Seeds of *Centranthus ruber* and *Valeriana officinalis* Contain Conjugated Linolenic Acids with Reported Antitumor Effects

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Abstract: Conjugated linolenic acids (CLNs) are naturally occurring fatty acids that are believed to have anticancer properties. In this study, we examined various plant seeds from herbs to discover seed oils containing CLNs. The ultraviolet spectra of total lipids from these seeds were measured. An absorption maximum around 270 nm was observed in seed oils belonging to the Valerianaceae family (*Centranthus ruber* and *Valeriana officinalis*). When the fatty acid compositions of these seed oils were measured, CLNs were detected. By silica column chromatography, neutral lipids (NLs), glycolipids, and phospholipids were eluted from seed oils of *C. ruber* and *V. officinalis*. Then, fatty acid compositions of these fractions were measured. This revealed that most of the CLNs in these seed oils existed in the NL fraction. When the NL fractions of these seed oils were reacted with lipase, CLNs showed good sensitivity to lipase hydrolysis. This suggested that the CLNs in the seed oils of *C. ruber* and *V. officinalis* existed predominantly at the sn-1,3 position of triacylglycerol and less at the sn-2 position. These results suggested that the CLNs from the seed oils of *C. ruber* and *V. officinalis* could easily be taken up by cancer cells as free fatty acids and had good potential as antitumor substances.

Key words: *Centranthus ruber*, conjugated linolenic acid, GC-MS, silica column chromatography, *Valeriana officinalis*
As described above, CLNs have potential applications in cancer prevention. However, only a few plants are known to contain CLNs in their seed oils (e.g., *Momordica charantia*). Therefore, discovering and researching new plants that contain CLNs in their seeds will be beneficial for cancer prevention. Although the therapeutic effects of volatile components of herbs have been actively studied, there is little information regarding the other components of herbs. In this study, we examined various medicinal and/or edible herb seeds in order to discover seed oils that contain CLNs. We reveal that several species of the Valerianaceae family contain CLNs. Furthermore, we also investigated the properties of plant seed oils that were discovered as sources of CLNs. We examined the ratio of CLNs in the various lipid fractions of the seed oils. In addition, we investigated the sensitivity of esterified CLNs of the seed oils to lipase hydrolysis. CLNs, as free fatty acids, show cytotoxic effects in cancer cells. Esterified fatty acids in the blood are hydrolyzed to free fatty acids by lipase before being ingested by the cell. Therefore, it is necessary to clarify the lipase sensitivity of the esterified CLNs in the seed oils.

Accordingly, in this study, we investigated the lipase sensitivities and bioavailabilities of esterified CLNs in seed oils of herbs in the Valerianaceae family.

### 2.2 Lipid extraction

Total lipids from plant seeds were extracted by the Bligh & Dyer method. Briefly, seeds were homogenized with nine volumes (w/v) of ice-cold saline. Next, a methanol and chloroform mixture (2:1, v/v) was added and mixed by vortexing for 2 min. The extract was mixed with chloroform and saline by vortexing for 2 min, to obtain a mixture of chloroform/methanol/water (1:1:0.9, v/v/v). The mixture was centrifuged for 5 min at 5°C and 3,000 rpm. The lower layer was transferred to another tube. Total lipids were dried under a stream of nitrogen gas and dissolved in a mixture of chloroform and methanol (2:1, v/v) at a final concentration of 10 mg/mL. The product was stored at -80°C until use.

### 2.3 Ultraviolet (UV) spectra of seed oils

To determine the characteristic structures of seed oils obtained from herb plants, UV spectra of total lipids from seed oils were measured. The total lipids of various seeds (*Alchemilla vulgaris*, *Arnica unalaschcensis*, *Centranthus ruber*, *Cynara scolymus*, *Echinops ritro*, *Hypericum perforatum*, *Hyssopus officinalis*, *Meliolus officinalis*, *Persicaria tinctoria*, *Salvia officinalis*, *Sanguisorba minor*, *Satureja hortensis*, *Symphymum officinale*, *Valeriana officinalis*, and *Verbascum thapsus*) were dissolved in ethanol (10 µg/mL), and UV spectra were measured using a spectrophotometer (V-530, JASCO, Tokyo, Japan).

