



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

Juree Kim¹ · Ji Hyun Kim² · Se In Bang³ · Hanna Shin⁴ · Eun Ju Cho³ · Sanghyun Lee^{1,5,6}

Received: 27 June 2021 / Revised: 21 February 2022 / Accepted: 21 February 2022 / Published online: 3 August 2022
© The Author(s) 2022, corrected publication 2022

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; Jirakitikul 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 particular, smaller particles in natural antioxidant enable the release of phenolic and flavonoids contents by increasing solubility structures (Zahirul Islam et al. 2019, 2020). Therefore, natural antioxidant enhancing 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 (Brannen 1975). Natural antioxidants such as tocopherol are safe, but their ability to block oxidative

J Kim and JH Kim contributed equally.

Communicated by Eun Jin Lee.

✉ Sanghyun Lee
slee@cau.ac.kr

¹ Department of Plant Science and Technology, Chung-Ang University, 17546 Anseong, Republic of Korea

² Department of Food Science, Gyeongsang National University, 52725 Jinju, Republic of Korea

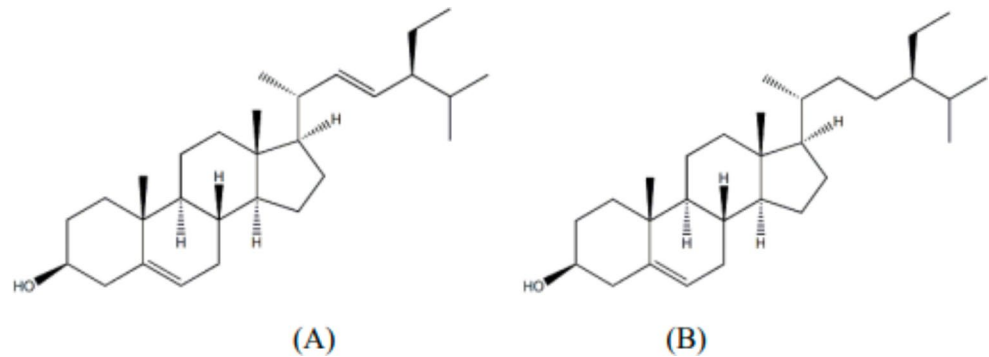
³ Department of Food Science and Nutrition, Pusan National University, 46241 Busan, Republic of Korea

⁴ Department of Forest Bioresources, National Institute of Forest Science, 16631 Suwon, Republic of Korea

⁵ BET Research Institute, Chung-Ang University, 17546 Anseong, Republic of Korea

⁶ Natural Product Institute of Science and Technology, 17546 Anseong, Republic of Korea

Fig. 1 Chemical structures of ST (A) and BS (B)



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), stigmasterol, 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. 1 A) and BS (Fig. 1B) have very similar chemical structures. Additionally, phytosterols exhibit a similar chemical structure to that of cholesterol, 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. 1 A) 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 (Ntanos 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, Ozel 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, $\cdot\text{OH}$, and $\text{O}_2^{\cdot-}$ 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.

2 Materials and methods

2.1 Plant materials

Dried powder samples from 18 different species of edible sprouts were obtained from 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 bumalda* DC. [Staphyleaceae] (SBD), *Morus alba* L. [Moraceae] (MAB), *Securinega suffruticosa* (Pall.) Rehder [Euphorbiaceae] (SST), *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 hardening in the Experimental Forest of National Institute of Forest 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 peroxide were obtained from Daejung Chemicals and Metals Co. Ltd. (Siheung, Korea) and Junsei (Tokyo, Japan), respectively. Phenazine methosulfate (PMS), nitrotriazolium 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). Chromatographic 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:

