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. nucifera* 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 CHCl₃-MeOH mixture (3:1, v/v, 4 L) to give fraction A (5.2 g). Fraction A was subjected to preparative MIDI HPCCC [CH₃Cl₂/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...
The B1–B4 were subjected to preparative HPLC, respectively, using a RP-column (Luna C18, 250 x 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) – C7H6O3; ESI-Q-TOF-MS: 137.0239 [M-H]−; 1H-NMR (500 MHz, CD3OD): δ 7.87 (2H, d, J = 8.8 Hz, H-2’, 6’), 6.80 (2H, d, J = 8.8 Hz, H-3’, 5’); 13C-NMR (125 MHz, CD3OD): 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) – C7H6O4; ESI-Q-TOF-MS: 153.0191 [M-H]−; 1H-NMR (500 MHz, CD3OD): 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); 13C-NMR (125 MHz, CD3OD): δ 170.24 (C-OOH), 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] – C9H8O3; ESI-Q-TOF-MS: 163.0397 [M-H]−; 1H-NMR (500 MHz, CD3OD): 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); 13C-NMR (125 MHz, CD3OD): 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] – C10H10O4; ESI-Q-TOF-MS: 193.0499 [M-H]−; 1H-NMR (500 MHz, CD3OD): 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, OCH3); 13C-NMR (125 MHz, CD3OD): 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 (OCH3).

**Compound 5** [(E)-Sinapate 4-O-β-D-glucopyranoside] – C17H22O10; ESI-Q-TOF-MS: 409.1112 [M+Na]++; 1H-NMR (500 MHz, CD3OD): 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, OCH3-3, 5), 3.77 (1H, dd, J = 12.0, 7.6 Hz, H-6’, 4’), 3.40 (2H, m, H-3’, 4’), 3.20 (1H, m, H-5’); 13C-NMR (125 MHz, CD3OD): 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), 117.71 (C-2), 117.69 (C-5), 115.73 (C-2).

**Compound 6** (L-Tryptophan) – C11H12N2O4; ESI-Q-TOF-MS: 205.0973 [M+H]++; 1H-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); 13C-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-8).
\[ \delta = 15.9 \text{ Hz}, H-4 \] 
\[ 6.52 (1H, d, J = 10.4 \text{ Hz}, H-5) \]
\[ 5.75 (1H, s, H-2) \]
\[ 4.10 (1H, m, H-3') \]
\[ 2.08 (3H, d, J = 11.1 \text{ Hz}, H-6) \]
\[ 2.03 (1H, ddd, J = 13.8, 7.0, 2.0 \text{ Hz}, H-4'\alpha) \]
\[ 1.85 (1H, ddd, J = 13.5, 7.0, 2.0 \text{ Hz}, H-2'\alpha) \]
\[ 1.72 (1H, dd, J = 13.8, 10.3 \text{ Hz}, H-4''\alpha) \]
\[ 1.65 (1H, ddd, J = 13.5, 10.8, 2.4 \text{ Hz}, H-3''\alpha) \]
\[ 1.14 (3H, s, H-9') \]
\[ 0.92 (3H, s, H-10') \]
\[ 13^C \text{ NMR (126 MHz, CD}_2\text{OD};} \]
\[ \delta = 169.58 \text{ (C-1)}, 119.2 \text{ (C-2)}, 151.58 \text{ (C-3)}, 135.16 \text{ (C-4)}, 131.87 \text{ (C-5)}, 103.04 \text{ (C-1'), 87.63 \text{ (C-5')}, 83.2 \text{ (C-8'), 78.07 \text{ (C-3')}, 77.97 \text{ (C-5')}, 77.14 \text{ (C-7'), 75.11 \text{ (C-2')}, 73.86 \text{ (C-7'), 71.65 \text{ (C-4')}, 62.75 \text{ (C-6'), 49.45 \text{ (C-1')}, 42.84 \text{ (C-2'), 42.79 \text{ (C-4'), 21.28 \text{ (C-6'), 19.70 \text{ (CH}_2\text{-9')}, 16.33 \text{ (CH}_3\text{-10')}.} \]

### 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), isoschaftoside (8), dihydrophaseic acid (9), dihydrophaseic acid 3′-O-β-d-glucopyranoside (10).

