Antioxidant activity of edible sprouts and phytosterol contents by HPLC/UV analysis

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Received: 27 June 2021 / Revised: 21 February 2022 / Accepted: 21 February 2022 / Published online: 3 August 2022
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Abstract
This study evaluated the in vitro radical scavenging activities of edible tree sprouts, particularly those of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), hydroxyl radical, and singlet oxide radical, to assess their antioxidant activities. Additionally, stigmasterol (ST) and β-sitosterol (BS) were analyzed using HPLC/UV. The edible sprouts of Eleutherococcus senticosus (ESC) and Morus alba (MAB) exhibited the highest DPPH scavenging activity among other edible sprouts. A reverse-phase column was used in an isocratic elution system, after which UV detection was performed at 210 nm. ST and BS analyses indicated that ESC sprouts contained the highest amounts of ST (9.99 mg·g⁻¹ extract), whereas MAB sprouts contained the highest concentrations of BS (14.69 mg·g⁻¹ extract). In conclusion, the highest antioxidant activity was observed in the edible sprouts with the highest phytosterol content. Therefore, our findings provide a theoretical basis for the development of plant-based functional foods or supplements with antioxidant properties.

Keywords Antioxidant activity · Edible sprouts · HPLC/UV · Phytosterol · Quantitative analysis

1 Introduction
Oxidative stress has been linked to various diseases such as diabetes, cancer, and dementia (Liguori et al. 2018). Particularly, the over-production of free radicals such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, hydroxyl radical (·OH), and singlet oxide radical (O₂⁻) are known to induce oxidative stress in a wide gamut of living organisms including humans (Liguori et al. 2018; Jakubczyk et al. 2020; Bouali et al. 2020; Yang et al. 2021; Jirakiat-tikul et al. 2021). Free radicals can oxidize several types of biomolecules including proteins, lipids, and cell membranes, resulting in cell death (Jakubczyk et al. 2020). In contrast, natural antioxidant compounds such as anthocyanin, β-carotene, lycopene, total phenolics, flavonoids, vitamin C, and the others can remove free radicals by donating electrons, protons, and hydrogen, to form stable structures (Fukuda and Nagata 1986; Zahirul Islam et al. 2019, 2020). In particularly, smaller particles in natural antioxidant defense mechanisms by increases of phenolic and flavonoids and reducing the risk of oxidative stress by interrupting free-radical oxidative reactions (Teleanu et al. 2019). Although synthetic antioxidants including BHT (3,5-di-tert-butyl-4-hydroxytoluene), BHA (β-hydroxy acid), PG (propyl gallate), and TBHQ (tert-butylhydroquinone) have been made, they have low thermal stability and may pose a risk of carcinogenesis (Branen 1975). Natural antioxidants such as tocopherol are safe, but their ability to block oxidative
chain reaction is low and is on the expensive side (Omaye et al. 1977). Many studies have focused on the development of natural products that are safe and have excellent antioxidant activity in various herbal medicines and edible plant extracts for the prevention of various diseases associated with oxidative stress (Kim et al. 1999a; Pohl and Lin 2018).

