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Chemical Constituents of *Nelumbo nucifera* Seeds

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Abstract – The phytochemical study for the extract of *Nelumbo nucifera* (Nymphaceae) seeds has led to the isolation of ten compounds including five simple phenolic compounds, two indole derivatives, a flavonoid glycoside, two abscisic acid derivatives. The interpretation of 1D and 2D NMR and ESI-Q-TOF-MS spectroscopic data revealed the chemical structures of isolates to be *p*-hydroxybenzoic acid (**1**), protocatechuic acid (**2**), (*E*)-*p*-coumaric acid (**3**), (*E*)-ferulic acid (**4**), (*E*)-sinapate-4-*O*-*β*-D-glucopyranoside (**5**), tryptophan (**6**), 3-indoleacetic acid (**7**), isoschaftoside (**8**), dihydrophaseic acid (**9**), dihydrophaseic acid 3'-*O*-*β*-D-glucopyranoside (**10**). To the best of our knowledge, **1** – **5** and **7** were identified for the first time from *N. nucifera* seeds, and the presence of dihydrophaseic acid (**9**) and its glucoside (**10**) were demonstrated secondly in this plant.
Keywords – *Nelumbo nucifera,* Seeds, Nymphaceae, Phytochemical study

Introduction

The seeds of *Nelumbo nucifera* Gaertner (Nymphaceae) have traditionally been used as antidepressant, tonic, antidiarrhea, antipyretic, diuretic and sedative in Korea.[1] The phytochemical studies have revealed that *N. nucufera* contained diverse constituents including alkaloids, flavonoids, sesquiterpenoids, essential oils, and the main studies have been focused on benzyl isoquinoline and aporphine alkaloids such as coclaurine, nuciferine and so on.[2] In the course of searching phytochemicals from the seeds of *N. nucifera*, ten compounds were isolated including five simple phenolic compounds (**1** – **5**), two indole derivatives (**6** – **7**), a flavonoid glycoside (**8**), two abscisic acid derivatives (**9** – **10**). Interestingly, the simple phenolic compounds (**1** – **5**) and an indole derivative (**7**) were identified for the first time from *N. nucifera* in the current study.

Experimental

General experimental procedure – The HPCCC instrument was a MIDI HPCCC (Dynamic Extractions, Berkshire, UK) possessing two set of a semi-preparative coil with total volume of 984 mL. The MIDI HPCCC was combined with a 2487 dual λ absorbance detector (Waters, MA, USA), a 1525 binary HPLC pump (Waters, MA, USA), a FC 204 fraction collector (Gilson, WI, USA) and a CCA-1111 circulatory temperature regulator (Eyela, Tokyo, Japan) to maintain the internal temperature at 30 °C. A Gilson HPLC (Gilson, Middleton, WI, USA) composed of binary pumps, a UV/Vis-155 detector and a GX-271 liquid handler was utilized to isolate compounds. Organic solvents for HPCCC and column chromatography were purchased from Daejung-Chemical and Metals Co. Ltd. (Kyunggi-Do, Korea) and deionized water was produced by Millipore Milli-Q water purification system (Millipore, USA). NMR spectra were recorded on a Bruker Ascend™ 500 spectrometer (Bruker, Germany), and a 6460 Q-TOF mass spectrometer (Agilent Technologies, CA, USA) was used to determine molecular formula.

Reagents and plant materials – Seeds of *Nelumbo nucifera* (500 g) was obtained from Human herb (Deagu, Korea), and the voucher specimen (CU-NeNu-16-07-11) was deposited at the herbarium of the College of Pharmacy, The Catholic University of Korea.

Extraction and isolation – Seeds of *N. nucifera* (500 g) were extracted with 25% aqueous ethanol (2 L × 90 min × 3 times) to yield 25% aqueous ethanol extract (36 g). The crude extract was absorbed to silica gel (120 g) and packed to glass column followed by elution of $CHCl_3$-MeOH mixture (3:1, v/v, 4 L) to give fraction A (5.2 g). Fraction A was subjected to preparative MIDI HPCCC [CH_2Cl_2/MeOH/Water (9:6:5, v/v/v), flow rate: 21.0 mL/min, normal-phased chromatography, detection at 280 nm] to give four sub-fractions (B1 – B4) and a stationary

