Selected Phytochemicals and Minerals in Box Thorn (Lycium chinense Miller) Leaves

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Contents of antioxidative vitamins (ascorbic acid, \( \beta \)-carotene and tocopherols), dietary fibers, rutin and various minerals in box thorn leaves collected at different seasons (from mid May to mid October) were determined. The leaves contained 131–192 (mg/100 g) of ascorbic acid. The ascorbic acid content was maximum in June and minimum in July. The content of \( \beta \)-carotene was high (8.7–13.8 mg/100 g), but no \( \alpha \)-carotene was found in the leaves. Among the tocopherols in the leaves, \( \alpha \)- and \( \beta \)-tocopherol were the major components. \( \alpha \)-Tocopherol was maximum in October (25.5 mg/100 g, on dry weight basis), and \( \beta \)-tocopherol, which is not an abundant type in nature, was high in July (27.2 mg/100 g), August (27.2 mg/100 g) and September (26.7 mg/100 g). The contents of dietary fiber (NDF, ADF, hemicellulose, lignin and cellulose) varied with the season. Differently from what has been known, box thorn leaves were not a rich source of rutin. Rutin was detected only in the leaves collected in May and October, and the contents were 0.45 and 0.38 mg/100 g, respectively. The leaves contained calcium (562–1316 mg/100 g), copper (0.26–1.20 mg/100 g), iron (9.42–21.77 mg/100 g), magnesium (403–657 mg/100 g), manganese (24.6–144.8 mg/100 g), potassium (1291–3046 mg/100 g) and zinc (3.2–24.3 mg/100 g). The contents of the individual minerals in the leaves varied greatly with the season.

Keywords: box thorn leaves, phytochemicals, minerals, seasonal changes

The most promising healthy food category to come along in years is phytochemicals. The fruits and leaves of box thorn (Lycium chinense Miller), which is a plant belonging to the family of Solanaceae, have long been used as foods, teas and/or medicines in the Orient. Especially, the leaves have great potential as an ingredient in healthy foods and teas because of their disease-preventive activity, clinical activity, and easy accessibility. The taste components and flavor compounds in box thorn leaves have been reported previously (Nishiyama, 1962; Yoshimura et al, 1969; Sannai et al, 1984; Kim et al, 1997).

It has been known that box thorn leaves are capable of abating or reducing the risk of certain diseases such as arteriosclerosis, essential arterial hypertension, diabetes and night blindness (Soga, 1985). Box thorn leaves have also been used for improvement of stamina, tranquilization activity, thirst quenching and antiaging activity (Soga, 1985). Park (1995) reported that a water extract or methanolic extract of box thorn leaves showed strong activity for scavenging superoxide anion radical. Park (1995) also reported that a water extract of box thorn leaves inhibited the activities of the angiotensin conversion enzyme (ACE).

The effective utilization of box thorn leaves as health food ingredients requires detailed information on the quality and quantity of biologically-active phytochemicals in the box thorn leaves collected at various seasons. However, this information is limited. Hansel and Huang (1977a, b) isolated and identified scopoletin, vanillic acid, betaine, lycium withanolide A and B in box thorn leaves. Box thorn leaves reportedly contain antioxidative vitamin such as ascorbic acid (Mizobuchi et al, 1964, 1969). However, the tocopherol content in box thorn leaves has not been reported. Moreover, another important antioxidative provitamin A, \( \beta \)-carotene, in box thorn leaves has not been studied. Mizobuchi et al (1969) reported that Japanese box thorn leaves contained 22–134 mg/100 g of rutin (on dry weight basis), a preventive phytochemical for hyperpiesia and stroke. No other paper on the rutin content in box thorn leaves has been published. The accuracy of the data on the content of rutin in box thorn leaves was suspicious because of the analytical method used. Dietary fibers, which are another important phytochemical, in box thorn leaves also have never been reported previously. Hashinaga et al (1971) reported that Japanese box thorn leaves contained 7.8 \( \mu \)g/100 g of Ge (on a dry weight basis). However, the information on the contents of other minerals in box thorn leaves was not available in literature.

Thus, the objective of this research was to obtain qualitative and quantitative information on biologically-active phytochemicals (ascorbic acid, \( \beta \)-carotene, tocopherols, dietary fibers and rutin) and various minerals in box thorn leaves collected at various seasons.

Materials and Methods

Materials Box thorn leaves were collected once a month from May 15, 1993 to October 15, 1993 at a box thorn
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farm located in Chungyang, Chongnam Province, Republic of Korea. The collected leaves were washed with 0.001% acetic acid solution to remove the possible residual pesticides and rinsed with water. The washed leaves were dried with hot air at 60°C and ground to pass through a 60 mesh sieve. The ground leaves were flushed with nitrogen and stored at -18°C until used. The ground leaves were used as samples for all investigation except for the ascorbic acid analysis. For the ascorbic acid analysis, freshly collected box thorn leaves without drying were used as samples. The moisture and ash contents of the box thorn leaves collected in May were 87.7 and 1.6%, respectively.