$$\text{DPPH radical scavenging activity (\%)} = (A-B)/A * 100.$$

(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

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 ^g	12.64 ± 0.99 ^h
APG	24.63 ± 1.93 ^g	88.46 ± 0.66 ^{ef}	20.63 ± 1.48 ^f
AKM	86.82 ± 4.96 ^a	88.64 ± 1.08 ^{ef}	25.91 ± 1.42 ^e
AET	68.41 ± 2.27 ^c	91.91 ± 1.83 ^{bc}	2.85 ± 0.48 ^j
ESF	52.91 ± 3.45 ^e	83.50 ± 0.99 ^{hi}	36.10 ± 1.77 ^c
ESC	88.51 ± 4.03 ^a	81.97 ± 1.75 ^j	19.18 ± 1.20 ^f
EGS	67.48 ± 2.80 ^c	94.89 ± 1.42 ^a	20.17 ± 1.04 ^f
KSL	80.50 ± 2.77 ^b	89.66 ± 0.68 ^{de}	24.75 ± 0.98 ^e
RVF	78.82 ± 3.19 ^b	77.31 ± 1.88 ^k	32.18 ± 1.55 ^d
SBD	46.14 ± 3.92 ^f	81.93 ± 1.14 ^j	25.34 ± 0.50 ^e
MAB	89.44 ± 3.24 ^a	84.18 ± 1.07 ^{gh}	24.89 ± 0.21 ^e
SST	70.31 ± 4.16 ^c	82.49 ± 1.23 ^{ij}	32.97 ± 1.71 ^d
CSS	80.78 ± 3.13 ^b	87.40 ± 1.20 ^f	37.46 ± 1.44 ^{bc}
EAT	60.07 ± 3.92 ^d	92.25 ± 1.77 ^b	16.41 ± 1.57 ^g
FMS	70.28 ± 3.58 ^c	89.38 ± 0.50 ^{de}	4.72 ± 1.56 ⁱ
PSR	58.12 ± 2.03 ^d	91.82 ± 0.67 ^{bc}	17.10 ± 1.26 ^g
TTC	81.63 ± 5.20 ^b	90.68 ± 0.78 ^{cd}	43.33 ± 1.03 ^a
LCS	78.54 ± 2.23 ^b	92.00 ± 0.21 ^{bc}	38.07 ± 3.50 ^b

All values are reported as means ± SD

^{a–g}Means with different letters are significantly different ($P < 0.05$), as determined by Duncan's multiple range test

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:

$$\cdot\text{OH radical scavenging activity (\%)} = (A-B)/A * 100.$$

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

2.6 O₂^{·-} radical scavenging activity

The O₂^{·-} 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). O₂^{·-} radical scavenging activity (%) was calculated as follows:

$$\text{O}_2^{\cdot-} \text{ radical scavenging activity (\%)} = (A-B)/A * 100.$$

(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 C₁₈ 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

(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 (Tungmunthum 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, $\cdot\text{OH}$, and O_2^- radical scavenging activities at a concentration of $50 \mu\text{g}\cdot\text{mL}^{-1}$ (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%) > TTC (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 $\cdot\text{OH}$ radical naturally occurs by oxidative metabolism in the body, and it is mainly generated from O_2^- radical and H_2O_2 in the body (Lipinski 2011; Jakubczyk et al. 2020). $\cdot\text{OH}$ 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, $\cdot\text{OH}$ radical is considered as strong ROS. To evaluate the $\cdot\text{OH}$ radical scavenging activities of edible sprouts, $\cdot\text{OH}$ radical is formed by the Fe^{3+} -EDTA and H_2O_2 (Fenton reaction) in this study. In addition, $\cdot\text{OH}$ radical causes 2-deoxyribose degradation and generates a malondialdehyde-like product (Gutteridge 1987). In our results, the $\cdot\text{OH}$ 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%), EAT (92.25%), FMS (89.38%), PSR (91.82%), TTC (90.68%), and LCS (92.00%). The $\cdot\text{OH}$ radical scavenging activities of all edible sprouts exceeded 80% except for RVF (77.31%). Therefore, edible sprouts exhibit $\cdot\text{OH}$ radical scavenging activity, suggesting their promising role as free radical scavengers.