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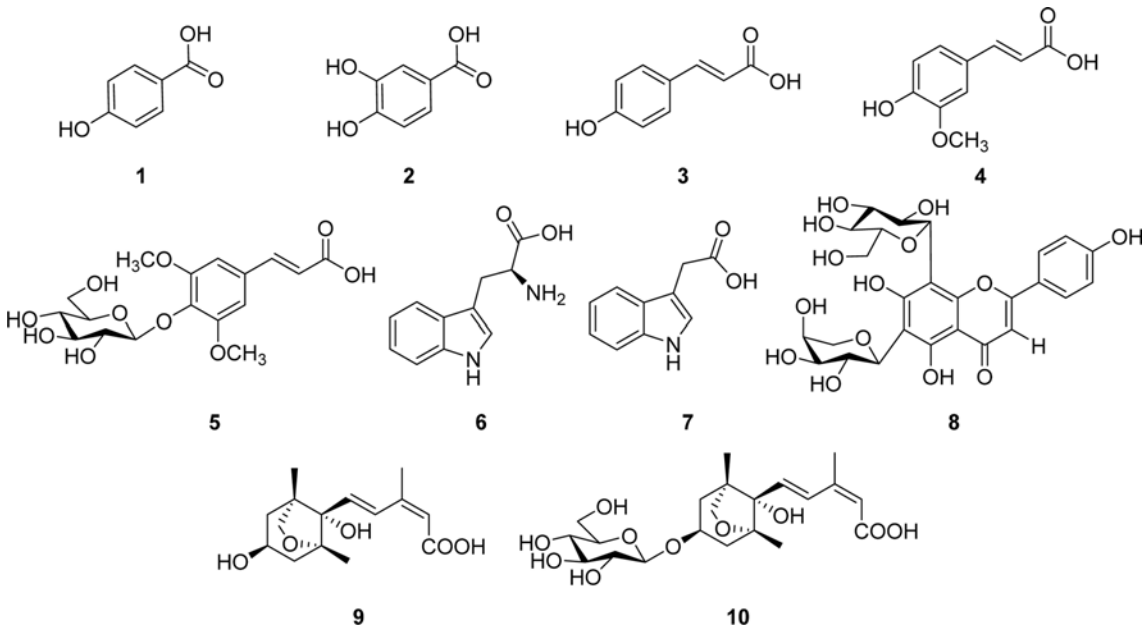

Fig. 1. The chemical structures of **1** – **10** from *Nelumbo nucifera* seeds.

phase fraction (BS). The B1 – B4 were subjected to preparative HPLC, respectively, using a RP-column (Luna C18, 250 × 21.2 mm I.D., Phenomenex) with a gradient elution of MeCN-Water mixture (10:90 → 70:30, v/v) to yield compounds **4** (1.2 mg), **3** (1.6 mg), **6** (3.1 mg) and **7** (1.4 mg), respectively. The sub-fraction BS was chromatographed on RP-HPLC using a gradient elution of MeCN-water mixture (10:90 → 70:30, v/v) to give **2** (2.0 mg), **10** (5.5 mg), **5** (2.1 mg), **8** (2.7 mg), **1** (4.8 mg) and **9** (3.3 mg).

Compound 1 (*p*-Hydroxybenzoic acid) – $C_7H_6O_3$; ESI-Q-TOF-MS: 137.0239 $[M-H]^-$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.87 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.80 (2H, d, *J* = 8.8 Hz, H-3', 5'); ^{13}C-NMR (125 MHz, CD_3OD): δ 170.09 (COOH), 163.38 (C-4), 133.00 (C-2, 6), 122.70 (C-1), 116.02 (C-3, 5).

Compound 2 (Protocatechuic acid) – $C_7H_6O_4$; ESI-Q-TOF-MS: 153.0191 $[M-H]^-$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.41 (1H, dd, *J* = 7.9, 2.2 Hz, H-6), 7.30 (1H, br s, H-2), 6.78 (1H, d, *J* = 7.9 Hz, H-5); ^{13}C-NMR (125 MHz, CD_3OD): δ 170.24 (COOH), 151.55 (C-4), 146.08 (C-3), 123.86 (C-6), 123.09 (C-1), 117.69 (C-5), 115.73 (C-2).