Plant sterols simply called phytosterols are unsaponifiable triterpenes with 27–30 carbons. More than 200 sterols have been already identified, namely campesterol, stigmasterol (ST), β-sitosterol (BS), stigmastanol, and cholesterol (Akhisa 1991). Phytosterols including plant stanols and sterols occur commonly in plants and differ only in their carbon side chain and/or the presence of a double bond (Patterson 2006). The only difference between ST and BS is the presence of a double bond (C22=C23) in ST and a single bond (C22-C23) in BS as shown in Fig. 1 (Griebel and Zeier 2010). Both ST (Fig. 1A) and BS (Fig. 1B) have very similar chemical structures. Additionally, phytosterols exhibit a similar chemical structure to that of cholesterols, and therefore competitively inhibit cholesterol absorption in the intestine, thus reducing cholesterol serum levels (Badole et al. 2011). Unlike fungal and animal cells, which contain only one major sterol, plant cells synthesize sterol mixtures in which ST and BS are predominant (Hartmann 1998). ST is an unsaturated plant sterol and one of the most abundant phytosterols in plants. This compound plays a significant role in maintaining the structure and physiological properties of the cell membrane (Ferrer et al. 2017). Additionally, ST (Fig. 1A) has been reported to neutralize viper venom (Gomes et al. 2007), regulates thyroid hormone and glucose levels (Panda et al. 2009), and possesses anti-cariogenic properties (Yu et al. 2007). BS (Fig. 1B) is a ubiquitous phytosterol in the plant kingdom and reportedly possesses various biological activities including anti-inflammatory, antioxidant, and anti-atherosclerosis properties (Ntanios and Jones 1998; Maruthappan and Shree 2010; Kamboj and Saluja 2011; Gupta et al. 2011). This compound has also been reported to improve the symptoms of rheumatoid arthritis, benign prostatic hypertrophy, and colon cancer (Bouic et al. 1996; Awad and Fink 2000; Awad et al. 2001). Referring to the review by Saeidnia et al. (2014), BS from herbal extracts shows not only antioxidant but also other outstanding effects, so edible sprouts also could be an important new natural source for antioxidant activity that can be used in herbal medicines or drugs. Foods and its additives as well as supplements rich in phytosterols have been marketed for decades, and people’s interest has been increasing. The richest sources of phytosterols are vegetable oils and products made from them. However, high intake of phytosterols increases the risk of heart disease, so it is important to consume adequate amounts of phytosterols (Yoo 2016).

Except for special cases such as vegetarians, the intake of phytosterols ranges between 200 and 300 mg·day⁻¹ depending on eating habits (Jesch and Carr 2017). Many scientists proved that the intake of 1.5-2.0 g·day⁻¹ of phytosterols can reduce LDL cholesterol levels by 10–15% (Ostlund Jr 2002; EFSA 2008). Specifically, FDA has approved that foods containing phytosterols reduced the risk of heart disease, particularly when consumed at least 1.3 g·day⁻¹ sterol, twice a day (EFSA 2008). Recently, several studies have been conducted on sprout plants in relation to the environment. Micromorphological characteristics may provide information in determining the response of the plant to environmental conditions (Sevik et al. 2021; Yucedag et al. 2021) examined the impacts of altitude and specific parameters on seed germination of Syrian juniper. In addition, Ozcel et al. (2021) identified the effects of UV-B radiation on germination and seedling characteristics of the seeds of Anatolian black pine. As such, edible sprouts have been studied recently, and it seems that the utilization potential is higher than that of the tree.

This study sought to evaluate the antioxidant activities of 18 species of edible tree sprouts, which are typically harvested and consumed in Korea during the spring season. To this end, we measured the DPPH, ·OH, and O₂⁻ radical scavenging activities of the edible sprouts, after which their ST and BS contents of each sprout were quantified using HPLC/UV to assess the relationship between their radical scavenging capacity and antioxidant contents.

![Fig. 1 Chemical structures of ST (A) and BS (B)](image-url)
2 Materials and methods

2.1 Plant materials

Dried powder samples from 18 different species of edible sprouts were obtained from the Department of Forest Biore-sources, National Institute of Forest Science, Suwon, Korea. The studied edible sprout species included Actinidia arguta Planch. [Actinidiaceae] (AAG), A. polygama Maxim. [Actinidiaceae] (APG), A. kolomikta Maxim. [Actinidiaceae] (AKM), Aralia elata (Miq.) Seem. [Araliaceae] (AET), Eleutherococcus sessiliflorus (Rupr. & Maxim.) S.Y.Hu [Araliaceae] (ESF), E. senticosus (Rupr. & Maxim.) Maxim. [Araliaceae] (ESC), E. gracilistylus (W.W.Sm.) S.Y.Hu [Araliaceae] (EGS), Kalopanax septemlobus (Thunb. ex A.Murr.) Koidz. [Araliaceae] (KSL), Rhus verniciflua Stokes [Anacardiaceae] (RVF), Staphylea humalda DC. [Staphyleaceae] (SB), Morus alba L. [Moraceae] (MAB), Securinega suffruticosa (Pall.) Rehder [Euphorbiaceae] (SS), Cedrela sinensis A. Juss. [Meliaceae] (CSS), Euonymus alatus (Thunb.) Sieb. [Celastraceae] (EAT), Fraxinus mandshurica Rupr. [Oleaceae] (FMS), Philadelphus schrenkii Rupr. [Hydrangeaceae] (PSR), Toxicodendron trichocarpum (Miq.) Kuntze [Anacardiaceae] (TTC), and Lycium chinense Mill. [Solanaceae] (LCS). Each tree sprouts were collected after budding and leaf-expanding before harden-ing in the Experimental Forest of National Institute of For-est Science located in Suwon, Korea in March-May, 2019. In this area, the soil was sandy loam and average temperature was 6.6 °C (March)-18.4 °C (May) (Weather Data Service, Korea Meteorological Administration). No irrigation and fertilization were conducted. At least two or three individuals for each species were selected to collect the edible sprouts. Collected sprouts were immediately cryopreserved and lyophilized for the subsequent analysis. All experiments and analysis were completed within six months after sample preparation. Voucher specimens were deposited at our department, Anseong, Korea.