Collection of box thorn leaves
For collecting box thorn leaves, a sector (ca. 40 m x 70 m) in a box thorn farm was arbitrarily divided and box thorn leaves were always collected in that same sector of the farm. All the branches of a box thorn tree, except 3-5 branches per tree which were saved for bearing fruit, were cut at the ground level within the sector. From the branches, the soft leaves at the top 5 cm were separately collected for a different purpose. One month after the previous collection, the newly grown branches were cut at the ground level, and the leaves were collected as described before. The remaining leaves were used for the samples. The amount of the collected leaves was 10-30 kg.

Ascorbic acid
Because ascorbic acid is very sensitive to oxidation, fresh box thorn leaves were used for ascorbic acid determination. The ascorbic acid in the leaves was extracted with 5% metaphosphoric acid and determined by an iodine method (Bessy & King, 1933).

β-Carotene
The sample was prepared according to the previously established method (JFHA, 1991). The prepared sample was injected into an HPLC. The HPLC system used was a Perkin Elmer, Model LC-50 pump (Perkin Elmer Co. Norwalk, CT), equipped with a UV-VIS spectrophotometer Model LC-290 (Perkin Elmer Co. Norwalk, CT). A µ-Bondapak C-18 column (Waters, Milford, MA) was used. The column temperature was maintained at 35°C. The flow rate of the mobile phase (methanol:chloroform, 96:4) was 1 ml/min. With authentic β-carotene and α-carotene, we found that the present HPLC method separated these carotenes well and that no α-carotene was present in the box thorn leaves. β-Carotene in the sample was quantitated at 453 nm with a standard curve of authentic β-carotene.

Tocopherols
Tocopherols in box thorn leaves were extracted three times with hexane. The hexane extracts were combined and dried with anhydrous sodium sulfate and then filtered. The solution was then concentrated under vacuum and passed through a 0.45 µm membrane filter. The filtered samples were injected into an HPLC for tocopherol analysis (Kim et al., 1994). The HPLC used was a Perkin Elmer, Model LC-50 pump, equipped with a UV-VIS spectrophotometer Model LC-290. A µ-Bondapak C-18 column was used. The flow rate of the mobile phase (2-propanol:hexane, 1.5:98.5) was 1.8 ml/min. Tocopherols in the sample were quantitated at 295 nm with standard curves of the authentic tocopherols.

Rutin
Rutin in box thorn leaves was determined by both visible spectrophotometry (Mizobuchi et al., 1969) and HPLC (Ohara et al., 1989). Before the extraction of rutin, interfering substances such as lipids, coloring compounds and others were first removed from the box thorn leaves with chloroform in a Soxhlet apparatus. Rutin was then extracted from the leaves with boiling methanol for 2 h. The extraction step was repeated twice more and the methanol extracts were combined.

For the visible spectrophotometric method, 1 g of metallic magnesium (turnings) and 1.5 ml of 22.5% HCl solution were added to 10 ml of methanolic extracts of box thorn leaves, and the sample was shaken vigorously. The sample was then allowed to stand for 20 min at room temperature and was filtered through filter paper. After filtration, the sample was allowed to stand for 10 min more, and the absorbance of the sample at 550 nm was measured. The content of rutin was calculated with a standard curve of authentic rutin.

Dietary fibers
Neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose and lignin were determined according to Van Soest and Wine (1967). Cellulose was determined according to Crampton and Maynard (1938).

Minerals
To measure the contents of individual minerals in box thorn leaves, 2 g samples wereashed in porcelain crucibles at 550-600°C in a muffle furnace for 4 h. The resulting ash was wetted with 2 ml of conc. HCl. The HCl was then evaporated on a hot plate. The sample was dissolved with 10 ml 0.5 N HNO₃ and transferred to a 50-ml volumetric flask and diluted to volume with 0.5 N HNO₃. The contents of copper, iron, manganese and zinc were determined using an inductively coupled plasma (ICP) emission spectrometer (Model JY 70PLUS, Jobin Yvon, France). The contents of calcium, potassium, magnesium were measured using an ion chromatograph (Model DX300, Dionex, Sunnyvale, CA, USA). The column used was a Dionex Ion Pac CS12 and the flow rate of eluent (20 mm methanesulfonic acid in deionized water) was 1.0 ml/min.

Statistical analysis
All content measurements in box thorn leaves were carried out in triplicate. Statistical analysis was accomplished with a Statistical Analysis System (SAS, 1985). Duncan's multiple range tests and coefficients of variation (CV) were used to ascertain the seasonal changes in the various phytochemicals in the box thorn leaves.

Results and Discussion
Antioxidative vitamins
Figures 1 and 2 and Table 1 show the contents of ascorbic acid, β-carotene and tocopherols in box thorn leaves collected at different seasons. It has been well-known that ascorbic acid, β-carotene and tocopherols have antioxidative and antiaging activity due to their ability for scavenging singlet oxygen, superoxide anion and other active oxygen (Nishikimi, 1975; Jung et al., 1991, 1995a,b). The box thorn leaves were rich in ascorbic acid, and the ascorbic acid contents in box thorn leaves varied with the
Fig. 1. Ascorbic acid content in box thorn leaves collected at different seasons. Bar with a different italic letter was significantly different (p<0.05).