Compound 3 [(*E*)-*p*-Coumaric acid] - $C_9H_8O_3$; ESI-Q-TOF-MS: 163.0397 $[M-H]^-$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.58 (1H, d, *J* = 15.9 Hz, H-7), 7.44 (2H, d, *J* = 8.6 Hz, H-2, 6), 6.79 (2H, d, *J* = 8.6 Hz, H-3, 5), 6.28 (1H, d, *J* = 15.9 Hz, H-8); ^{13}C-NMR (125 MHz, CD_3OD): δ 171.2 (C-9), 161.16 (C-4), 146.49 (C-7), 131.06 (C-2, 6), 127.26 (C-1), 116.79 (C-3, 5), 115.82 (C-8).

Compound 4 [(*E*)-Ferulic acid] – $C_{10}H_{10}O_4$; ESI-Q-TOF-MS: 193.0499 $[M-H]^-$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.57 (1H, d, *J* = 15.9 Hz, H-7), 7.17 (1H, d, *J* = 1.9 Hz, H-2), 7.05 (1H, dd, *J* = 8.2, 1.9 Hz, H-6), 6.80 (1H, d, *J* = 8.2 Hz, H-5), 6.31 (1H, d, *J* = 15.9 Hz, H-8), 3.89 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, CD_3OD): δ 150.40 (C-3), 149.36 (C-4), 146.48 (C-7), 127.91 (C-1), 123.90 (C-6), 116.43 (C-5), 114.55 (C-8), 111.57 (C-2), 56.40 (OCH_3).

Compound 5 [(*E*)-Sinapate 4-*O*-*β*-D-glucopyranoside] – $C_{17}H_{22}O_{10}$; ESI-Q-TOF-MS: 409.1112 $[M+Na]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.60 (1H, d, *J* = 15.9 Hz, H-7), 6.94 (2H, s, H-2, 6), 6.45 (1H, d, *J* = 15.9 Hz, H-8), 4.98 (1H, d, *J* = 7.6 Hz, H-1'), 3.88 (6H, s, OCH_3-3, 5), 3.77 (1H, dd, *J* = 12.0, 2.3 Hz, H-6'a), 3.65 (1H, dd, *J* = 12.0, 5.3 Hz, H-6'b), 3.48 (1H, m, H-2'), 3.40 (2H, m, H-3', 4'), 3.40 (2H, m, H-3', 4'), 3.20 (1H, m, H-5'); ^{13}C-NMR (125 MHz, CD_3OD): δ 170.50 (C-9), 154.58 (C-3), 154.58 (C-5), 146.12 (C-7), 137.95 (C-4), 132.27 (C-1), 119.13 (C-8), 107.13 (C-2), 107.13 (C-6), 104.81 (C-1'), 78.42 (C-5'), 77.84 (C-3'), 75.7 (C-2'), 71.31 (C-4'), 62.52 (C-6'), 57.06 (OCH_3-3, 5).

Compound 6 (L-Tryptophan) – $C_{11}H_{12}N_2O_4$; ESI-Q-TOF-MS: 205.0973 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO): δ 11.06 (1H, brs, NH-1), 7.57 (1H, d, *J* = 8.0 Hz, H-4), 7.37 (1H, d, *J* = 8.0 Hz, H-7), 7.22 (1H, brs, H-2), 7.09 (1H, t, *J* = 7.4 Hz, H-6), 7.00 (1H, t, *J* = 7.4 Hz, H-5), 4.06 (1H, m, H-9), 3.28 (1H, dd, *J* = 15.2, 5.1 Hz, H-8a), 3.21 (1H, dd, *J* = 15.2, 6.8 Hz, H-8b); ^{13}C-NMR (125 MHz, DMSO): δ 170.94 (C-10), 136.30 (C-7a), 127.06 (C-3a), 124.85 (C-2), 121.15 (C-6), 118.58 (C-5), 118.25

(C-4), 111.50 (C-7), 107.04 (C-3), 52.92 (C-9), 26.29 (C-8).

Compound 7 (3-Indoleacetic acid) – $C_{10}H_9NO_2$; ESI-Q-TOF-MS: 176.0719 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO): δ 7.54 (1H, dt, *J* = 8.1, 1.0 Hz, H-4), 7.34 (1H, dt, *J* = 8.1, 1.0 Hz, H-7), 7.18 (1H, s, H-2), 7.10 (1H, ddd, *J* = 8.1, 7.0, 1.0 Hz, H-6), 7.01 (1H, ddd, *J* = 8.1, 7.0, 1.0 Hz, H-5), 3.65 (2H, d, *J* = 0.8 Hz, H-8); ^{13}C-NMR (125 MHz, DMSO): δ 178.05 (C-9), 138.15 (C-7a), 128.52 (C-3), 124.91 (C-6), 122.58 (C-5), 119.95 (C-4), 119.32 (C-2), 112.32 (C-7), 109.49 (C-3a), 33.52 (C-8).

