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Development of an Analytical Approach for the Utilization of Edible Tree Sprouts

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Abstract – This study evaluated the general nutritional ingredients such as crude fats, crude ashes, crude proteins, total polyphenols, and total flavonoids in 18 kinds of edible tree sprouts. The tree sprouts of *Philadelphus schrenckii*, *Lycium chinense*, and *Morus alba* had the highest crude fat, crude ash, and crude protein content, respectively. The tree sprouts of *Cedrela sinensis* (CSS) with high $ABTS^+$ radical scavenging activities had a high content of total polyphenols (175.65 mg/g ext.) and total flavonoids (75.18 mg/g ext.). The simultaneous determination of flavonoids such as rutin, isoquercitrin, quercitrin, afzelin, and quercetin in CSS was conducted using high-performance liquid chromatography with a wavelength of 270 nm. Among the flavonoids, the content of quercitrin in CSS was the highest at 59.28 mg/g ext. This study also aids the quality control of many edible tree sprouts by analyzing the general components, total polyphenols, and total flavonoids.
Keywords – *Cedrela sinensis*, Edible sprout, Anti-oxidant activity, Quercitrin, Total polyphenol, Total flavonoid

Introduction

The intake of high-fat, high-calorie foods is increasing due to recent westernized eating habits. In addition, the intake of fruits, vegetables, and grains has decreased, resulting in an increased incidence of chronic diseases among adults. As a result, there is a great interest in illness prevention, which has led to increased consumption of plant materials and vegetables with large amounts of phytochemicals. The chemical composition and nutritional value of plants vary according to their variety and growth stage, with variable protein, fats, fibers, and bioactive components.[1] Recently, various health benefits of polyphenols and flavonoids have been reported.[2,3] Barley sprouts, oat sprouts, and soybean sprouts have been known to contain phytochemicals that are different from those found in fully grown plants.[4,5]

Tree sprouts are nutritious and beneficial food products. Edible tree sprouts contain large amounts of polyphenols, active substances, and enzymes involved in chemical reactions in vivo.[6] They are known to reduce the risk of viral infection, inflammation, cancer, diabetes, and obesity.[7-11] The aforementioned studies, as well as the growing interest of the general public in food and health, drives the demand for comprehensive and reliable information on food quality and raw materials.[12] Therefore, sprouts are being increasing used in many ways.

This study investigated the general ingredients of edible tree sprouts that are most commonly consumed for foods. These edible tree sprouts are highly effective tree sprouts used as raw materials for functional foods. In addition, total polyphenols and total flavonoids were analyzed, and their simultaneous determination of flavonoids was conducted to utilize the tree sprouts as raw materials in functional food. Finally, the study focus on the development of an analytical approach for the utilization of edible tree sprouts.

Experimental

Plant materials – The tree sprouts of *Actinidia arguta* (AAG), *Aralia elata* (AET), *Actinidia kolomikta* (AKM), *Actinidia polygama* (APG), *Cedrela sinensis* (CSS, Fig. 1), *Euonymus alatus* (EAT), *Eleutherococcus gracilistylus* (EGS), *Eleutherococcus senticosus* (ESC), *Eleutherococcus sessiliflorus* (ESF), *Fraxinus mandshurica* (FMS), *Kalopanax septemlobus* (KSL), *Lycium chinense* (LCS), *Morus*

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Fig. 1. The sprout of CSS.