### 2.4 Gas chromatography

A known amount of heptadecanoic acid (C17:0) was added to the total lipids of seeds as an internal standard, and the mixture was methylated with trimethylsilyldiazomethane for 30 min and sodium methoxide/methanol (diluted to ca. 1 mol/L by hexane) for 10 min at room temperature to prepare fatty acid methyl esters. The product was subjected to gas chromatography (GC-4000, GL Science, Tokyo, Japan) using a flame ionization detector and an InertCap Pure-WAX fused silica capillary column (30 m × 0.25 mm, 0.25-µm film thickness, GL science). Helium was used as the carrier gas. The injector and detector temperatures were 250°C, and the column oven temperature was increased by 30°C/min from 50°C to 200°C and then held constant for 36 min. Then, the temperature was increased by 20°C/min from 200°C to 220°C. Then, the tem-
temperature was increased by 1°C/min from 220°C to 240°C and then held constant for 3 min.

2.5 Structure determination of CLNs

To determine the positional isomerism of CLNs, the structures of the fatty acids were further analyzed by analyzing 4,4-dimethyloxazoline (DMOX) derivatives by gas chromatography-mass spectrometry (GC-MS)\(^\text{19}\). DMOX derivatives were prepared after freeing the fatty acids from total lipids. The free fatty acids were mixed with 2-amino-2-methyl-1-propanol in a test tube, which was then purged with nitrogen gas, screw-capped, and heated at 170°C. After 30 min, the tube was then redisolved in an adequate amount of \(n\)-hexane. GC-El/MS was performed using a GCMS-QP2010 Ultra (Shimadzu, Kyoto, Japan) equipped with an InertCap Pure-WAX fused silica capillary column (30 m × 0.25 mm, 0.25-µm film thickness; GL Science). Helium was used as the carrier gas. The injector and detector temperatures were both 250°C. The temperature of the ion source was 200°C.

2.6 Silica column chromatography

Neutral lipids (NL), glycolipids (GL) and phospholipids (PL) were eluted from total lipids by silica column chromatography, as described previously\(^\text{20}\). In brief, 150 mg of total lipids from seeds were poured into a silica column with 5 mL of chloroform. Then, the NL, GL and PL fractions were eluted using 175 mL of chloroform, 700 mL of acetone and 175 mL of methanol, respectively. These fractions were evaporated and weighed to calculate the content of each fraction. Moreover, the fatty acid compositions of these fractions were measured by gas chromatography.

Fig. 2 UV spectra of total lipids in various herb seeds.
2.7 Lipase hydrolysis of esterified CLNs

The NL fraction (10 mg) was added to a mixture of 0.8 mL of 1M Tris-HCl (pH 8.0), 0.2 mL of 22% CaCl₂, and 0.1 mL of 1% sodium cholate. Then, this mixture was emulsified by sonication. 0.1 mL of enzyme liquid (100 mg of pancreatic in 1 mL of 1M Tris-HCl (pH 8.0)) was added and shaken (20 min, 40°C). The reaction was stopped by adding 1 mL of 6N HCl and 1 mL of ethanol. Then, 2 mL of n-hexane was added and mixed by vortexing for 2 min. The mixture was centrifuged for 5 min at room temperature and 1,000 rpm. The upper layer was dried under a stream of nitrogen gas and dissolved in ethanol. The hydrolyzed fatty acids were methylated with trimethylsilyldiazomethane for 30 min at room temperature, and the products were subjected to gas chromatography.¹⁷

2.8 Statistical analysis

Results are expressed as means ± standard deviations (SDs). In experiments in which only two groups were compared, the data were analyzed by Student’s t-tests. If multiple groups were run, the data were analyzed by one-way analysis of variance with Turkey’s post-hoc tests. Differences with P values of less than 0.05 were considered significant.

3 Results

3.1 UV spectra of the seed oils

UV spectra of the seed oils are shown in Fig. 2. Three absorption peaks around 270 nm were observed for seed oils.