Compound 8 (Isoschaftoside) – $C_{26}H_{28}O_{14}$; ESI-Q-TOF-MS: 587.1375 $[M+Na]^+$; ^{1}H-NMR (500 MHz, DMSO): δ 8.03 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.89 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.82 (1H, s, H-3), 4.75 (1H, d, *J* = 10.4 Hz, H-1'''), 4.71 (1H, d, *J* = 9.5 Hz, H-1''), 3.88 (1H, m, H-2'''), 3.83 (1H, m, H-2''), 3.82 (1H, m, H-5''a), 3.80 (1H, m, H-4''), 3.74 (1H, m, H-6'''a), 3.64 (1H, m, H-5''b), 3.51 (1H, m, H-6'''b), 3.46 (1H, m, H-3''), 3.39 (1H, m, H-4'''), 3.28 (1H, m, H-3'''), 3.23 (1H, m, H-5'''); ^{13}C-NMR (125 MHz, DMSO): δ 182.31 (C-4), 164.10 (C-7), 161.20 (C-2), 160.89 (C-4'), 158.17 (C-5), 155.06 (C-9), 129.04 (C-2', 6'), 121.52 (C-1'), 115.80 (C-3', 5'), 108.08 (C-6), 105.10 (C-8), 103.71 (C-3), 102.59 (C-10), 81.90 (C-5'''), 78.85 (C-3'''), 74.14 (C-1''), 73.79 (C-3''), 73.24 (C-1'''), 70.86 (C-2'''), 70.51 (C-4'''), 70.09 (C-5''), 69.58 (C-2''), 68.39 (C-4''), 61.17 (C-6''').

Compound 9 (Dihydrophaseic acid) – $C_9H_8O_3$; ESI-Q-TOF-MS: 281.1390 $[M-H]^-$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.98 (1H, d, *J* = 15.9 Hz, H-4), 6.52 (1H, d, *J* = 15.9 Hz, H-5), 5.76 (1H, s, H-2), 4.10 (1H, m, H-3'), 3.80 (1H, dd, *J* = 7.4, 2.0 Hz, H-7'$_{ax}$), 3.71 (1H, d, *J* = 7.4 Hz, H-7'$_{eq}$), 2.08 (3H, d, *J* = 1.1 Hz, H-6), 2.03 (1H, ddd, *J* = 13.8, 7.0, 2.0 Hz, H-4'$_{ax}$), 1.85 (1H, ddd, *J* = 13.5, 7.0, 2.0 Hz, H-2'$_{ax}$), 1.72 (1H, dd, *J* = 13.8, 10.3 Hz, H-4'$_{eq}$), 1.65 (1H, ddd, *J* = 13.5, 10.8, 2.3 Hz, H-2'$_{eq}$), 1.14 (3H, s, H-9'), 0.92 (3H, s, H-10'); ^{13}C-NMR (126 MHz, CD_3OD): δ 169.58 (C-1), 151.53 (C-3), 135.22 (C-5), 131.79 (C-4), 119.21 (C-2), 87.81 (C-5'), 83.24 (C-8'), 77.27 (C-7'), 66.01 (C-3'), 49.85 (C-1'), 45.99 (C-4'), 44.52 (C-2'), 21.25 (C-6), 19.65 (C-9'), 16.36 (C-10').