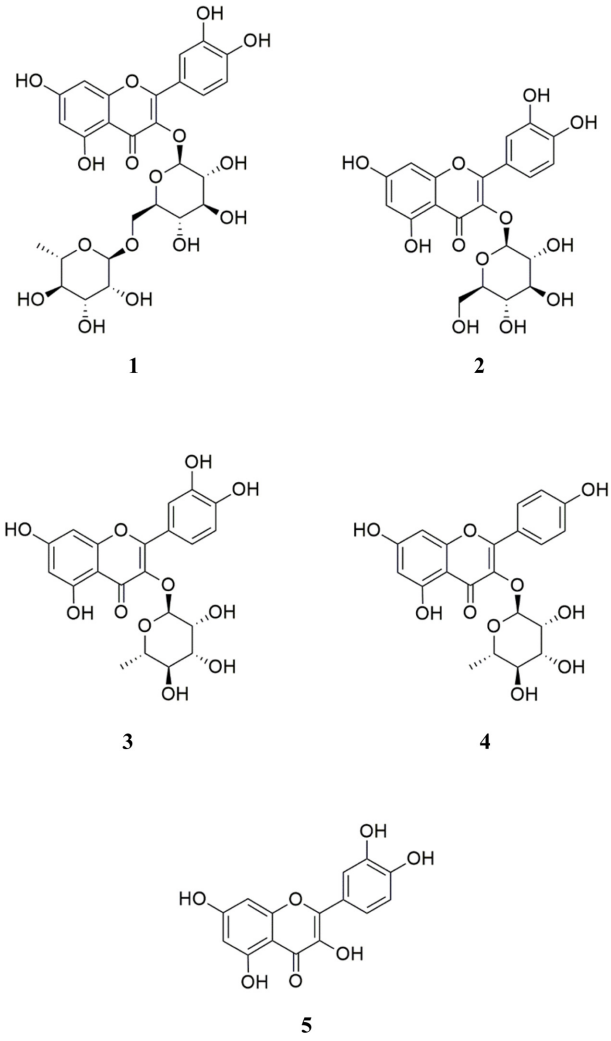

Fig. 2. Chemical structures of rutin (**1**), isoquercitrin (**2**), quercitrin (**3**), afzelin (**4**), and quercetin (**5**).

alba (MAB), *Philadelphus schrenkii* (PSR), *Rhus trichocarpa* (RTC), *Rhus verniciflua* (RVF), *Staphylea bumalda* (SBD), and *Securinega suffruticosa* (SST) were provided by the Division of Special Forest Products, National Institute of Forest Science, Suwon, Republic of Korea. Dried powder of the tree sprouts was obtained and used for the analyses. Voucher specimens were stored in the herbarium of the Department of Botanical Engineering, Chung-Ang University, Republic of Korea.

Instruments and reagents – An Epoch (VT, USA) microplate spectrophotometer was used for the analysis. The reagents used were 2N Folin–ciocalteu reagent, sodium carbonate, aluminum (III) chloride hexahydrate, and ethanol (EtOH), from Sigma (MA, USA). High Performance Liquid Chromatography (HPLC) analysis was conducted using an Agilent 1260 Infinity II Quat (CA, USA) with pumps, autosamplers, and Diode array detector (DAD) WR detectors. HPLC-grade solvents such as acetic acid, water, acetonitrile (ACN), and methanol (MeOH) were purchased from J.T. Baker (Phillipsburg, USA). The standard compounds of rutin, isoquercitrin, quercitrin, afzelin, and quercetin were obtained from the Natural Products Institute of Science and Technology (www.nist.re.kr), Anseong, Republic of Korea (Fig. 2).

Sample and flavonoid preparation – Eighteen edible tree sprout powders were used for general ingredient analysis. The samples (10 g each) were extracted with EtOH as the solvent for 3 h under reflux for total polyphenols and total flavonoids. The CSS extract was dissolved in MeOH and filtered using a 0.45-μm PVDF membrane filter for quantitative analysis. Each sample was dissolved in MeOH at a concentration of 12.5 mg/mL. Flavonoids such as rutin, isoquercitrin, quercitrin, afzelin, and quercetin were dissolved in 1 mL of MeOH and filtered through a 0.45-μm PVDF membrane filter.

General ingredient analysis – Crude fat was extracted using an automatic Soxhlet extraction system (Buchi B-811 extracted system, Switzerland). Two grams of ground sample was extracted for 2 h in 200 mL of *n*-hexane, and dried at 105 °C. After cooling for 1 h, the weight of the extracted crude fat was measured and calculated as the

crude fat content.[13] The crude ash content was determined by burning in a muffle furnace at a temperature of 550 ºC.[14] The crude protein content was analyzed according to the Kjeldahl method using the Buchi B-339 auto Kjeldahl system (Buchi Labortechnik AG, Flawil, Switzerland). That is, 0.5 g of catalyst ($CuSO_4$:K_2SO_4 = 1:9) was added to 3 mg of milled sample. The mixture was hydrolyzed by adding 6 mL of sulfuric acid. The nitrogen coefficient was calculated using a distillation apparatus, and the crude protein content was calculated by multiplying the nitrogen reduction coefficient by 6.25.[15]