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Fig. 2 GC chromatograms of fatty acid methyl esters in the seeds of Centranthus ruber, Valeriana officinalis, and Momordica charantia. α-ESA: α-eleostearic acid, CPA: catalpic acid, β-ESA: β-eleostearic acid.
oils from *Centranthus ruber* and *Valeriana officinalis*. In *Centranthus ruber* seed oil, the absorption maximum was at 268 nm, followed by peaks at 259 and 279 nm. In *Valeriana officinalis* seed oil, the absorption maximum was at 270 nm, followed by peaks at 261 and 281 nm. However, no characteristic absorbance was observed in seed oils of the other herbs. This result suggested that *Centranthus ruber* and *Valeriana officinalis* contained conjugated trienoic fatty acids in their seed oils. In addition, these findings implied that the major conjugated fatty acid isomers in these seed oils differed from each other.

### 3.2 Fatty acid composition of total lipids from seed oils

The fatty acid compositions of the total lipids obtained from *Centranthus ruber* and *Valeriana officinalis*, in which we observed characteristic absorbance at 270 nm, were measured by GC (Table 1). The fatty acid composition of seed oil obtained from *Momordica charantia*, which has already been reported to contain CLNs, was also measured for comparison. The results suggested that CLNs were present in these seed oils (Fig. 3). As shown in Fig. 3, peaks based on α-eleostearic acid (α-ESA; 9Z,11E,13E-18:3) and β-eleostearic acid (β-ESA; 9E,11E,13E-18:3), which are types of CLNs, were observed at retention times (Rts) of 49 and 52 min, respectively, on chromatograms from *Momordica charantia* seed oil. At Rts of 49 and 52 min, peaks were also found in *Valeriana officinalis* seed oil. In *Centranthus ruber* seed oils, three peaks were found at Rts of 49, 50, and 52 min on the chromatograms. The peak found at an Rt of 50 min was the same Rt of catalpic acid (CPA; 9E,11E,13Z-octadecatrienoic acid). Therefore, these three peaks were expected to represent α-ESA, CPA, and β-ESA, respectively. To further confirm the identified of these fatty acids, the structure of these fatty acids were further analyzed using GC-MS. The spectra of the DMOX derivative of these fatty acids detected in *Centranthus ruber* are shown in Fig. 4. Fatty acid methyl esters of total lipids from *Centranthus ruber* were converted to DMOX derivatives and analyzed using GC-MS, in which a fragment due to the presence of a conjugated double bond was apparent. The GC-MS spectra of the DMOX derivatives of compounds A, B, and C (expected as α-ESA, CPA, and β-ESA, respectively) had fragments of m/z 113 and 126 due to fatty acid DMOX derivatives (Fig. 4). The presence of α-ESA, CPA, and β-ESA was confirmed by the positions of the double bonds (Fig. 4). The GC-MS spectrum of the DMOX derivative of compound A had a molecular ion with an m/z ratio of 331 and fragments of m/z 196 and 274 due to allylic cleavage of the fatty acid chain. The mass spectrum also exhibited differences of 12 mass units between fragments of m/z 196 and 208, between fragments of m/z 222 and 234, and between fragments of

<table>
<thead>
<tr>
<th></th>
<th><em>C. ruber</em></th>
<th><em>V. officinalis</em></th>
<th><em>M. charantia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>total lipid contents (% of the wet weight)</td>
<td>31.6 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.5 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.5 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>fatty acids (mg/g lipids)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C16:0</td>
<td>49.6 ± 1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.1 ± 2.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.6 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:0</td>
<td>33.8 ± 1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15.9 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>379.6 ± 27.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:1</td>
<td>54.2 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.5 ± 5.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.8 ± 9.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:2</td>
<td>377.5 ± 6.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>512.1 ± 34.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.0 ± 11.9&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>C18:3</td>
<td>N.D.</td>
<td>6.9 ± 0.4</td>
<td>N.D.</td>
</tr>
<tr>
<td>α-ESA</td>
<td>79.1 ± 1.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>225.0 ± 15.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>418.1 ± 65.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CPA</td>
<td>19.2 ± 0.8</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>β-ESA</td>
<td>237.1 ± 6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.1 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total CLN</td>
<td>335.4 ± 8.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>232.1 ± 15.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>419.5 ± 65.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>others</td>
<td>6.9 ± 1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.4 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.1 ± 10.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

