroscopic evidences and comparison with literature values, compound **7** was determined to be (+)-2,3-*trans*-dihydrokaempferol 3-*O*-α-L-rhamnopyranoside.

Compound **8** was isolated as dark purple amorphous powder and its molecular formula was established as $C_8H_8O_5$ based on the positive mode of ESI-QTOF MS (*m/z* 185.0443 $[M+H]^+$). The ^1H NMR spectrum showed a singlet at δ_H 7.04 (2H, s, H-2, 6), assignable to symmetrical protons at H-2 and H-6, and a methoxy signal at δ_H 3.81 (3H, s, -OCH_3). The ^{13}C NMR spectrum also showed six aromatic carbon signals at δ_C 121.6 (C-1), 110.2 (C-2, 6), 146.6 (C-3, 5), 139.9 (C-4), a methoxy carbon signal at δ_c 52.4 and a carbonyl carbon signal at δc 169.2. Based on above data with the comparison to the literature values, compound **8** was identified as methyl gallate.

Compound **9** was obtained as white amorphous powder, and displayed molecular ion peaks at *m/z* 426.3862 $[M+H]^+$ on the positive ion mode HRFAB-MS showing molecular formula of $C_{30}H_{50}O$. The ^1H NMR spectrum exhibited seven methyl groups at δ_H 0.77 (3H, s, H-23), 0.81 (3H, s, H-24), 0.92 (3H, s, H-25), 0.94 (3H, s, H-26), 1.01 (3H, s, H-27), 0.74 (3H, s, H-28) and 1.66 (3H, s, H-

30), two germinal-coupled vinyl protons at 4.54 (1H, m, H-29a), 4.66 (1H, d, J = 2.4 Hz, H-29b), an oxygenated methine proton signals at δ_H 3.16 (1H, dd, J = 11.5, 5.2 Hz, H-3). The ^{13}C NMR detected thirty carbon signals showing characteristic two vinyl carbons (δ_C 151.2, C-20; 109.5, C-29), an oxygenated carbon (δ_C 79.2, C-3). Therefore, compound **9** was determined to be lupeol based on the spectroscopic evidences and literature values.

The molecular formula of compound **10** was determined to be $C_{31}H_{48}O_3$ by LRFAB-MS. The ^{1}H NMR spectrum exhibited six methyl groups at δ_H 0.90 (3H, s, H-23), 0.93 (3H, s, H-24), 0.95 (3H, s, H-25), 0.99 (3H, s, H-26), 1.04 (3H, s, H-27) and 1.66 (3H, s, H-30), a methoxy group at δ_H 3.65 (3H, s, 28-OC$\underline{H}_3$) and two germinal-coupled vinyl protons at 4.58 (1H, m, H-29a), 4.71 (1H, m). In addition, the ^{13}C NMR observed thirty carbon signals including two vinyl carbons (δ_C 150.5, C-20; 109.7, C-29), two carbonyl carbons at δ_C 218.2 (C-3) and 176.7 (C-28). From the above spectroscopic data and comparing them with published values, compound **10** was elucidated to be methyl lup-20(29)-en-3-on-28-oate.

Compound **11** was isolated as white amorphous powder, and its molecular formula was determined to be $C_{30}H_{50}O$ by HR-FABMS spectroscopy. The ^{1}H NMR showed eight methyl signals at δ_H 0.98 (3H, s, H-23), 0.77 (3H, s, H-24), 0.92 (3H, s, H-25), 0.95 (3H, s, H-26), 1.11 (3H, s, H-27), 0.81 (3H, s, H-28), 0.85 (6H, s, H-29, H-30), an oxygenated methine signal at δ_H 3.20 (1H, dd, J = 10.2, 4.5 Hz, H-3) and a olefinic proton at δ_H 5.16 (1H, t, J = 3.6 Hz, H-12). The ^{13}C NMR revealed thirty carbons containing two olefinic carbons at δ_C 121.9 (C-12), 145.4 (C-13) and an oxygenated carbon at δ_C 76.6 (C-3). On the basis of above spectroscopic data and comparing them with published values, compound **11** was identified to be β-amyrin.

The MS, ^{1}H and ^{13}C NMR data were close to compound **12** except for the two olefinic carbon signals at δ_C 124.6 (C-12), 139.8 (C-13) which was characteristic in ursan skeleton. Thus, compound **12** was determined to be a-amyrin by comparing spectroscopic data with those of published values.