2.2 Reagents and instruments

DPPH and 2-deoxy-ribose were obtained from Sigma (St. Louis, MO, USA); FeSO₄·7H₂O-EDTA and hydrogen per-oxide were obtained from Daejung Chemicals and Metals Co. Ltd. (Siheung, Korea) and Junsei (Tokyo, Japan), respecti-vely. Phenazine methosulfate (PMS), nitrotertazolium blue chloride (NBT), and NADH disodium salt were obtained from Bio Basic Co. (Toronto, Canada). Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were obtained from Acros Organics (Fair Lawn, NJ, USA) and Kanto Chemical Co. Inc (Tokyo, Japan), respectively. ST (Fig. 1 A) and BS (Fig. 1B) were obtained from Natural Product Institute of Science and Technology (www.nist.re.kr; Anseong, Korea). The solvents used for HPLC were HPLC grade and were purchased from J. T. Baker (Phillipsburg, PA, USA). The methanol (MeOH) and ethanol (EtOH) used in our experiments were of analytical grade and were purchased from Samchun Pure Chemicals (Pyeongtaek, Korea). Chromato-graphic analysis was performed using an HPLC system (Agilent Technology 1290 Infinity II, CA, USA) equipped with a pump, auto-sampler, and UV detector.

2.3 Preparation of samples and stock solutions

The powdered samples from all 18 species of edible sprouts (10 g each) were extracted using EtOH (200 mL) for 3 h under constant reflux; this procedure was repeated three times. After extraction, the samples were filtered and evaporated to obtain an extract. The weights of the extract were the following: AAG (0.9 g), AET (1.3 g), AKM (0.8 g), APG (2.3 g), CSS (1.3 g), EAT (2.0 g), EGS (0.6 g), ESC (0.6 g), ESF (1.8 g), FMS (5.0 g), KSL (1.7 g), LCS (2.9 g), MAB (1.0 g), PSR (3.1 g), RVF (2.2 g), SBD (1.2 g), SST (2.3 g), and TTC (4.4 g). An experimental stock solution was prepared by dissolving 20 mg of each edible sprout extract in MeOH under sonication for 20 min and filtered using a 0.45-µm PVDF membrane filter. Afterward, standard stock solutions were prepared by dissolving 1 mg of ST and BS in MeOH under sonication for 20 min. The solutions were then filtered as described above.

2.4 Measurement of DPPH radical scavenging activity

Reaction mixtures (200 µL) were prepared by combining 100 µL of each sample and 100 µL of 60 µM DPPH solution in 96 well plates as described by Hatano et al. (1989). The mixtures were then incubated in the dark for 30 min. Sample absorbance at 540 nm was measured using a microplate reader (Thermo Fisher Scientific, Vantaa, Finland). EtOH was used as a control (blank). The DPPH radical scavenging activity of the sample (%) was calculated as follows:

\[
\text{DPPH radical scavenging activity (\%) = } \left( \frac{A - B}{A} \right) \times 100.
\]

(A: Absorbance of the control; B: Absorbance of the sample)