The O_2^- radical is generated from H_2O_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 $\cdot\text{OH}$ 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 $\cdot\text{OH}$, 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:

Fig. 2 HPLC chromatogram of ST and BS

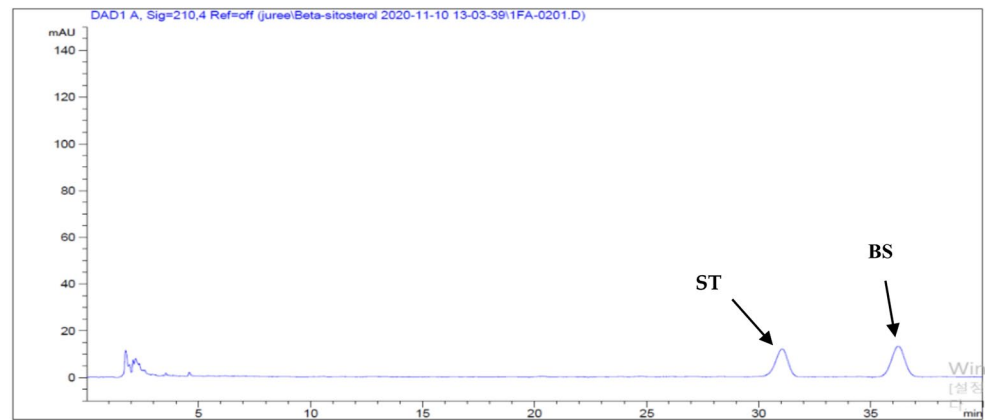
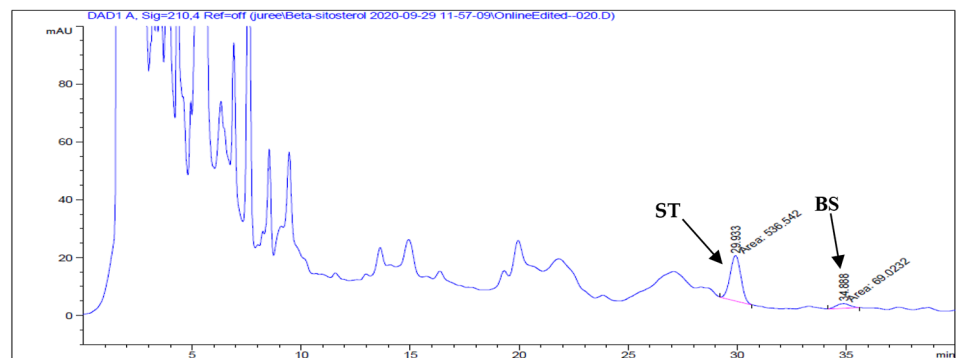
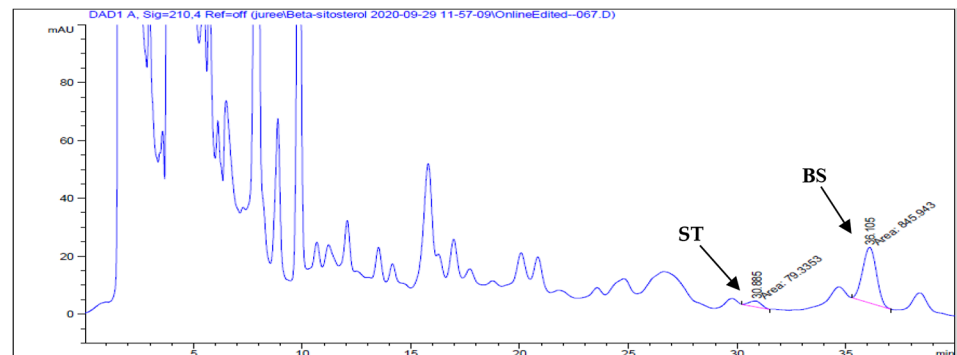


Fig. 3 HPLC chromatograms of the ESC (A) and MAB (B) EtOH extracts



(A)



(B)

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

^a Y = peak area, X = concentration of standards ($\text{mg} \cdot \text{mL}^{-1}$)

^b r^2 = correlation coefficient based on five data points in the calibration curves