The positive ion mode HRFAB-MS showed pseudomolecular ion peak at *m/z* 426.3853 $[M+H]^+$ for compound **13** giving molecular formula of $C_{30}H_{50}O$. The ^{1}H NMR showed eight methyl signals at δ_H 1.08 (3H, s, H-23), 1.17 (3H, s, H-24), 0.88 (3H, s, H-25), 1.13 (3H, s, H-26), 1.04 (3H, s, H-27), 1.19 (3H, s, H-28), 1.02 (3H, s, H-29), 0.98 (3H, s, H-30), an oxygenated methine signal at δ_H): δ 3.50 (1H, m, H-3) and a vinyl proton at δ_H 5.66 (1H, m, H-12). In ^{13}C NMR, thirty carbon resonances were observed including an oxygenated methine carbon at δ_C 76.6 (C-3) and two olefinic carbons at δ_C 141.8 (C-5), 122.3 (C-6). According to MS, MS, ^{1}H and ^{13}C NMR of compound **13** and comparing them with literature values, compound **13** was elucidated as 3β-hydroxy-D:B-friedo-olean-5-ene.

Acknowledgements

This work was supported by a National Institute of Biological Resources (NIBR) grant funded by the Korean government (ME).

References

(1) Tangmouo, J. G.; Ho, R.; Lannang, A. M.; Komguem, J.; Lontsi, A.T.; Lontsi, D.; Hostettmann, K. *Phytochem. Lett*. **2009**, *2*, 192-195.
(2) Singh, S.; Joshi, H. *Asian J. Res. Pharm. Sci*. **2011**, 1, 55-58.
(3) Kim, H. H.; Kim, D. S.; Kim, S. W.; Lim, S. H.; Kim, D. K.; Shin, T. Y.; Kim, S. H. *Int. J. Mol. Med*. **2013**, *32*, 945-951.
(4) Lee, J. H.; Lee, Y. B.; Seo, W. D.; Kang, S. T.; Lim, J.; W.; Cho, K. M. *Prev. Nutr. Food Sci.* **2012**, *17*, 141-151.
(5) Xue, Y. L.; Miyakawa, T.; Hayashi, Y.; Okamoto, K.; Hu, F.; Mitani, N.; Furihata, K.; Sawano, Y.; Tanokura, M. *J. Agric. Food Chem*. **2011**, *59*, 6011-6017.
(6) Duan, J.; Chen, V. L.; Dong, Q.; Ding, K.; Fang, J. *Int. J. Biol. Macromol*. **2010**, *46*, 465-470.
(7) Mori-Yasumoto, K.; Izumoto, R.; Fuchino, H.; Ooi, T.; Agatsuma, Y.; Kusumi, T.; Satake, M.; Sekita, S. *Bioorg. Med. Chem*. **2012**, *20*, 5215-5219.
(8) Kim, J. E.; Kim, S. S.; Hyun, C. G.; Lee, N. H. *Int. J. Pharmacol*. **2012**, *8*, 410-415.
(9) Saito, A.; Emoto, M.; Tanaka, A.; Doi, Y.; Shoji, K.; Mizushina, Y.; Ikawa, H.; Yoshida, H.; Matsuura, N.; Nakajima, N. *Tetrahedron.* **2004**, *60*, 12043-12049.
(10) Braca, A.; Politi, M.; Sanogo, R.; Sanou, H.; Morelli, I.; Pizza, C.; De Tommasi, N. *J. Agric. Food Chem*. **2003**, *51*, 6689-6695.
(11) Drewes, S. E.; Taylor, C. W.; Cunningham, A. B. *Phytochemistry* **1992**, *31*, 1073-1075.
(12) Fujiwara, M.; Yagi, N.; Miyazawa, M. *Nat. Prod. Res*. **2011**, *25*, 1540-1548.
(13) Mahajan, A.; Pai, N. *J. Chem. Pharm. Res*. **2010**, *2*, 97-103.
(14) da Silva, D. A.; Alves, V. G.; Franco, D. M.; Ribeiro, L. C.; de Souza, M. C.; Kato, L.; de Carvalho, J. E.; Kohn, L. K.; de Oliveira, C. M.; da Silva, C. C. *Nat. Prod. Res*. **2012**, *26*, 364-369.
(15) Jin, Q. L.; Ko, H. J.; Chang, Y. S.; Woo E. R. *Nat. Prod. Sci.* **2013**, *19*, 269-274.
(16) Xu, J.; Li, Z.; Luo, J.; Yang, F.; Liu, T.; Liu, M.; Qiu, W. W.; Tang, J. *J. Med. Chem*. **2012**, *55*, 3122-3134.
(17) Jin, Q.; Jin, H. G.; Kim, A. R.; Woo, E. R. *Helv. Chim. Acta*. **2012**, *95*, 1445-1460.
(18) Wang, Y.; Xiang, L.; Chen, M.; Zhang, Z.; He, X. *J. Mol. Catal. B. Enzym*. **2012**, *83*, 51-56.
(19) Gonzalez, A. G.; Ferro, E. E.; Ravelo, A. G. *Phytochemistry* **1987**, *26*, 2785-2788.

Received October 2, 2014
Revised January 26, 2015
Accepted January 26, 2015