Compound 10 (Dihydrophaseic acid 3'-*O*-*β*-D-glucopyranoside) – $C_{21}H_{32}O_{10}$; ESI-Q-TOF-MS: 467.1890 $[M+Na]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.98 (1H, d, *J* = 15.9 Hz, H-4), 6.52 (1H, d, *J* = 15.9 Hz, H-5), 5.75 (1H, br s, H-2), 4.35 (1H, d, *J* = 7.8 Hz, H-1''), 4.25 (1H, tt, *J* = 10.5, 7.0 Hz, H-3'), 3.86 (1H, dd, *J* = 11.8, 1.4 Hz, H-6''a), 3.79 (1H, dd, *J* = 7.4, 1.9 Hz, H-7'$_{exo}$), 3.75 (1H, d, *J* = 7.4 Hz, H-7'$_{endo}$), 3.65 (1H, dd, *J* = 11.8, 5.3 Hz, H-6''b), 3.34 (1H, m, H-3''), 3.27 (1H, m, H-5''), 3.26 (1H, m, H-4''), 3.12 (1H, dd, *J* = 9.1, 7.8 Hz, H-2''), 2.18 (1H, ddd, *J* = 13.7, 7.0, 1.9 Hz, H-4'$_{ax}$), 2.07 (3H, d, *J* = 1.1 Hz, H-6), 1.97 (1H, ddd, *J* = 13.7, 7.0, 1.9 Hz, H-2'$_{ax}$), 1.81 (1H, m, H-4'$_{eq}$), 1.78 (1H, m, H-2'$_{eq}$), 1.16 (3H, s, CH_3-9'), 0.93 (3H, s, CH_3-10'); ^{13}C-NMR (125 MHz, CD_3OD): δ 169.58 (C-1), 119.2 (C-2), 151.58 (C-3), 135.16 (C-4), 131.87 (C-5), 103.04 (C-1''), 87.63 (C-5'), 83.2 (C-8'), 78.07 (C-3''), 77.97 (C-5''), 77.14 (C-7'), 75.11 (C-2''), 73.86 (C-3'), 71.65 (C-4''), 62.75 (C-6''), 49.45 (C-1'), 42.84 (C-2'), 42.79 (C-4'), 21.28 (C-6), 19.70 (CH_3-9'), 16.33 (CH_3-10').

Result and Discussion

Phytochemical study of *N. nucifera* seeds extract led to ten known compounds including five simple phenolic compounds (**1** – **5**), two indole derivatives (**6** – **7**), a flavonoid glycoside (**8**) and two abscisic acid derivatives (**9** – **10**). The interpretation of ^{1}H and ^{13}C NMR and ESI-Q-TOF-MS spectroscopic data revealed the chemical structures of isolates to be *p*-hydroxybenzoic acid (**1**), protocatechuic acid (**2**), (*E*)-*p*-coumaric acid (**3**), (*E*)-ferulic acid (**4**), (*E*)-sinapate-4-*O*-*β*-D-glucopyranoside (**5**), tryptophan (**6**), 3-indoleacetic acid (**7**). Isoshaftoside (**8**), dihydrophaseic acid (**9**), dihydrophaseic acid 3'-*O*-*β*-D-glucopyranoside (**10**).

The molecular formula of **1** determined to be $C_7H_6O_3$ from the ESI-Q-TOF-MS spectrum, and the ^{1}H NMR of **1** showed 1,4-disubstituted benzene ring at δ_H 7.87 (2H, d, *J* = 8.8 Hz, H-2, 6) and 6.80 (2H, d, *J* = 8.8 Hz, H-3, 5), and ^{13}C NMR showed a carbonyl resonance at δ_C 170.09 as well as four signals assignable to 1,4-disubstituted benzene ring. Based on the spectroscopic data, compound **1** was elucidated to be *p*-hydroxybenzoic acid.[3]

Compound **2** was isolated amorphous colorless powder and its molecular formula was deduced to be $C_7H_6O_4$ by pseudomolecular ion at *m/z* 153.0191 $[M-H]^-$ from ESI-Q-TOF-MS spectrum. The ^{1}H NMR of **2** showed an 1,3,4-trisubstituted benzene structure at δ_H 7.41 (1H, dd, *J* = 7.9, 2.2 Hz, H-6), 7.30 (1H, brs, H-2), 6.78 (1H, d, *J* = 7.9 Hz, H-5) and ^{13}C NMR of **2** displayed a carbonyl signal at δ_C 170.24 along with six sp^2 carbons. From the spectroscopic evidences and literature data, compound **2** was determined to be protocatechuic acid.[4]

The ESI-Q-TOF-MS spectrum of **3** revealed the molecular formula of **3** to be $C_9H_8O_3$ from the pseudomolecular ion peak at *m/z* 163.0397 $[M-H]^-$. The ^{1}H NMR of **1** showed an 1,4-disubstituted benzene ring at δ_H 7.44 (2H, d, *J* = 8.6 Hz, H-2, 6), 6.79 (2H, d, *J* = 8.6 Hz, H-3, 5), and two *trans*-coupled olefinic protons at δ_H δ 7.58 (1H, d, *J* = 15.9 Hz, H-7), 6.28 (1H, d, *J* = 15.9 Hz, H-8). The