The molecular formula of 1 determined to be \(C_{31}H_{52}O_{14}\) from the ESI-Q-TOF-MS spectrum, and the \(^1\)H NMR of 1 showed 1,4-disubstituted benzene ring at \(\delta_{ll} 7.87\) (2H, d, \(J = 8.8 \text{ Hz}, H-2, 6\) and 6.80 (2H, d, \(J = 8.8 \text{ Hz}, H-1, 3\), 5), and \(^13\)C NMR showed a carbonyl resonance at \(\delta_{c} 170.09\) 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.

Compound 2 was isolated amorphous colorless powder and its molecular formula was deduced to be \(C_{27}H_{36}O_{14}\) 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 \(\delta_{ll} 7.41\) (1H, d, \(J = 7.9, 2.2 \text{ Hz}, H-6), 7.30 (1H, brs, H-2'), 6.78 (1H, d, \(J = 7.9 \text{ Hz}, H-5)\) and \(^13\)C NMR of 2 displayed a carbonyl signal at \(\delta_{c} 170.24\) along with six \(sp^2\) carbons. From the spectroscopic evidences and literature data, compound 2 was determined to be protocatechuic acid.

The ESI-Q-TOF-MS spectrum of 3 revealed the molecular formula of 3 to be \(C_{24}H_{30}O_{12}\) from the pseudomolecular ion peak at \(m/z\) 163.0397 [M-H]−. The \(^1\)H NMR of 3 showed an 1,4-disubstituted benzene ring at \(\delta_{ll} 7.44\) (2H, d, \(J = 8.6 \text{ Hz}, H-2, 6), 6.79 (2H, d, \(J = 8.6 \text{ Hz}, H-3, 5\), and two \(trans\)-coupled olefinic protons at \(\delta_{ll} 7.58\) (1H, d, \(J = 15.9 \text{ Hz}, H-7), 6.28 (1H, d, \(J = 15.9 \text{ Hz}, H-8). The
\(^{13}\)C NMR showed a carbonyl resonance at \(\delta_c 171.2\) 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 \(p\)-cumaric acid.\(^5\)

The \(^1\)H NMR of 4 was showed resonances for an 1,3,4-trisubstituted benzene ring at \(\delta_H 7.17\) (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 \(\delta_H 7.57\) (1H, d, \(J = 15.9\) Hz, H-7), 6.31 (1H, d, \(J = 15.9\) Hz, H-8) and a methoxy resonance at \(\delta_H 3.89\) (3H, s, 4-OCH\(_3\)), 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 \(C_{19}H_{20}O_{12}\) from the pseudomolecular peak at \(m/z 409.1112\) [M+Na]. The \(^1\)H NMR of 5 exhibited a singlet at \(\delta_H 6.94\), two trans-coupled olefinic protons at \(\delta_H 7.60\) (1H, d, \(J = 15.9\) Hz, H-7), 6.45 (1H, d, \(J = 15.9\) Hz, H-8), two methoxy proton resonances at \(\delta_H 3.88\) (6H, s, OCH\(_3\)-3, 5), which was characteristic for sinapic acid resonances. In addition, an anomeric proton signal was found at \(\delta_H 4.98\) (1H, d, \(J = 7.6\) Hz, H-1') derived from sugar moiety. The \(^13\)C NMR showed structures for sinapic acid and a glucose moiety and these were good agreement with previously reported values of \((E)\)-sinapate 4-\(\beta\)-D-glucopyranoside.\(^7\)

The \(^1\)H NMR of 6 showed an mono-substituted indole skeleton at \(\delta_H 7.57\) (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 \(\delta_H 3.28\) (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 \(\delta_H 4.06\) (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-amino-3-propionic 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-amino-3-propionic 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 \(C_{28}H_{32}O_{14}\) 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 \(\delta_H 8.03\) (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 \(\delta_H 6.82\) (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-3''). 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 \(C_{22}H_{22}O_{10}\) 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 \(\delta_H 2.08\) (3H, d, \(J = 1.1\) Hz, H-6), three olefinic protons at \(\delta_H 7.98\) (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 anomic proton resonance at \(\delta_H 4.33\) (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 \(\delta_c 103.04\) (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'-\(O\)-\(\beta\)-D-glucopyranoside.\(^11\)

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

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