2.5 Measurement of ·OH scavenging activity

The ·OH radical scavenging activity was measured according to Gutteridge (1987). Reaction mixtures (2 mL) containing each of the edible sprout extracts, 10 mM FeSO₄·7H₂O-EDTA, 10 mM 2-deoxyribose, and 10 mM hydrogen peroxide were incubated in the dark at 37°C for 4 h. Both 1% TBA and 2.8% TCA solutions were added...
to the mixture and then incubated in the dark at 100°C for another 20 min. Sample absorbances were measured at 490 nm using a microplate reader. Phosphate buffered saline was used as a control (blank). The ·OH radical scavenging activity of the sample (%) was calculated as follows:

\[
\text{·OH radical scavenging activity (\%)} = \frac{(A - B)}{A} \times 100.
\]

(A: Absorbance of the control; B: Absorbance of the sample)

### 2.6 \(O_2^-\) radical scavenging activity

The \(O_2^-\) radical scavenging activity of the edible sprout extracts was assessed according to Ewing and Janero (1995). Reaction mixtures (1,200 µL) containing each of the edible sprout extracts, 0.1 M Tris-HCl, 100 µM PMS, 500 µM NBT, and 500 µM NADH. These mixtures were then incubated at room temperature in a dark room for 10 min. Sample absorbance was measured at 560 nm using a microplate reader. Distilled water was used as a control (blank). \(O_2^-\) radical scavenging activity (%) was calculated as follows:

\[
\text{\(O_2^-\) radical scavenging activity (\%)} = \frac{(A - B)}{A} \times 100.
\]

(A: Absorbance of the control; B: Absorbance of the samples)

### 2.7 HPLC conditions

Quantification of ST and BS was performed in an isocratic elution HPLC system using a reverse phase YMC-Pack Pro C\(_{18}\) column (4.6×150 mm, 5 µm), which was maintained at room temperature throughout the experiment. UV detection was conducted at 210 nm. The mobile phase consisted of MeOH (A) and acetonitrile (B) and the isocratic elution system was composed of 30% A and 70% B for 40 min. The injection volume was 10 µL and the flow rate was 1 mL·min\(^{-1}\). All injections were performed in triplicate.

### 2.8 Calibration curves

Standard stock solutions of ST and BS were prepared by dissolving compounds in MeOH (1 mg·mL\(^{-1}\)). To construct the calibration curve, the working solutions were prepared by serially diluting the stock solutions. The calibration functions of ST and BS were calculated using the peak area (Y) and concentration (X, mg·mL\(^{-1}\)) and were reported as mean ± standard deviation (SD) \((n = 3)\).

### 2.9 Statistical analysis

All results in Table 1 were reported as mean ± SD. The differences in the scavenging activities of each of the free radicals and edible sprouts analyzed herein were evaluated via analysis of variance (ANOVA) followed by Duncan's Multiple Range test. A P-value < 0.05 was considered statistically significant.

### 3 Results and discussion

Free radicals are highly reactive due to its one or more unpaired electrons (Liguori et al. 2018; Jakubczyk et al. 2020). Free radicals react to donating or accepting electrons or hydrogens from other molecules to accomplish a more stable state, attacking other biomolecules along the process.