Total polyphenol content analysis – The extract of tree sprouts (1 mg each) was analyzed by modifying the Folin-Ciocalteu method.[16] First 60 μL of 2N Folin-Ciocalteu phenol reagent (St. Lewis, Sigma-Aldrich, USA) were added to 60 μL of the extract. After adding 60 μL of 15% Na_2CO_3 to the solution for 30 min, the absorbance was measured at 760 nm using a microplate reader (Epoch, BioTek, Winooski, VT, USA). Finally, a calibration curve was prepared using gallic acid as the standard to quantify the total polyphenol content.

Total flavonoid content analysis – The extract of tree sprouts (1 mg each) was analyzed by modifying the Woisky & Salatino method.[17] The extract was applied from 1 mg/mL to 100 μL, After adding 100 μL of 2% $AlCl_3$ to the solution incubated at 25 ºC for 10 min, and absorbance was measured at 430 nm using a microplate reader (Epoch, BioTek, Winooski, VT, USA). A calibration curve was created using quercetin as the standard compound, and the total flavonoid content was determined.

$ABTS^+$ radical scavenging activity – The extract of tree sprouts was analyzed by modifying the Heo et al.[18] According to the experimental method, $ABTS^+$ and potassium persulfate solution were mixed and diluted with distilled water so that the absorbance value was 1.25 ± 0.04 mg/mL. After storing in the dark for 24 h, each prepared sample was added to the radical stock solution. After storing for 30 min, the residual radical concentration was measured at 734 nm using a microplate reader (Epoch, BioTek, Winooski, Vietnam, USA). Ascorbic acid (Acs) was used as the positive control. Concentration ranges from 0.5 to 0.025 mg/mL. The scavenging properties (IC_{50}) of the extract are shown in Fig. 3, and it is expressed as the concentration required to reduce the absorbance of the control group by 50% using only the solvent.

HPLC conditions – Quantitative analysis was conducted using reverse phase HPLC with an INNO C18 column (250 × 4.6 mm, 5 μm). The injection volume was 10 μL, and a DAD was used. The column temperature was maintained at 25 ºC, and the flow rate was set to 1 mL/min. The detector wavelength was set at 270 nm. The analysis was conducted with a gradient elution system using a mobile phase composed of 0.5% acetic acid in water (A) and ACN (B). The gradient elution system performed as follows; 0 min-95% A, 10 min-70% A, 25 min-70% A, 30 min-20% A, 35 min-100% B, 40 min-100% B, 50 min-95% A, and 55 min-95% A.

Calibration curves – A standard stock solution was prepared by dissolving the compound in MeOH (1 mg/mL). The working solutions for constructing the calibration curve were prepared by serially diluting the stock solutions to the desired concentrations. The analyte content was determined from the corresponding calibration curves. The calibration function of the standard was calculated using peak area (Y) and, concentration (X, mg/mL), and mean values ± standard deviation (n = 5) are presented.

Results and Discussion

The consumption of bioactive compounds present in plant-derived foods is a promising strategy to strengthen immunity and to prevent illnesses. However, substantial amounts of time and resources are needed to find materials containing effective bioactive compounds. Many phytochemicals are known to have antibacterial and immune-enhancing effects. These biological activities suggest that these compounds have great potential for treating various problems in the human body.[19]

Table 1 presents the results of the general ingredient analysis for the 18 edible tree sprouts. The crude fat