Fig. 2. β-Carotene content in box thorn leaves collected at different seasons. Bar with a different italic letter was significantly different (p<0.05).

Table 1. Tocopherol content in box thorn leaves collected at different seasons.

<table>
<thead>
<tr>
<th>Tocopherols</th>
<th>15 May</th>
<th>15 Jun</th>
<th>15 Jul</th>
<th>15 Aug</th>
<th>15 Sep</th>
<th>15 Oct</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol</td>
<td>15.2b</td>
<td>11.6c</td>
<td>10.8f</td>
<td>12.9d</td>
<td>13.4d</td>
<td>25.5c</td>
</tr>
<tr>
<td>β-Tocopherol</td>
<td>16.5d</td>
<td>15.0c</td>
<td>27.2a</td>
<td>27.2a</td>
<td>26.7b</td>
<td>22.4c</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>δ-Tocopherol</td>
<td>0.8a</td>
<td>0.2c</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
</tr>
</tbody>
</table>

The measurement was done in triplicate and the coefficient of variation (CV) was 1.28%.
Means in the same row with different superscript letters are significantly different (p<0.05).
ND: Not detected.
trace: The content was too small to be calculated.

The ranges of ascorbic acid content in box thorn leaves at different seasons were 131-192 mg/g (dry weight basis). The ascorbic acid content was high in May, June and October but low in July, August and September. The present results were consistent with those previously reported (Mizobuchi et al., 1969). They reported that the ascorbic acid content in Japanese box thorn leaves was high in May, June, October and November but low in July and August.

The content of β-carotene (8.7-13.8 mg/100 g, on dry weight basis) in the leaves varied greatly with the different seasons (Fig. 2). α-Carotene was not present at a detectable level in box thorn leaves. The provitamin activity of α-carotene is half that of β-carotene. The β-carotene content in the leaves was maximum in July. The present data indicated that box thorn leaves might be a possible source for the extraction of commercial carotenes. At present, carotenes on the market are chemically synthetic β-carotene, carotene extracted from carrots, carotene extracted from algae Dunaliella, and carotene from palm oil. Carrot carotene, Dunaliella carotene and palm oil carotene contain 30-50, 6-10 and 30-35% α-carotene in addition to β-carotene.

Among the tocopherols in the leaves, α- and β-tocopherol were the major components and their contents differed with the season (Table 1). It is also interesting to note that β-tocopherol, which is not an abundant type in nature, is the major component among the tocopherols in box thorn leaves. β-Tocopherol was high in July (27.2 mg/100 g), August (27.2 mg/100 g) and September (26.7 mg/100 g). α-Tocopherol was maximum in October (25.5 mg/100 g).

Dietary fibers It has been generally known that high dietary fiber intake is associated with bowel regularity, modulation of blood glucose levels, cholesterol metabolism and low risk of colon cancer development. However, the preventative and therapeutic natures of dietary fibers are reportedly different with the types and sources of the fibers (Kritchevsky, 1988; Stark & Madar, 1994). Thus we believed that information on the types of fibers and their contents in the box thorn leaves was indispensable for effective utilization of the leaves as health food ingredients. The contents of NDF, ADF, lignin, hemicellulose and cellulose varied with the season (Table 2). The present data showed that the ranges of the different fibers in the box thorn leaves were 37.8-48.1 (g/100 g) for NDF, 15.4-26.5 (g/100 g) for ADF, 21.7-29.7 (g/100 g) for lignin, 13.2-17.5 (g/100 g) for cellulose. The contents of NDF, ADF and...
Means in the same row with different superscript letters are significantly different (p<0.05).

The measurements were done in triplicate and the coefficients of variation (CV) for NDF, ADF, lignin, hemicellulose and cellulose were 4.61, 4.32, 8.18, 9.35 and 4.61%, respectively.

Means in the same row with different superscript letters are significantly different (p<0.05).

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and iron.

In summary, box thorn leaves were rich in antioxidative and antiaging vitamins such as ascorbic acid, β-carotene and tocopherols. However, α-carotene was not found in the leaves. It was also interesting to note that β-tocopherol, which is not an abundant type in nature, was the major component among the tocopherols. Box thorn leaves were also rich in various dietary fibers and important minerals. The high contents of these phytochemicals and minerals in box thorn leaves might be related, at least to some extent, to the preventive and therapeutic activity of the box thorn leaves. Based on the high performance liquid chromatographic analysis, the rutin content in the leaves was not high. Nevertheless, the present data provide important information on the contents and seasonal changes of selected phytochemicals and minerals in the box thorn leaves. We believed that these present data should aid the related food industry in the design and formulation of healthy foods or functional foods with box thorn leaves.

References