### Table 1 Radical scavenging activities of edible sprouts

| Sample | Scavenging activity (%) | \(DPPH\) | ·OH | \(O_2^-\) |
|--------|------------------------|---------|-----|---------|
| AAG    | 70.85 ± 2.66\(^c\)    | 84.09 ± 0.47\(^f\) | 12.64 ± 0.99\(^h\) |
| APG    | 24.63 ± 1.93\(^d\)    | 88.46 ± 0.66\(^e\) | 20.63 ± 1.48\(^e\) |
| AKM    | 86.82 ± 4.96\(^a\)    | 88.64 ± 1.08\(^d\) | 25.91 ± 1.42\(^d\) |
| AET    | 68.41 ± 2.27\(^b\)    | 91.91 ± 1.83\(^d\) | 2.85 ± 0.48\(^c\) |
| ESP    | 52.91 ± 3.45\(^a\)    | 83.50 ± 0.99\(^e\) | 36.10 ± 1.77\(^e\) |
| ESC    | 88.51 ± 4.03\(^a\)    | 81.97 ± 1.75\(^d\) | 19.18 ± 1.20\(^e\) |
| EGS    | 67.48 ± 2.80\(^b\)    | 94.89 ± 1.42\(^d\) | 20.17 ± 1.04\(^d\) |
| KSL    | 80.50 ± 2.77\(^b\)    | 89.66 ± 0.68\(^e\) | 24.75 ± 0.98\(^e\) |
| RVF    | 78.82 ± 3.19\(^b\)    | 77.31 ± 1.88\(^d\) | 32.18 ± 1.55\(^d\) |
| SBD    | 46.14 ± 3.92\(^f\)    | 81.93 ± 1.14\(^e\) | 25.34 ± 0.50\(^c\) |
| MAB    | 89.44 ± 3.24\(^a\)    | 84.18 ± 1.07\(^d\) | 24.89 ± 0.21\(^e\) |
| SST    | 70.31 ± 4.16\(^c\)    | 82.49 ± 1.23\(^b\) | 32.97 ± 1.71\(^d\) |
| CSS    | 80.78 ± 3.13\(^b\)    | 87.40 ± 1.20\(^f\) | 37.46 ± 1.44\(^b\) |
| EAT    | 60.07 ± 3.92\(^d\)    | 92.25 ± 1.77\(^h\) | 16.41 ± 1.57\(^b\) |
| FMS    | 70.28 ± 3.58\(^c\)    | 89.38 ± 0.50\(^b\) | 4.72 ± 1.56\(^d\) |
| PSR    | 58.12 ± 2.03\(^d\)    | 91.82 ± 0.67\(^c\) | 17.10 ± 1.26\(^d\) |
| TTC    | 81.63 ± 5.20\(^b\)    | 90.68 ± 0.78\(^c\) | 43.33 ± 1.03\(^d\) |
| LCS    | 78.54 ± 2.23\(^b\)    | 92.00 ± 0.21\(^b\) | 38.07 ± 3.50\(^b\) |

All values are reported as means ± SD

\(^a\#\) Means with different letters are significantly different \((P < 0.05)\), as determined by Duncan's multiple range test.
(Jakubczyk et al. 2020). However, the body has antioxidant defense system such as antioxidant enzymes including glutathione peroxidase, superoxide dismutase, and catalase, to counteract free radical damages in the body (Birben et al. 2012). Additionally, consumption of natural products with antioxidant activity plays a significant role in the antioxidant defense system of body (Peng et al. 2014).

Many edible natural products were reported to possess antioxidant activity by radical scavenging activities with no side effects and toxicity compared with the synthetic antioxidants (Kahl and Kappus 1993; Prakash et al. 2012). These edible natural products mainly contain compounds such as flavonoids and polyphenols, and they are widely known to exhibit antioxidant activities (Tungmunnithum et al. 2018). Therefore, many studies focus on development of natural antioxidants from edible natural plants for treating and preventing oxidative stress-related diseases (Chanwitheesuk et al. 2005). Since ancient times, edible sprouts that are often consumed during spring season in Korea have been usually blanched and eaten, and it has pleasant flavor. The sprouts of *Rosa multiflora* are said to help children grow taller, so they were used as snacks for children in the past. EAT sprouts were eaten in spring, dried and consumed as tea, and decoction was applied to abscess. Edible tree sprouts consumed in spring season in Korea have been known to possess several health benefits. For example, CSS sprouts have antioxidant and whitening effects (Kim et al. 2010). KSL has been widely long used for its anti-inflammatory and anti-pain activities. Recently, several varieties of KSL sprouts showed antioxidant activity (Song et al. 2015). Moreover, sprouts of *Pinus densiflora* showed antioxidant and anti-inflammatory effects (Cho et al. 2009). Therefore, this study examined the antioxidant activity of 18 different edible sprout extracts by assessing their in vitro DPPH, .OH, and O$_2^-$ radical scavenging activities at a concentration of 50 µg·mL$^{-1}$ (Table 1).