Table 3 ST and BS contents in the edible sprouts

Sample	Content ($\text{mg} \cdot \text{g}^{-1}$ extract)	
	ST	BS
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 ($9.99 \text{ mg} \cdot \text{g}^{-1}$ extract) and MAB ($14.69 \text{ mg} \cdot \text{g}^{-1}$ 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 stigmastanol by HPLC/UV using a wavelength of 205 nm, and the contents of ST and BS in *Achyranthes aspera* were 8.29 and $10.12 \mu\text{g} \cdot \text{g}^{-1}$, respectively. Xu et al. (2014) developed HPLC method and quantified lupenone and BS in *Musa basjoo* by isocratic elution system using acetonitrile:MeOH (50:50, v/v), and the contents of BS ranged from 209.0 to $1183.8 \mu\text{g} \cdot \text{g}^{-1}$ 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 $0.22 \text{ mg} \cdot \text{g}^{-1}$ DW when they used Soxhlet extraction method for 48 h using 85% MeOH. Moreover, Shilajan and Swar (2013) quantified the BS in *Rhododendron arboretum* leaves and flowers using high-performance thin-layer chromatographic (HPTLC) method. Generally, the contents of leaves (1.02 – $1.26 \text{ mg} \cdot \text{g}^{-1}$) were higher than that of flowers (0.53 – $0.92 \text{ mg} \cdot \text{g}^{-1}$), and BS concentrations in this plant exhibited slight regional variations. Additionally, Shailajan and Deepti (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 $0.51 \text{ mg} \cdot \text{g}^{-1}$, 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 $59.09 \text{ mg} \cdot \text{g}^{-1}$ 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 – $5.67 \text{ g} \cdot 100 \text{ g}^{-1}$ 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 (Brekhsman 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 anti-oxidant activity data and wrote manuscript; SL designed experiments and wrote manuscript.

