## Supplementary Materials: Quantitative Determination of Alkaloids in Lotus Flower (Flower Buds of Nelumbo nucifera) and Their Melanogenesis Inhibitory Activity

Toshio Morikawa, Niichiro Kitagawa, Genzoh Tanabe, Kiyofumi Ninomiya, Shuhei Okugawa, Chiaki Motai, Iyori Kamei, Masayuki Yoshikawa, I-Jung Lee ${ }^{5}$ and Osamu Muraoka


Figure S1. Monitoring for chemical transformation of $\mathbf{2}$ into its ammonium carbamate salt (2") and to methyl carbamate (2a) by HPLC analysis.

Table S1. Contents of alkaloids ( $\mathbf{( 1 0} \mathbf{- 1 0}$ ) in the methanol extracts from the leaf, fruit, and embryo parts of $N$. nucifera.

| Sample <br> No. | Part | Loss of Drying <br> a (\%) | Extraction Yield ${ }^{\text {b }}$ (\%) | Contents (mg/g in Dry Material) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | 1 | 2 |  | 3 | 4 | 5 |
| NN-9 | Leaf | 9.8 | 19.0 | 0.24 | 0.05 |  | 0.38 | 0.14 | n.d. ${ }^{\text {c }}$ |
| NN-10 | Fruit | 10.9 | 10.9 | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ |  | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ |
| NN-11 | Fruit | 9.3 | 10.0 | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ |  | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ |
| NN-12 | Embryo | 8.4 | 32.8 | 0.02 | n.d. ${ }^{\text {c }}$ |  | n.d. c | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ |
|  |  |  | Contents ( $\mathrm{mg} / \mathrm{g}$ in dry material) |  |  |  |  |  | Total ${ }^{\text {a }}$ |
|  |  |  | 6 | 7 | 8 | 9 | 10 |  |  |
|  |  |  | 0.16 | 0.25 | 0.16 | n.d. ${ }^{\text {c }}$ | c 0.07 |  | 1.20 |
|  |  |  | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ | c n.d. ${ }^{\text {c }}$ |  | n.d. ${ }^{\text {c }}$ |
|  |  |  | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ | n.d. ${ }^{\text {c }}$ | c n.d. ${ }^{\text {c }}$ |  | n.d. ${ }^{\text {c }}$ |
|  |  |  | 0.25 | n.d. ${ }^{\text {c }}$ | 0.20 | n.d. ${ }^{\text {c }}$ | c 0.17 |  | 0.64 |

${ }^{\text {a }}$ Each powdered sample was dried at $105^{\circ} \mathrm{C}$ for 8 h ; ${ }^{\text {b }}$ each powdered sample was extracted two times with methanol under reflux for 120 min and ${ }^{\text {c less than }}$ the quantitation limit.

Table S2. Contents of alkaloids (1-10) in the methanol extracts from the leaf (NN-9), fruit (NN-10 and 11), and embryo (NN-12) of $N$. nucifera.

| Sample <br> No. | Inhibition (\%) |  |  |  |  | $\begin{gathered} \mathrm{IC}_{50} \\ (\mu \mathrm{~g} / \mathrm{mL}) \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $0 \mu \mathrm{~g} / \mathrm{mL}$ | $3 \mu \mathrm{~g} / \mathrm{mL}$ | $10 \mu \mathrm{~g} / \mathrm{mL}$ | $30 \mu \mathrm{~g} / \mathrm{mL}$ | $100 \mu \mathrm{~g} / \mathrm{mL}$ |  |
| NN-9 | $\begin{gathered} 0.0 \pm 4.9 \\ (100.0 \pm 3.4) \end{gathered}$ | $\begin{gathered} 15.0 \pm 5.7 \\ \left(66.2 \pm 2.6^{*}\right) \end{gathered}$ | $\begin{gathered} 7.6 \pm 6.3 \\ \left(58.8 \pm 1.9^{*}\right) \end{gathered}$ | $\begin{gathered} 15.6 \pm 8.0 \\ \left(51.7 \pm 3.1^{\#}\right) \end{gathered}$ | $\left(39.5 \pm 1.7^{*}\right)$ | >100 |
| NN-10 | $\begin{gathered} 0.0 \pm 3.6 \\ (100.0 \pm 4.9) \end{gathered}$ | $\begin{gathered} 22.7 \pm 9.2 \\ (112.2 \pm 3.2) \end{gathered}$ | $\begin{gathered} 8.4 \pm 4.8 \\ (103.9 \pm 4.9) \end{gathered}$ | $\begin{gathered} 17.5 \pm 5.6 \\ (119.7 \pm 3.8) \end{gathered}$ | $\begin{gathered} 11.6 \pm 1.5 \\ (126.8 \pm 4.9) \end{gathered}$ | >100 |
| NN-11 | $\begin{gathered} 0.0 \pm 7.6 \\ (100.0 \pm 2.1) \end{gathered}$ | $\begin{gathered} 11.9 \pm 6.8 \\ (99.3 \pm 2.8) \end{gathered}$ | $\begin{gathered} 2.8 \pm 4.1 \\ (92.2 \pm 5.9) \end{gathered}$ | $\begin{gathered} 22.8 \pm 4.9 \\ (102.8 \pm 2.2) \end{gathered}$ | $\begin{aligned} & 42.8 \pm 4.8^{* *} \\ & (113.2 \pm 6.9) \end{aligned}$ | >100 |
| NN-12 | $\begin{gathered} 0.0 \pm 10.9 \\ (100.0 \pm 5.9) \end{gathered}$ | $\begin{gathered} 30.8 \pm 15.4 \\ (107.8 \pm 5.0) \end{gathered}$ | $\begin{gathered} 88.0 \pm 5.0 * * \\ (95.5 \pm 5.8) \\ \hline \end{gathered}$ | $\begin{gathered} 92.9 \pm 2.8^{* *} \\ (84.8 \pm 4.5) \\ \hline \end{gathered}$ | $\begin{aligned} & 71.6 \pm 1.9^{* *} \\ & \left(56.2 \pm 2.7^{* *}\right) \end{aligned}$ | 4.5 |

Each value represents the mean $\pm$ S.E.M. $(n=4)$; asterisks denote significant differences from the control group, ${ }^{* *} p<0.01$.; "cytotoxic effects were observed, and values in parentheses indicate cell viability (\%) in MTT assay.