^{13}C NMR showed a carbonyl resonance at δ_C 171.2 and as well as four signals assignable to 1,4-disubstituted benzene ring and two sp^2 carbon resonances. Based on the spectroscopic data and comparison of published literature data, compound **3** was identified to be *p*-coumaric acid.[5]

The ^{1}H NMR of **4** was showed resonances for an 1,3,4-trisubsituted benzene ring at δ_H 7.17 (1H, d, J = 1.9 Hz, H-2), 7.05 (1H, dd, J = 8.2, 1.9 Hz, H-6), 6.80 (1H, d, J = 8.2 Hz, H-5), two trans-coupled olefinic protons at δ_H 7.57 (1H, d, J = 15.9 Hz, H-7), 6.31 (1H, d, J = 15.9 Hz, H-8) and a methoxy resonance at δ_H 3.89 (3H, s, 4-OC$\underline{H_3}$), which was typical for (*E*)-ferulic acid. The ^{13}C NMR and ESI-Q-TOF-MS spectra of **4** provided the further spectroscopic evidences for (*E*)-ferulic acid, which were confirmed by literature data.[6]

The molecular formula of **5** was determined to be $C_{17}H_{22}O_{10}$ from the psuedomolecular ion peak at *m/z* 409.1112 $[M+Na]^+$. The ^{1}H NMR of **5** exhibited a singlet at δ_H 6.94, two trans-coupled olefinic protons at δ_H 7.60 (1H, d, J = 15.9 Hz, H-7), 6.45 (1H, d, J = 15.9 Hz, H-8), two methoxy proton resonances at δ_H 3.88 (6H, s, OCH_3-3, 5), which was characteristic for sinapic acid resonances. In addition, an anomeric proton signal was found at δ_H 4.98 (1H, d, J = 7.6 Hz, H-1') derived from sugar moiety. The ^{13}C NMR of **5** showed structures for sinapic acid and a glucose moiety and these were good agreement with previously reported values of (*E*)-sinapate 4-*O*-*β*-D-glucopyranoside.[7]

The ^{1}H NMR of **6** showed an mono-substituted indole skeleton at δ_H 7.57 (1H, d, J = 8.0 Hz, H-4), 7.37 (1H, d, J = 8.0 Hz, H-7), 7.22 (1H, brs, H-2), 7.09 (1H, t, J = 7.4 Hz, H-6), 7.00 (1H, t, J = 7.4 Hz, H-5), and a methylene resonance at δ_H 3.28 (1H, dd, J = 15.2, 5.1 Hz, H-8a) and 3.21 (1H, dd, J = 15.2, 6.8 Hz, H-8b), and a methine proton signal at δ_H 4.06 (1H, m, H-9). The 2D NMR spectra including HSQC and HBMC as well as ^{13}C NMR revealed that compound **6** possessed a 2-aminopropionic acid which was linked to C-3 position of indole moiety. Comparing spectroscopic data of **6** with literature values, it was determined to be L-tryptophane.[8]

The spectroscopic data of **7** was similar to those of **6** except a 2-aminopropionic acid moiety was replaced by an acetic acid moiety. Therefore, compound **7** was identified to be 3-indoleacetic acid. The chemical structure of indoleacetic acid were further confirmed by comparing **7** with authentic compound.

Compound **8** isolated as a yellowish amorphous powder and its molecular formula was confirmed to be $C_{26}H_{28}O_{14}$ from the pseudomolecular ion peak at *m/z* 587.1375 $[M+Na]^+$ from ESI-Q-TOF-MS. The ^{1}H NMR of **8** showed resonances for 1,4-disubstituted benzene ring at δ_H 8.03 (2H, d, J = 8.8 Hz, H-2', 6'), 6.89 (2H, d, J = 8.8 Hz, H-3', 5') and an sp^2 proton signal at δ_H 6.82 (1H, s, H-3). Furthermore, two doublets derived from two sugar moieties were observed at 4.75 (1H, d, J = 10.4 Hz, H-1'''), 4.71 (1H, d, J = 9.5 Hz, H-1''). From the ^{1}H NMR spectrum, it was deduced that the structure of **8** was an apigenin 6-*C* and 8-*C* diglycoside because the large coupling constants than those of *O*-glycoside form as well as there were no typical H-6 and H-8 resonances. The ^{13}C NMR, 2D NMR experiment (HSQC, HMBC) and comparing them with published literature values, compound **8** was elucidated to be isoschaftoside (apigenin 6-*C*-arabinosyl-8-*C*-glucoside).[9]