Table 1. Contents of general ingredients

Tree sprout	Crude fat (%)	Crude ash (%)	Crude protein (%)
AAG	3.24	9.10	28.40
AET	5.13	7.81	28.90
AKM	3.40	4.80	27.20
APG	2.78	8.55	29.10
CSS	2.79	7.78	21.32
EAT	3.09	8.46	25.39
EGS	4.61	7.83	26.07
ESC	2.41	9.35	16.20
ESF	1.57	8.51	28.28
FMS	3.22	5.10	20.46
KSL	2.34	8.35	27.81
LCS	3.51	17.03	30.60
MAB	2.78	7.14	35.17
PSR	5.84	7.01	16.91
RTC	3.96	4.50	16.54
RVF	4.38	8.75	28.65
SBD	4.35	6.40	24.48
SST	2.03	7.34	36.75

content in PSR was the highest (5.84%), followed by AET (5.13%) and EGS (4.61%). In plants with relatively low crude fat content, ESF, SST, and KPT had 1.57%, 2.03%, and 2.34%, respectively. Crude ash analyses showed that LCS had the highest value (17.03%), followed by ESC (9.35%) and AAG (9.10%). In plants with relatively low crude protein content, RTC, AKM, and FMS had 4.50%, 4.80%, and 5.10%, respectively. Crude protein analysis showed that SST had the highest value (36.75%), followed by MAB (35.75%) and LCS (30.60%). In plants with relatively low crude protein content, the ESC, RTC, and PSR had 16.20%, 16.54%, and 16.91%, respectively.

Table 2 shows the total polyphenol and total flavonoid contents of 18 edible tree sprouts. With regard to total polyphenol content, RTC had the highest at 242.0 mg/g, followed by FMS at 200.0 mg/g, and CSS at 175.6 mg/g. In a study by Kasmi et al., the total polyphenol content of *Fraxinus angustifolia* was 23.5 mg/g.[20] The difference in total polyphenol content observed in this study is thought to be different due to various factors such as plant species, extraction parts, and UV detection. Tree sprouts with relatively low total polyphenol content were determined to have a total polyphenol content of 5.3 mg/g. Meanwhile, Itidel et al. reported a total polyphenol concentration of 48.3 mg/g for the same genus, *Rhus pentaphylla*.[21] Therefore, the difference in the total polyphenol content of RVF could be attributed to the differences in the extraction site and detection method.

Table 2. Contents of total polyphenol and total flavonoid

Tree sprout	Total polyphenol (mg/g ext.)	Total flavonoid (mg/g ext.)
AAG	66.96 ± 1.50	65.04 ± 3.74
AET	141.76 ± 4.78	36.60 ± 0.75
AKM	94.12 ± 2.97	43.03 ± 2.38
APG	73.77 ± 1.02	25.54 ± 0.75
CSS	175.65 ± 8.58	75.18 ± 1.74
EAT	74.15 ± 2.43	62.99 ± 1.19
EGS	136.58 ± 6.03	50.85 ± 1.40
ESC	127.62 ± 7.06	40.49 ± 0.96
ESF	100.49 ± 4.42	25.59 ± 0.18
FMS	199.99 ± 1.69	41.53 ± 1.00
KSL	95.34 ± 5.75	31.81 ± 0.52
LCS	87.62 ± 7.11	37.78 ± 0.47
MAB	71.23 ± 1.33	69.62 ± 2.37
PSR	80.19 ± 3.31	27.47 ± 0.49
RTC	242.01 ± 1.90	11.83 ± 0.15
RVF	166.19 ± 4.50	57.80 ± 0.52
SBD	54.00 ± 8.11	53.96 ± 3.35
SST	120.02 ± 1.57	31.32 ± 0.05

CSS had the highest total flavonoid concentration at 75.2 mg/g, followed by MBA (69.6 mg/g) and AAG (65.0 mg/g). Although Ahmad et al. reported that the total flavonoid content of *Cedrela serrata*, a plant of the same genus, of 113.5 mg/g. The difference could be attributable to a different method of total flavonoid detection and experimental conditions such as extraction time.[22] RTC (11.8 mg/g) is a tree sprout with a low total flavonoid concentration. Even though RTC was found to have the greatest overall polyphenol content (242.0 mg/g), the total flavonoid concentration was low. In the study of Park et al. the total polyphenol content of plants of the genus *Rhus* was 42.1 mg/g and the total flavonoid content was 7.5 mg/g, showing similar results to ours. The total polyphenol content was high while the total flavonoid content was low.[23]

The desirable conditions for good raw materials for functional foods include good texture, taste, and nutrition.