Declarations

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

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Akhisa T (1991) Naturally occurring sterols and related compounds from plants. In: Patterson GW, Nes WD (eds) *Physiology and Biochemistry of Sterols*. American Oil Chemists' Society, Champaign, IL, pp 172–228
- Arouca A, Grassi-Kassisse DM (2013) *Eleutherococcus senticosus*: Studies and effects. *Health* 2013. <https://doi.org/10.4236/health.2013.59205>
- Awad AB, Fink CS (2000) Phytosterols as anticancer dietary components: Evidence and mechanism of action. *J Nutr* 130:2127–2130. <https://doi.org/10.1093/jn/130.9.2127>
- Badole M, Dighe V, Charegaonkar G (2011) Simultaneous quantification of β -amyrin and stigmasterol in *Putranjiva roxburghii* Wall. by high-performance thin-layer chromatography. *Int J Pharma Bio Sci* 2:346–352
- Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidative stress and antioxidant defense. *World Allergy Organ J* 5:9–19. <https://doi.org/10.1097/WOX.0b013e3182439613>
- Bouali I, Tsafouros A, Ntanos E, Albouchi A, Boukhchina S, Rousos PA (2020) Inter-cultivar and temporal variation of phenolic compounds, antioxidant activity and carbohydrate composition of pecan (*Carya illinoensis*) kernels grown in Tunisia. *Hortic Environ Biotechnol* 61:183–196. <https://doi.org/10.1007/s13580-019-00188-8>
- Böszörményi A, Szarka S, Hethelyi E, Gyurjan I, Laszlo M, Simándi B, Szőke É, Lemberkovics É (2009) Triterpenes in traditional and supercritical-fluid extracts of *Morus alba* leaf and stem bark. *Acta Chromatogr* 21:659–669. <https://doi.org/10.1556/achrom.21.2009.4.11>
- Bouic PJD, Etsebeth S, Liebenberg RW, Albrecht CF, Pegel K, Van Jaarsveld PP (1996) β -Sitosterol and β -sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination. *Int J Immunopharmacol* 18:693–700. [https://doi.org/10.1016/S0192-0561\(97\)85551-8](https://doi.org/10.1016/S0192-0561(97)85551-8)
- Branen AL (1975) Toxicological and biochemistry of butylated hydroxytoluene, butylated hydroxyanisole. *J Am Oil Chem Soc* 52:59–63. <https://doi.org/10.1007/BF02901825>
- Brekhamann IL, Dardymov ID (1969) New substances of plant origin which increase nonspecific resistance. *Annu Rev Pharmacol* 9:419–430. <https://doi.org/10.1146/annurev.pa.09.040169.002223>
- Chan EWC, Lye PY, Wong SK (2016) Phytochemistry, pharmacology, and clinical trials of *Morus alba*. *Chin J Nat Med* 14:17–30. <https://doi.org/10.3724/SPJ.1009.2016.00017>
- Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N (2005) Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chem* 92:491–497. <https://doi.org/10.1016/j.foodchem.2004.07.035>
- Chen F, Nakashima N, Kimura I, Kimura M (1995) Hypoglycemic activity and mechanisms of extracts from mulberry leaves (*Folium mori*) and cortex mori radices in streptozotocin-induced diabetic mice. *Akugaku Zasshi* 115:476–482. https://doi.org/10.1248/yakushi1947.115.6_476
- Cho EK, Song HJ, Cho HE, Kim M, Choi IS, Choi YJ (2009) Inhibitory effects of ethanol extracts from pine buds (*Pinus densiflora*) on angiotensin converting enzyme, xanthine oxidase and nitric oxide synthesis. *J Life Sci* 19:1629–1636. <https://doi.org/10.5352/JLS.2009.19.11.1629>
- European Food Safety Authority (EFSA) (2008) Plant sterols and blood cholesterol. Scientific substantiation of a health claim related to plant sterols and lower/reduced blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006. *EFSA J* 781:1–12. <https://doi.org/10.2903/j.efsa.2008.781>
- Ewing JF, Janero DR (1995) Microplate superoxide dismutase assay employing a nonenzymatic superoxide generator. *Anal Biochem* 232:243–248. <https://doi.org/10.1006/abio.1995.0014>
- Ferrer A, Altabella T, Arró M, Boronat A (2017) Emerging roles for conjugated sterols in plants. *Prog Lipid Res* 67:27–37. <https://doi.org/10.1016/j.plipres.2017.06.002>
- Fukuda Y, Nagata M (1986) Chemical aspects of the antioxidative activity of roasted sesame seed oil and the effect of using the oil for frying. *Agric Biol Chem* 50:857–861. <https://doi.org/10.1271/bbb1961.50.857>
- Giuffrè AM (2019) Bergamot (*Citrus bergamia*, Risso): The effects of cultivar and harvest date on functional properties of juice and cloudy juice. *Antioxidants* 8:221. <https://doi.org/10.3390/antiox8070221>
- Giuffrè AM, Caracciolo M, Zappia C, Capocasale M, Poiana M (2018) Effect of heating on chemical parameters of extra virgin olive oil, pomace olive oil, soybean oil and palm oil. *Ital J Food Sci* 30:715–739. <https://doi.org/10.14674/IJFS-1269>