α-ESA; α-eleostearic acid, CPA; catalpic acid, β-ESA; β-eleostearic acid, n = 3, mean ± SD, N.D.; not detected. Means in a row with different letters are significantly different at p < 0.05.

m/z 248 and 260, indicating the presence of conjugated double bonds at carbon atoms 9, 11, and 13. These results showed that these compounds were 9,11,13-CLNs. Similarly, compounds B and C were also identified as 9,11,13-CLNs. In addition, because the earliest and latest 9,11,13-CLNs had the same Rts for α-ESA and β-ESA from Momordica charantia, respectively, the GC-EI/MS spectra of the DMOX derivatives of these fatty acids obtained from Centranthus ruber were identified as α-ESA and β-ESA, respectively. Furthermore, because the second to last 9,11,13-CLN had the same retention time as CPA (standard), the fatty acids obtained from Centranthus ruber were identified as CPAs. The same results were obtained for α-ESA and β-ESA obtained from Valeriana officinalis (data not shown).

As shown in Table 1, total lipid contents of seeds from Centranthus ruber, Valeriana officinalis, and Momordica charantia were 31.6%, 27.5%, and 41.5% of the wet weight, respectively. With regard to fatty acid composition, Centranthus ruber seed oil contained 79.1 mg α-ESA/g lipids, 19.2 mg CPA/g lipids, and 237.1 mg β-ESA/g lipids, whereas Valeriana officinalis seed oil contained 225.0 mg lipids.

\[
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\]
C. ruber and V. officinalis Seeds Contain Conjugated Linolenic Acid


The content of β-ESA in Centranthus ruber seed oil was much higher than that in other seed oils, although total CLN contents in Centranthus ruber and V officinalis seed oils were lower than those in Momordica charantia seed oil. Therefore, this showed that Centranthus ruber and Valeriana officinalis contained CLNs in their seed oils.

### 3.3 Proportions of NLs, GLs, and PLs and the fatty acid compositions of the seeds

To examine the properties of CLN-containing seed oils in detail, NLs, GLs, and PLs were eluted from the seed oils obtained from Centranthus ruber, Valeriana officinalis, and Momordica charantia by silica column chromatography. The proportions of NLs, GLs, and PLs to total lipids in the seeds are shown in Table 2. The seed oils consisted largely of NLs. In particular, Centranthus ruber, Valeriana officinalis, and Momordica charantia contained 91.5%, 94.3%, and 97.2% NLs, respectively. The fatty acid compositions of the NL, GL, and PL fractions are shown in Table 3.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Ratio (weight %) of the NL, GL, and PL fractions of total lipids in seeds of Centranthus ruber, Valeriana officinalis, and Momordica charantia.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. ruber</td>
</tr>
<tr>
<td>NL</td>
<td>91.5 ± 0.5</td>
</tr>
<tr>
<td>GL</td>
<td>6.6 ± 0.5</td>
</tr>
<tr>
<td>PL</td>
<td>1.9 ± 0.1</td>
</tr>
</tbody>
</table>

NL: Neutral lipids; GL: Glycolipids; PL: Phospholipids; n = 3, mean ± SD. Means in a column with different letters are significantly different at p < 0.05.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Fatty acid composition of lipid fractions obtained from Centranthus ruber, Valeriana officinalis, and Momordica charantia.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. ruber</td>
</tr>
<tr>
<td>NL</td>
<td>GL</td>
</tr>
<tr>
<td>C16:0</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.7 ± 0.1</td>
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<tr>
<td>C18:1</td>
<td>6.2 ± 0.3</td>
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<tr>
<td>C18:2</td>
<td>43.5 ± 0.5</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>α-ESA</td>
<td>9.2 ± 0.3</td>
</tr>
<tr>
<td>CPA</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>β-ESA</td>
<td>26.9 ± 0.5</td>
</tr>
<tr>
<td>Others</td>
<td>2.8 ± 0.1</td>
</tr>
</tbody>
</table>