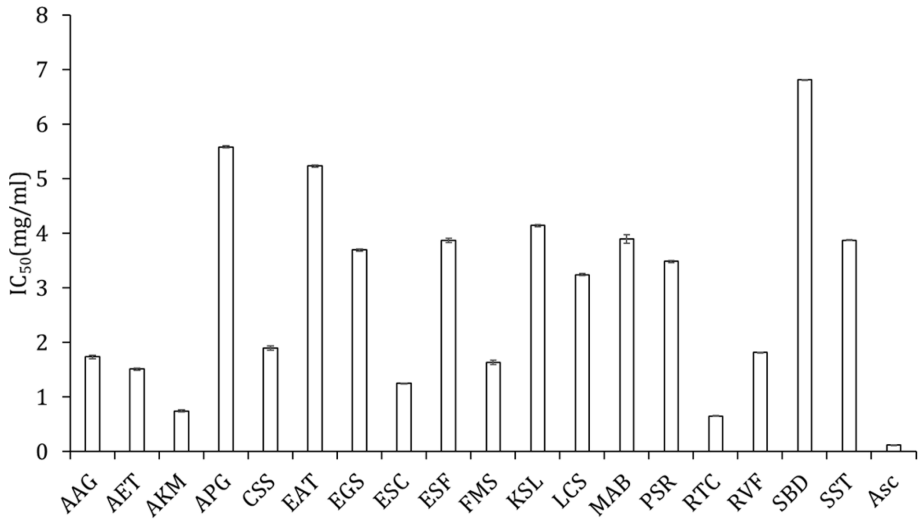

Fig. 3. $ABTS^{+}$ radical scavenging activity of edible tree sprouts.

In this study, the most usable ingredients were selected by focusing on nutrition. Many functional ingredients determine the quality of edible tree sprouts. With respect to the total polyphenol and total flavonoid content, the most effective edible tree sprout was CSS. It had the highest total flavonoid content. CSS is a plant of the family Meliaceae that is native to Asia. Previous studies on CSS have found anti-oxidant and anti-bacterial properties, and CSS has been reported to contain carotene, vitamin B, vitamin C, and substantial levels of calcium and potassium.[24-26] In addition, it has been reported that CSS contains phenolic compounds such as rutin, isoquercitrin, quercitrin, afzelin, and quercetin.[27,28] The $ABTS^+$ radical scavenging IC_{50} values of 18 tree sprout extracts were measured. When the IC_{50} of Asc was 0.119 mg/mL, the highest values were shown in the order of RTC (0.654 mg/mL), AKM (0.735 mg/mL), and CSS (0.937 mg/mL) (Fig. 3).

Several flavonoids are the main components of the CSS. These substances act as therapeutic agents for various diseases and play an essential role in improving immunity. Quantitative analysis of flavonoids such as rutin, isoquercitrin, quercitrin, afzelin, and quercetin in CSS was performed using reversed-phase HPLC with a gradient elution system. HPLC analysis showed good separation of flavonoids, and the DAD was found to be effective in detecting flavonoid compounds. The detection of flavonoids such as rutin, isoquercitrin, quercitrin, afzelin, and quercetin was optimized at a wavelength of 270 nm. Calibration curves were generated by linearly plotting the peak area versus the concentration and were analyzed using linear regression. The linear regression coefficient (r^2) for the standard was 0.9996–1 (Table 3). Fig. 4 shows the HPLC chromatogram and CSS chromatogram of rutin, isoquercitrin, quercitrin, afzelin, and quercetin. The highest content of quercitrin (59.28 mg/g)