- Gomes A, Saha A, Chatterjee I, Chakravarty AK (2007) Viper and cobra venom neutralization by β -sitosterol and stigmasterol isolated from the root extract of *Pluchea indica* Less. (Asteraceae). *Phytomedicine* 14:637–643. <https://doi.org/10.1016/j.phymed.2006.12.020>
- Griebel T, Zeier J (2010) A role for β -sitosterol to stigmasterol conversion in plant-pathogen interactions. *Plant J* 63:254–268. <https://doi.org/10.1111/j.1365-3113X.2010.04235.x>
- Gupta R, Sharma AK, Dobhal MP, Sharma MC, Gupta RS (2011) Antidiabetic and antioxidant potential of β -sitosterol in streptozotocin-induced experimental hyperglycemia. *J Diabetes* 3:29–37. <https://doi.org/10.1111/j.1753-0407.2010.00107.x>
- Gutteridge JM (1987) Ferrous-salt-promoted damage to deoxyribose and benzoate. The increased effectiveness of hydroxyl-radical scavengers in the presence of EDTA. *Biochem J* 243:709–714. <https://doi.org/10.1042/bj2430709>
- Hartmann MA (1998) Plant sterols and the membrane environment. *Trends Plant Sci* 3:170–175. [https://doi.org/10.1016/S1360-1385\(98\)01233-3](https://doi.org/10.1016/S1360-1385(98)01233-3)
- Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T, Yoshida T, Okuda T (1989) Effects of the interaction of tannins with co-existing substances, VI. Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. *Chem Pharm Bull* 37:2016–2021. <https://doi.org/10.1248/cpb.37.2016>
- Jakubczyk K, Dec K, Kalduńska J, Kawczuga D, Kochman J, Janda K (2020) Reactive oxygen species - sources, functions, oxidative damage. *Pol Merkur Lekarski* 48:124–127
- Jesch ED, Carr TP (2017) Food ingredients that inhibit cholesterol absorption. *Prev Nutr Food Sci* 22:67–80. <https://doi.org/10.3746/pnf.2017.22.2.67>
- Jirakiattikul Y, Rithichai P, Kwanthong P, Itharat A (2021) Effect of jasmonic acid elicitation period on enhancement of bioactive compounds and antioxidant activity in callus cultures of *Hibiscus sabdariffa* Linn. *Hortic Environ Biotechnol* 62:629–636. <https://doi.org/10.1007/s13580-021-00332-3>
- Kahl R, Kappus H (1993) Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. *Z Lebensm Unters Forsch* 196:329–338. <https://doi.org/10.1007/bf01197931>
- Kamboj A, Saluja AK (2011) Isolation of stigmasterol and β -sitosterol from ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae). *Int J Pharm Pharmaceut Sci* 3:94–96
- Kim HJ, Cha JY, Choi ML, Cho YS (2000) Antioxidative activities by water-soluble extracts of *Morus alba* and *Cudrania tricuspidata*. *J Korean Soc Agric Chem Biotechnol* 43:148–152
- Kim MH, Kim MC, Park JS, Park EJ, Lee JO (1999a) Determination of antioxidants contents in various plants used as tea materials. *Kor J Food Sci Technol* 31:273–279
- Kim MJ, Kwon YS, Yu CY (2005) Antioxidative compounds in extracts of *Eleutherococcus senticosus* Max. plantlets. *Korean J Med Crop Sci* 13:194–198
- Kim SY, Gao JJ, Lee WC, Ryu KS, Lee KR, Kim YC (1999b) Antioxidative flavonoids from the leaves of *Morus alba*. *Arch Pharm Res* 22:81–85. <https://doi.org/10.1007/BF02976442>
- Kim SY, Kim CR, Kim HM, Kong M, Lee JH, Lee HJ, Lim MS, Jo NR, Park SN (2010) Antioxidant activity and whitening effect of *Cedrela sinensis* A. Juss shoots extracts. *J Soc Cosmet Sci Korea* 36:175–182
- Lee DG, Lee J, Kim KT, Lee SW, Kim YO, Cho IH, Kim HJ, Park CG, Lee S (2018) High-performance liquid chromatography analysis of phytosterols in *Panax ginseng* root grown under different conditions. *J Ginseng Res* 42:16–20. <https://doi.org/10.1016/j.jgr.2016.10.004>
- Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P (2018) Oxidative stress, aging, and diseases. *Clin Interv Aging* 13:757–772. <https://doi.org/10.2147/CIA.S158513>