Table 3. In Centranthus ruber, the NL fraction contained 9.2% α-ESA, 2.3% CPA, and 26.9% β-ESA. The GL fraction contained 3.4% α-ESA, 0.8% CPA, and 11.8% β-ESA. The PL fraction contained 0.7% α-ESA and 1.1% β-ESA. The total CLN contents in NLs, GLs, and PLs in Centranthus ruber were 38.3% ± 0.5%, 16.0% ± 1.0%, and 1.9% ± 0.1%, respectively, with significantly higher concentrations in NLs than in the other fractions. In Valeriana officinalis, the NL fraction contained 32.4% α-ESA and 0.4% β-ESA. The GL fraction contained 21.0% α-ESA, and the PL fraction contained 0.7% α-ESA. The total CLNs content in NLs, GLs, and PLs in Valeriana officinalis were 32.8% ± 0.3%, 21.0% ± 1.2%, and 0.7% ± 0.1%, respectively, with significantly higher concentrations in NLs than in the other fractions. In Momordica charantia, the NL fraction contained 62.9% α-ESA and 1.7% β-ESA. The GL fraction contained 18.6% α-ESA and 3.1% β-ESA. The PL fraction contained 1.9% α-ESA and 0.3% β-ESA. The total CLN contents in NLs, GLs, and PLs in Momordica charantia were 64.6% ± 0.4%, 21.6% ± 2.6%, and 2.2% ± 0.6%, respectively, with significantly higher concentrations in NL than in the other fractions. These results suggested the CLNs in these seed oils mostly existed as triacylglycerol.

### 3.4 Lipase hydrolysis of esterified CLNs

Our analysis of the CLNs in seed oils obtained from Centranthus ruber, Valeriana officinalis, and Momordica charantia suggested that these CLNs mostly existed as triacylglycerol. Therefore, to examine the lipase sensitivity of the esterified CLNs in the seed oils, the fatty acid compositions of total NLs and hydrolyzed NLs (fatty acids that were connected at the sn-1,3 position) obtained from these seed oils were compared (Fig. 5). The α-ESA contents of Momordica charantia decreased significantly after hydrolysis.
In contrast, the α-ESA contents of Valeriana officinalis increased significantly after hydrolysis. In Centranthus ruber, the α-ESA content did not differ significantly after hydrolysis. The CPA contents of Centranthus ruber also showed no significant differences after hydrolysis. The β-ESA contents of Momordica charantia before and after hydrolysis were 1.7% ± 0.2% and 0.9% ± 0.1%, respectively, indicating a significant decrease after hydrolysis. In contrast, those of Valeriana officinalis before and after hydrolysis were 0.4% ± 0.0% and 0.9% ± 0.2%, respectively, indicating a significant increase after hydrolysis. In Centranthus ruber, the β-ESA contents before and after hydrolysis were 26.9% ± 0.5% and 25.4% ± 2.0%, respectively, with no significant difference between the two groups. These results suggested that the CLNs in the seed oils of Centranthus ruber and Valeriana officinalis had higher lipase sensitivity than those of Momordica charantia.

4 Discussion

In this study, we investigated the compounds found in seed oils obtained from various herbs in order to discover seed oils that contained CLNs. First, UV spectra of these seed oils were measured, and absorption maxima around 270 nm were observed from seed oils of Centranthus ruber and Valeriana officinalis. Conjugated trienoic fatty acids...
acids have characteristic absorption maxima at around 270 nm. Therefore, this suggested that these seed oils contained conjugated trienoic fatty acids. To confirm the presence of CLNs in the seed oils of *Centranthus ruber* and *Valeriana officinalis*, the fatty acid compositions of the total lipid contents were measured by GC and GC-MS. This revealed that *Centranthus ruber* seed oil contained α-ESA, CPA, and β-ESA, whereas *Valeriana officinalis* seed oil contained α-ESA and β-ESA.