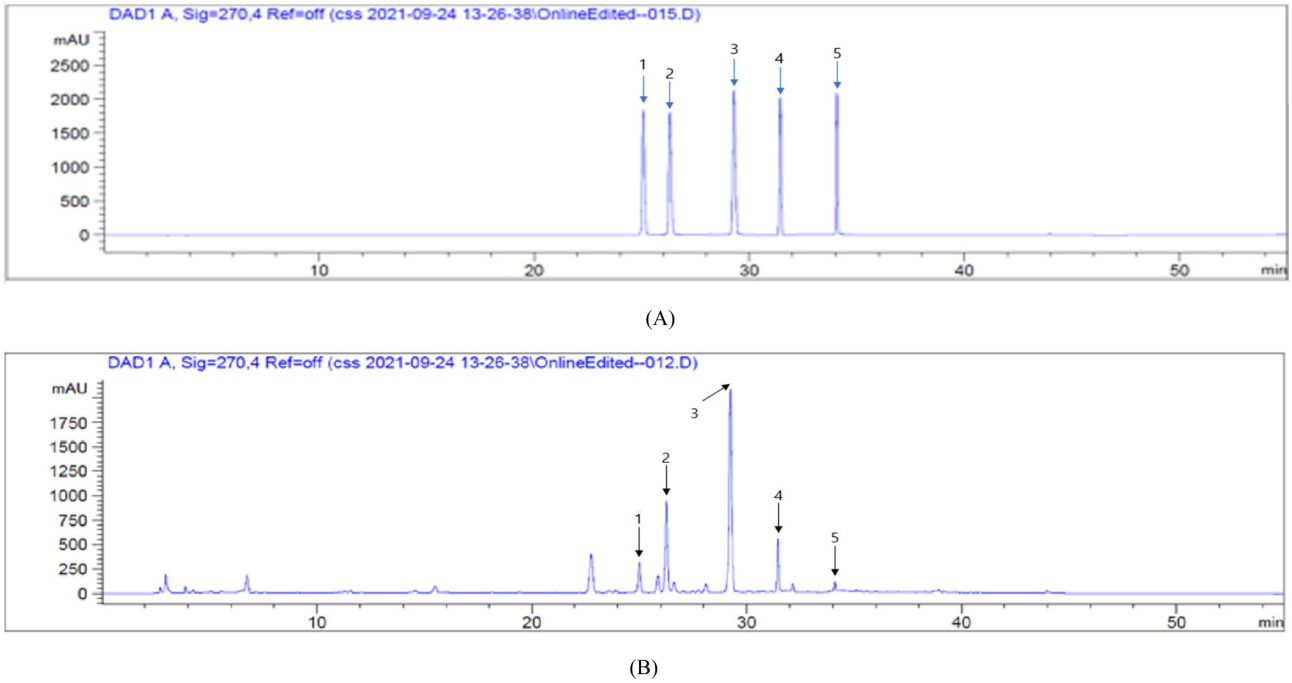

Fig. 4. HPLC chromatograms of standard flavonoids (A) and CSS extract (B).

Table 3. Calibration curves of flavonoids

Compound	t_R	Calibration equation [a]	Correlation factor, r^2 [b]
1	24.9	Y = 14.502X + 76.129	0.9996
2	26.2	Y = 19.331X + 121.31	0.9999
3	28.1	Y = 23.718X + 17.792	0.9998
4	31.4	Y = 23.267X + 153.07	1.0000
5	34.1	Y = 21.52X + 141.45	0.9996

[a] Y = peak area, X = concentration of standards (mg/mL)
[b] r^2 = correlation coefficient based on three data points in the calibration curves

Table 4. Contents of flavonoids in CSS

Compound	Content (mg/g ext.)
1	13.06 ± 0.08
2	30.42 ± 0.09
3	59.28 ± 0.33
4	9.21 ± 0.07
5	0.92 ± 0.01

was found in CSS, followed by isoquercitrin (30.42 mg/g) (Table 4). In addition, in previous studies, there were effects of isoquercitrin on cell damage caused by hydrogen peroxide[29] and the production of detoxifying enzyme proteins in liver tissue genes.[30,31] According to Kim, CSS showed an excellent ability to restore cell damage and hepatotoxicity.[32] Thus, it was confirmed that the general ingredient, total flavonoid, and total polyphenol analyses were helpful in selecting functional food development materials.

In this study, we proposed the selection of substances with better potency using general ingredient, total polyphenol, and total flavonoid analyses. This study will also provide useful information for the quality control of many edible tree sprouts and will help to develop basic analytical methods for the undeveloped edible tree sprouts.

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