- Lipinski B (2011) Hydroxyl radical and its scavengers in health and disease. *Oxid Med Cell Longev* 2011:809696. <https://doi.org/10.1155/2011/809696>
- Liu T, Li Z, Li R, Cui Y, Zhao Y, Yu Z (2019) Composition analysis and antioxidant activities of the *Rhus typhina* L. stem. *J Pharm Anal* 9:332–338. <https://doi.org/10.1016/j.jpha.2019.01.002>
- Maruthappan V, Shree KS (2010) Hypolipidemic activity of haritaki (*Terminalia chebula*) in atherogenic diet induced hyperlipidemic rats. *J Adv Pharm Technol Res* 1:229–235
- Nachimuthu K, Palaniswamy C (2013) Physicochemical analysis and HPLC-PDA method for quantification of stigmasterol in *Dipteracanthus patulus*. (Jacq) Nees *J Pharm Res* 7:741–746. <https://doi.org/10.1016/j.jopr.2013.08.014>
- Narayanaswamy N, Balakrishnan KP (2011) Evaluation of some medicinal plants for their antioxidant properties. *Int J Pharm Tech Res* 3:381–385
- Niu HS, Liu IM, Cheng JT, Lin CL, Hsu FL (2008) Hypoglycemic effect of syringin from *Eleutherococcus senticosus* in streptozotocin-induced diabetic rats. *Planta Med* 74:109–113. <https://doi.org/10.1055/s-2008-1034275>
- Ntanos FY, Jones PJ (1998) Effects of variable dietary sitostanol concentrations on plasma lipid profile and phytosterol metabolism in hamsters. *Biochim Biophys Acta* 1390:237–244. [https://doi.org/10.1016/S0005-2760\(97\)00196-3](https://doi.org/10.1016/S0005-2760(97)00196-3)
- Omaye ST, Reddy KA, Cross CE (1977) Effect of butylated hydroxytoluene and other antioxidants on mouse lung metabolism. *J Toxicol Environ Health* 3:829–836. <https://doi.org/10.1080/15287397709529617>
- Ostlund RE Jr (2002) Phytosterols in human nutrition. *Ann Rev Nutr* 22:533–549. <https://doi.org/10.1146/annurev.nutr.22.020702.075220>
- Ozel HB, Aisha AESA, Cetin M, Sevik H, Cetin IZ (2021) The effects of increased exposure time to UV-B radiation on germination and seedling development of Anatolian black pine seeds. *Environ Monit Assess* 193:1–11. <https://doi.org/10.1007/s10661-021-09178-9>
- Panda S, Jafri M, Kar A, Meheta BK (2009) Thyroid inhibitory, anti-peroxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. *Fitoterapia* 80:123–126. <https://doi.org/10.1016/j.fitote.2008.12.002>
- Park JS, Oh CH, Koh HY, Choi DS (2002) Antimutagenic effect of extract of *Eleutherococcus senticosus* Maxim. *Korean J Food Sci Technol* 34:1110–1114
- Patterson CA (2006) Phytosterols and stanols. *Agriculture and Agri-Food Canada, Government of Canada*, pp 1–4
- Peng C, Wang X, Chen J, Jiao R, Wang L, Li YM, Zuo Y, Liu Y, Lei L, Ma KY, Huang Y, Chen ZY (2014) Biology of ageing and role of dietary antioxidants. *Biomed Res Int* 2014:831841. <https://doi.org/10.1155/2014/831841>
- Pohl F, Lin PKT (2018) The potential use of plant natural products and plant extracts with antioxidant properties for the prevention/treatment of neurodegenerative diseases: In vitro, in vivo and clinical trials. *Molecules* 23:3283. <https://doi.org/10.3390/molecules23123283>
- Prakash D, Upadhyay G, Gupta C, Pushpangadan P, Singh KK (2012) Antioxidant and free radical scavenging activities of some promising wild edible fruits. *Int Food Res J* 19:1109–1116
- Robak J, Gryglewski RJ (1988) Flavonoids are scavengers of superoxide anions. *Biochem Pharmacol* 37:837–841. [https://doi.org/10.1016/0006-2952\(88\)90169-4](https://doi.org/10.1016/0006-2952(88)90169-4)
- Saeidnia S, Manayi A, Gohari AR, Abdollahi M (2014) The story of beta-sitosterol-a review. *Eur J Med Plants* 590–609. <https://doi.org/10.9734/EJMP/2014/7764>