The molecular formula of **9** was determined to be $C_9H_8O_3$ by ESI-Q-TOF-MS (*m/z* 281.1390 $[M-H]^-$) and the ^{1}H NMR showed a characteristic skeleton corresponding to 3-methyl-penta-2,4-dienoic moiety at δ_H 2.08 (3H, d, J = 1.1 Hz, H-6), three olefinic protons at δ_H 7.98 (1H, d, J = 15.9 Hz, H-4), 6.52 (1H, d, J = 15.9 Hz, H-5), 5.76 (1H, s, H-2). The 1D and 2D NMR revealed additional functional groups including two CH_3 groups, two -CH_2- moieties, an oxymethylene group, a secondary oxymethine, two oxygenated quaternary carbons and a quaternary carbon. Based on the spectroscopic evidences of **9** and comparing them with those of published values, the structure of **9** was confirmed to be dihydrophaseic acid.[10]

The spectroscopic data of **10** were very similar to those of compound **9**, which indicated that **10** was a dihydrophaseic acid derivative. The difference between **9** and **10** was what an additional sugar moiety was linked to dihydrophaseic acid moiety. The mass value (*m/z* 467.1890 $[M+Na]^+$) was +162 amu higher than that of dihydrophaseic acid and ^{1}H NMR observed an anomeric proton resonance at δ_H 4.33 (1H, d, J = 7.8 Hz, H-1'). The identity of sugar moiety was determined to be glucose according to the six carbon resonances at δ_C 103.04 (C-1''), 78.07 (C-3''), 77.97 (C-5''), 75.11 (C-2''), 71.65 (C-4''), 62.75 (C-6''). Therefore, compound **10** was identified to be dihydrophaseic acid 3'-*O*-*β*-D-glucopyranoside.[11]

To the best of our knowledge, **1** – **5** and **7** was identified firstly from *N. nucifera*, and the presence of dihydrophaseic acid (**9**) and its glucoside (**10**) were determined secondly in this plant.

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References

(1) The Compilation Committee of Pharmacognosy Textbook. Pharmacognosy; Dong-Myeung press: Korea, **2015**, pp 358-360.
(2) Sharma, B. R.; Gautam, L. N.; Adhikari, D.; Karki, R. *Phytother. Res.* **2017**, *31*, 3-26.
(3) Dhakal, R. C.; Rajbhandari, M.; Kalauni, S. K.; Awale, S.; Gewali, M. B. *J. Nepal Chem. Soc.* **2008/2009**, *23*, 89-92.
(4) Flamini, G.; Antognoli, E.; Morelli, I. *Phytochemistry* **2001**, *57*, 559-564.
(5) Nguyen, D. H.; Zhao, B. T.; Le, D. D.; Kim, K. Y.; Kim, Y. H.; Yoon, Y. H.; Ko, J. Y.; Woo, K. S.; Woo, M. H. *Nat. Prod. Sci.* **2016**, *22*, 140-145.
(6) Woo, K. W.; Lee, K. R. *Nat. Prod. Sci.* **2013**, *19*, 221-226.
(7) Wolfram, K.; Schmidt, J.; Wray, V.; Milkowski, C.; Schliemann, W.; Strack, D. *Phytochemistry* **2010**, *71*, 1076-1084.
(8) Kim, C. S.; Kim, K. H.; Lee, K. R. *Nat. Prod. Sci.* **2014**, *20*, 86-90.
(9) Xie, C.; Veitch, N. C.; Houghton, P. J.; Simmonds, M. S. *Chem. Pharm. Bull*. **2003**, *51*, 1204-1207.
(10) Seo, J. H.; Choi, Y. H.; Yoo, M. Y.; Hong, K. S.; Lee, B. H.; Yon, G. H.; Kim, Y. S.; Kim, Y. K.; Ryu, S. Y. *Kor. J. Pharmacogn.* **2006**, *37*, 290-297.
(11) Youn, U. J.; Lee, J.; Nam, J. W.; Lee, Y. J.; Seo, E. K. *Bull. Korean Chem. Soc*. **2011**, *32*, 4083-4085.

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