DPPH is a stable free radical and it has violet color. Antioxidants directly react with DPPH radical by transferring electrons or hydrogen donor resulting in a color change from violet to yellow (Hatano et al. 1989). Therefore, DPPH assay can predict and evaluate the antioxidant activities of samples (Narayanaswamy and Balakrishnan 2011). In the previous studies, DPPH radical scavenging assay used to investigate antioxidant activity of many different matrices such as the hydrophilic vegetable extract, vegetable oils, bergamot juice, and the other natural extracts (Giuffrè et al. 2018; Giuffrè 2019). Interestingly, the DPPH radical scavenging activities of the extracts varied widely from 24.63% in APG to 89.44% in MAB [MAB (89.44%) > ESC (88.51%) > AKM (86.82%) > TCC (81.63%) > CSS (80.78%) > KSL (80.50%) > RVF (78.82%) > LCS (78.54%) > AAG (70.85%) > SST (70.31%) > FMS (70.28%) > AET (68.41%) > EGS (67.48%) > EAT (60.07%) > PSR (58.12%) > ESF (52.91%) > SBD (46.14%) > APG (24.63%)]. Particularly, MAB and ESC exhibited the highest DPPH radical scavenging activities (89.44% and 88.51%, respectively) among the 18 edible sprout species. MAB is a fast-growing mulberry species that belongs to the Moraceae family. Traditionally, its fruits have been used as food and medicinal ingredient, and dried MAB leaves are used as tea. On the other hand, ESC, also known commonly as “Siberian ginseng”, is a species in the Araliaceae family whose fruits and stems are widely known for their therapeutic properties. Several studies have reported the health benefits of MAB and ESC for the treatment of oxidative stress-related diseases such as diabetes, cancer, and inflammation, suggesting that these plants possess strong antioxidant properties (Kim et al. 1999b, 2005; Arouca and Grassi-Kassisse 2013; Chan et al. 2016).

The .OH radical naturally occurs by oxidative metabolism in the body, and it is mainly generated from O$_2^-$ radical and H$_2$O$_2$ in the body (Lipinski 2011; Jakubczyk et al. 2020). .OH quickly reacts with other biomolecules such as lipids, proteins, and DNA, and there is no specific enzyme to remove it in humans (Lipinski 2011). Therefore, .OH radical is considered as strong ROS. To evaluate the .OH radical scavenging activities of edible sprouts, .OH radical is formed by the Fe$^{3+}$-EDTA and H$_2$O$_2$ (Fenton reaction) in this study. In addition, .OH radical causes 2-deoxyribose degradation and generates a malondialdehyde-like product (Gutteridge 1987). In our results, the .OH radical scavenging activities of the edible sprouts were as follows: AAG (84.09%), APG (88.46%), AKM (88.64%), AET (91.91%), ESF (83.50%), ESC (81.97%), ESC (81.97%), EGS (94.89%), KSL (89.66%), RVF (77.31%), SBD (81.93%), MAB (84.18%), SST (82.49%), CSS (87.40%), AAG (92.25%), FMS (89.38%), PSR (91.82%), TTC (90.68%), and LCS (92.00%). The .OH radical scavenging activities of all edible sprouts exceeded 80% except for RVF (77.31%). Therefore, edible sprouts exhibit .OH radical scavenging activity, suggesting their promising role as free radical scavengers.

The O$_2^-$ radical is generated from H$_2$O$_2$ by dismutation reaction in the body (Jakubczyk et al. 2020). In particularly, O$_2^-$ radical directly reacts with lipid peroxidation and produces other ROS such as .OH radical (Robak and Gryglewski 1988). Therefore, O$_2^-$ radical is very harmful species. In the O$_2^-$ radical assay, PMS/NADH system generates O$_2^-$ radical and reduces NBT into a purple-colored formazan (Ewing and Janero 1995). In this study, the O$_2^-$ radical scavenging activities of the edible sprouts analyzed herein were generally lower than those of .OH, ranging from 2.85% in AET to 43.33% in TTC. The O$_2^-$ radical scavenging activities of edible sprouts are in the following order:
several bioactive compounds such as gallic acid, methyl gallate, 1-O-galloyl-β-d-glucose, and 1,2,3,4,6-penta-O-galloyl-β-d-glucose (Liu et al. 2019).