In *Centranthus ruber* seed oil, the absorption maximum was 268 nm, followed by peaks at 259 and 279 nm. In *Valeriana officinalis* seed oil, the absorption maximum was at 270 nm, followed by peaks at 261 and 281 nm. In a previous study, the three absorption peaks of α-ESA were found to exist at slightly shorter wavelengths than those of α-ESA. Similarly, in this study, the absorption peaks of *Centranthus ruber* seed oil were at shorter wavelengths than those of *Valeriana officinalis*. This result was consistent with data showing that the major CLNs in seed oils of *Centranthus ruber* and *Valeriana officinalis* were β-ESA and α-ESA, respectively.

In a previous study, the cytotoxic effects of CPA in cancer cells were found to be particularly strong for various CLN isomers. Therefore, *Centranthus ruber* is likely to have strong antitumor effects. In addition, *Centranthus ruber* seed oil contained abundant amounts of β-ESA. Although some plant seed oils (e.g., *Momordica charantia* and *Vernicia fordita*) contain β-ESA, the contents are lower than those in *Centranthus ruber*. To the best of our knowledge, no seed oils that contain β-ESA as the major component of CLNs have been reported. Notably, however, the cytotoxic effects of β-ESA on cancer cells are stronger than those of α-ESA. Therefore, *Centranthus ruber* seed oils may have promising applications in the treatment of cancer owing to their potent antitumor effects compared with other plant seed oils that have been previously reported to have CLNs.

From lipid fractionation by silica column chromatography, we found that the seed oils of *Centranthus ruber* and *Valeriana officinalis* contained primarily NLs. In addition, the major components in NLs were confirmed to be triacylglycerols by thin-layer chromatography (data not shown). These results demonstrated that the CLNs in these seed oils mostly existed as triacylglycerols. Triacylglycerols in blood must be hydrolyzed to free fatty acids by lipase before being taken up by cells. To exhibit cytotoxic effect, CLNs must be taken up by cancer cells as free fatty acids. Therefore, it is necessary to clarify the lipase sensitivity of esterified CLNs in the seed oils. We found the binding site between CLNs and glycerol to evaluate the availability of NLs in these seed oils for cancer prevention. LPL hydrolyzes esterified fatty acids in the blood at the sn-1,3 position of triacylglycerol. In this study, we hydrolyzed esterified fatty acids in the NL fraction using pancreatin lipase, which is also known to hydrolyze the sn-1,3 position of triacylglycerol, similarly to LPL. Then, to examine the distribution of the esterified CLNs in the seed oils, we compared CLN contents of total NLs and hydrolyzed NLs (CLNs connected at the sn-1,3 position) with *Momordica charantia*, the α-ESA and β-ESA contents were significantly decreased after hydrolysis, whereas in *Valeriana officinalis*, the α-ESA and β-ESA contents were significantly increased after hydrolysis. In *Centranthus ruber*, the contents of CLNs were not changed significantly. Thus, this suggested that more CLNs existed at the sn-2 position than at the sn-1,3 position in *Momordica charantia*, whereas more CLNs existed at the sn-1,3 position than at the sn-2 position in *Valeriana officinalis*. Additionally, CLNs were evenly distributed between the sn-1,3 and sn-2 positions in *Centranthus ruber*. Therefore, CLNs from *Centranthus ruber* and *Valeriana officinalis* were likely to be taken up by cancer cells more effectively than CLNs from *Momordica charantia*.

As shown in Table 1, total CLN contents of *Centranthus ruber* valeriana, *Valeriana offi*...
for the presence of CLNs in their seed oils. We found that Centranthus ruber and Valeriana officinalis contained CLNs in their seed oils. The CLN contents in these seeds were higher in the NL fraction than in the other fractions. These CLNs were found to be relatively well hydrolyzed by pancreatic lipase. These results indicated that the CLNs in the seed oils of Centranthus ruber and Valeriana officinalis could easily be taken up by cancer cells and had potential applications as antitumor agents.

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