- Sevik H, Cetin M, Ozel HB, Erbek A, Cetin IZ (2021) The effect of climate on leaf micromorphological characteristics in some broad-leaved species. *Environ Dev Sustain* 23:6395–6407. <https://doi.org/10.1007/s10668-020-00877-w>
- Shailajan S, Deepti G (2014) Pharmacognostic and phytochemical evaluation of *Chrysophyllum cainito* Linn. leaves. *Int J Pharm Sci Rev Res* 26:106–111
- Shilajan S, Swar G (2013) Simultaneous estimation of three triterpenoids-ursolic acid, β -sitosterol and lupeol from flowers, leaves and formulations of *Rhododendron arboreum* Smith. using validated HPTLC method. *Int J Green Pharm* 7:206–210. <https://doi.org/10.4103/0973-8258.120219>
- Song JH, Kim H, Kim MS, Kim SH, Lee JT (2015) Bioactive component analysis and antioxidant activity in fresh sprout of new *Kalopanax septemlobus* cultivar. *J Korean For Soc* 104:397–402. <https://doi.org/10.14578/jkfs.2015.104.3.397>
- Talreja T, Kumar M, Goswami A, Gahlot G, Jinger SK, Sharma T (2017) Qualitative and quantitative estimation of phytosterols in *Achyranthes aspera* and *Cissus quadrangularis* by HPLC. *Pharma Innov* 6:76–79
- Teleanu RI, Chircov C, Grumezescu AM, Volceanov A, Teleanu DM (2019) Antioxidant therapies for neuroprotection-a review. *J Clin Med* 8:1659. <https://doi.org/10.3390/jcm8101659>
- Tungmunthum D, Thongboonyou A, Pholboon A, Yangsabai A (2018) Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Med (Basel)* 5:93. <https://doi.org/10.3390/medicines5030093>
- Xu F, Wu H, Wang X, Yang Y, Wang Y, Qian H, Zhang Y (2014) RP-HPLC characterization of lupenone and β -sitosterol in Rhizoma *Musae* and evaluation of the anti-diabetic activity of lupenone in diabetic Sprague-Dawley rats. *Molecules* 19:14114–14127. <https://doi.org/10.3390/molecules190914114>
- Yang W-K, Kim D-S, Son E, Lee YM, Park Y-C, Lee G-J, Kim S-H (2021) Reduction of allergy effects of peanut sprout extract in a systemic anaphylaxis food allergy mouse model. *Hortic Environ Biotechnol* 62:637–647. <https://doi.org/10.1007/s13580-021-00336-z>
- Yoo EG (2016) Sitosterolemia: a review and update of pathophysiology, clinical spectrum, diagnosis, and management. *Ann Pediatr Endocrinol Metab* 21:7–14. <https://doi.org/10.6065/apem.2016.21.1.7>
- Yu HH, Moon HD, Hwang JY, Kim SY, Jeong SI, Jeon BH, You YO (2007) Isolation of anti-cariogenic agent, stigmasterol, from *Aralia continentalis*. *Korean J Oriental Physiol Pathol* 21:70–75
- Yucedag C, Cetin M, Ozel HB, Aisha AESA, Alrabiti OBM, JAMA AMOA (2021) The impacts of altitude and seed pretreatments on seedling emergence of *Syrian juniper* (*Juniperus drupacea* (Labill.) Ant. et Kotschy). *Ecol Process* 10:1–6. <https://doi.org/10.1186/s13717-020-00276-z>
- Zahirul Islam M, An HG, Lee YT (2019) Comparative analyses on the bioactive compounds and in vitro antioxidant capacity of tea infusions prepared from selected medicinal fruits. *Chem Biodivers* 16:e1900459. <https://doi.org/10.1002/cbdv.201900459>
- Zahirul Islam M, Cho DK, Lee YT (2020) Bioactive compounds and antioxidant capacity of tea infusion prepared from whole and ground medicinal herb parts. *CYTA J Food* 1:116–121. <https://doi.org/10.1080/19476337.2019.1702104>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.