Two antioxidant phytosterols, ST (Fig. 1A) and BS (Fig. 1B), were quantified via HPLC analyses. The HPLC chromatogram exhibited separation and retention times of 31.0 and 36.2 min for ST and BS, respectively (Fig. 2). ST and BS were effectively detected at a 210 nm wavelength. Table 2 details the standard calibration curves of ST and BS. Each calibration curve was determined by plotting the peak area against the prepared concentrations, followed by

TTC (43.33%) > LCS (38.07%) > CSS (37.46%) > ESF (36.10%) > SST (32.97%) > RVF (32.18%) > AKM (25.91%) > SBD (25.34%) > MAB (24.89%) > KSL (24.75%) > APG (20.63%) > EGS (20.17%) > ESC (19.18%) > PSR (17.10%) > EAT (16.41%) > AAG (12.64%) > FMS (4.72%) > AET (2.85%). Particularly, the \( \text{O}_2^- \) radical scavenging activity of TTC (43.33%) was the highest among the edible sprout species analyzed herein. A previous study demonstrated the outstanding in vitro antioxidant activity of \textit{Rhus typhina} stems (i.e., a tree species belongs to the same genus as TTC), which was linked to several bioactive compounds such as gallic acid, methyl gallate, 1-O-galloyl-β-d-glucose, and 1,2,3,4,6-penta-O-galloyl-β-d-glucose (Liu et al. 2019).

Two antioxidant phytosterols, ST (Fig. 1 A) and BS (Fig. 1B), were quantified via HPLC analyses. The HPLC chromatogram exhibited separation and retention times of 31.0 and 36.2 min for ST and BS, respectively (Fig. 2). ST and BS were effectively detected at a 210 nm wavelength. Table 2 details the standard calibration curves of ST and BS. Each calibration curve was determined by plotting the peak area against the prepared concentrations, followed by
Table 2  Calibration curves of ST and BS

| Compound | $b$ | Calibration equation | Correlation factor, $r^2$ |
|----------|-----|-----------------------|--------------------------|
| ST       | 31.0 | $Y = 2.7049X + 8.0902$ | 0.9996                   |
| BS       | 36.2 | $Y = 2.8381X + 9.7838$ | 0.9999                   |

$Y =$ peak area, $X =$ concentration of standards (mg mL$^{-1}$) 
$r^2 =$ correlation coefficient based on five data points in the calibration curves

Table 3  ST and BS contents in the edible sprouts

| Sample | ST (mg·g$^{-1}$ extract) | BS (mg·g$^{-1}$ extract) |
|--------|--------------------------|--------------------------|
| AAG    | 2.00±0.15                | 8.12±0.09                |
| APG    | 3.00±0.24                | 9.20±0.8                 |
| AKM    | 1.07±0.22                | 10.35±0.15               |
| AET    | 2.65±0.37                | 0.58±0.08                |
| ESF    | 6.84±0.73                | 3.81±0.68                |
| ESC    | 9.99±0.31                | 0.96±0.25                |
| EGS    | 3.03±0.81                | 0.07±0.01                |
| KSL    | 8.91±0.12                | 2.02±0.12                |
| RVF    | 1.04±0.20                | 9.52±0.61                |
| SBD    | 2.45±0.24                | 4.07±0.56                |
| MAB    | 1.56±0.23                | 14.69±0.60               |
| SST    | 1.20±0.01                | 4.39±0.66                |
| CSS    | 1.28±0.49                | 14.40±0.95               |
| EAT    | 0.73±0.13                | 9.17±0.66                |
| FMS    | 0.07±0.07                | 3.10±0.56                |
| PSR    | 4.72±0.77                | 0.24±0.15                |
| TTC    | 0.11±0.05                | 1.83±0.27                |
| LCS    | 0.74±0.12                | 2.59±0.60                |

linear regression analysis. Both ST and BS exhibited good correlation coefficients ($r^2$) of 0.9996 and 0.9999, respectively. The peaks of ST and BS were identified in the HPLC chromatograms of the 18 edible sprout species studied herein, and the content of each compound was calculated using the calibration equation. The contents of ST and BS in the edible sprouts were summarized in Table 3. The concentrations of BS were generally higher than those of ST in the studied edible sprouts; however, this may vary in a species dependent manner. The highest ST and BS contents were observed in ESC (9.99 mg·g$^{-1}$ extract) and MAB (14.69 mg·g$^{-1}$ extract), respectively (Table 3).

Many studies on the simultaneous analysis of ST and BS have been conducted, and our isocratic HPLC method seems to be also suitable for quantitative analysis. Talreja et al. (2017) simultaneously analyzed ST, BS, campesterol and stigmasterol by HPLC/UV using a wavelength of 205 nm, and the contents of ST and BS in Achyranthes aspera were 8.29 and 10.12 µg·g$^{-1}$, respectively. Xu et al. (2014) developed HPLC method and quantified lupenone and BS in Musa basjoo by isocratic elution system using acetoniitrile:MeOH (50:50, v/v), and the contents of BS ranged from 209.0 to 1183.8 µg·g$^{-1}$ from different origin. Nachimuthu and Palaniswamy (2013) reported that the content of ST in the leaves of Dipteracanthus patulus used in traditional medicine in India was 0.22 mg·g$^{-1}$ DW when they used Soxhlet extraction method for 48 h using 85% MeOH. Moreover, Shilajain and Swar (2013) quantified the BS in Rhododendron arboreum leaves and flowers using high-performance thin-layer chromatographic (HPTLC) method. Generally, the contents of leaves (1.02–1.26 mg·g$^{-1}$) were higher than that of flowers (0.53–0.92 mg·g$^{-1}$), and BS concentrations in this plant exhibited slight regional variations. Additionally, Shailajain and Deepi (2014) evaluated plant pharmacognostic parameters and quantified the BS in Chrysophyllum cainito leaves from the Sion and Matunga regions using HPTLC methods. The content of both regions was 0.26 and 0.51 mg·g$^{-1}$, respectively. The BS content of MAB and other species in our study was higher than that in the aforementioned study but lower than that of Panax ginseng roots (straight ginseng, red ginseng, and white ginseng) analyzed by Lee et al. (2018) (9.18 to 59.09 mg·g$^{-1}$ DW). Böszörményi et al. (2009) measured content of BS of leaves and stem bark of MAB by several extraction methods, and analytical supercritical-fluid extraction was the best method for extraction of the most BS (5.50–5.67 g·100 g$^{-1}$ extract).

ESC has been reported to contain various components such as lignan, coumarin, diterpene, and triterpenoid in the leaves and fruits (Ovodov et al. 1996). In particular, ESC has excellent adaptogenic activity as well as antioxidant, antimutagenic, and hypoglycemic effects (Brekhmann and Dardymov 1969; Park et al. 2002; Niu et al. 2008). The leaves of MAB are known to prevent and treat diabetes and quench thirst as a traditional herbal medicine. It is well known to contain flavones, steroids, vitamin, triterpenes, minerals, and amino acids (Chen et al. 1995; Kim et al. 2000) analyzed in vitro antioxidant activity against MAB and Cudrania tricuspidata, the leaves of MAB showed antioxidant activity in free radical level of DPPH.

4 Conclusions

Oxidative stress is closely related to various diseases such as diabetes, cancer, and dementia along with the aging process. Due to the limitations of existing synthetic or natural antioxidants, it is necessary to find natural products which are safe and have excellent antioxidant activity. This study evaluated the in vitro radical scavenging activities of edible sprouts that are often traditionally consumed during spring in Korea. Two sterols with similar structures, ST and BS, have been reported to have various effects including anti-inflammatory, anti-atherosclerosis, and anti-cariogenic as well as antioxidant effect. The contents of ST and BS were also calculated using HPLC/UV. Several edible sprout species have been found to possess antioxidant properties. In
particular, both ESC and MAB exhibited an outstanding DPPH radical scavenging activity compared to the other edible sprout species, which coincided with their high ST and BS contents, respectively. Additionally, the ST and BS analyses conducted herein could serve as a methodological framework to evaluate the free radical scavenging activities of other plants. Our findings provide a theoretical basis for the development of plant-based functional food and supplements with antioxidant properties.

Acknowledgements This research was supported by the Chung-Ang University Research Grant in 2020 and the Research Program for Forest Science & Technology Development of the National Institute of Forest Science (Project No. FG0403-2018-03).

Author Contribution JK performed HPLC analysis and wrote manuscript; JHK and SIB performed anti-oxidant activity experiments; HS analyzed the data and material preparation; EJC analyzes antioxidant activity data and wrote manuscript; SL designed experiments and wrote manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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