

Article

The Electrical Conductivity and Drying Method Changed the Secondary Metabolite Content and Photoprotective Effects of Hydroponically Cultivated *Agastache rugosa* Kuntze

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Abstract: Many studies have examined how various cultivation methods and elicitors elevate target crop values. Post-harvest treatments, such as the drying method, may alter the final product's characteristics. We investigated the effect of nutrient solution concentration during the hydroponic cultivation and drying method (cold air drying and freeze drying) after harvesting *Agastache rugosa* Kuntze, an herb used for folk medicine and as a deodorant, spice, and tea in East Asia. To explore the properties of *A. rugosa*, we determined its growth parameters, secondary metabolites, and photoprotective effects. We observed the highest growth and yields in *A. rugosa* grown under 4.0 dS·m^{−1} electrical conductivity (EC) conditions. However, the EC 2.0 group showed higher antioxidative properties than those grown in EC 1.5 and 4.0 conditions. Drying conditions varied the content and ratios of major active compounds (rosmarinic acid, tilianin, and acacetin) in *A. rugosa*. Cold air drying caused rosmarinic acid transmutation to tilianin and acacetin, and tilianin showed a stronger positive correlation with antioxidative and photoprotective activities than rosmarinic acid. The increased tilianin content in cold-air-dried *A. rugosa* accompanied the improved photoprotective effects in dermal cell lines. This study reveals the effects of cultivation and drying methods in *A. rugosa* production, and proposes alternate industrial production techniques.

Keywords: *Agastache rugosa* Kuntze; electrical conductivity; drying method; secondary metabolite; antioxidation; photoprotective effect



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1. Introduction

Agastache rugosa Kuntze is a perennial and edible herb that belongs to the *Labiatae* family that is widely distributed in China, Korea, and Japan [1]. *A. rugosa* leaves and flowers have a unique aroma, and so it has been used as a deodorant, spice, and tea throughout East Asia [2,3]. *A. rugosa* is a unique food ingredient and a folk medicinal herb with multiple potencies [4]. Previous studies revealed that *A. rugosa* has antiatherogenic, antimicrobial, antitumor, and anti-inflammation effects and may mitigate human immunodeficiency virus (HIV), hyperlipidemia, and postmenopausal osteoporosis symptoms [5–11]. The antioxidative activity of *A. rugosa* via upregulating heme oxygenase-1 (HO-1), glutathione (GSH), and superoxide dismutase (SOD) are also well verified [9].

Recently, *A. rugosa* has received attention in the cosmetic market because of its complex potencies for dermal cells [12]. Ultraviolet (UV) light attacks skin cells and promotes the production of reactive oxygen species (ROS). *A. rugosa* treatment enhances the protective efficacy against UV damage due to its ROS scavenging activities [4,13,14]. Moreover, *A. rugosa* downregulates matrix metalloproteinase (MMP)-related genes and promotes anti-photoaging, anti-wrinkle, and skin-healing potentials via procollagen production [13,15].

Rosmarinic acid (RA) and tilianin are the major phenylpropanoids found in *A. rugosa* plants, and other phenolic compounds and monoterpenes may contribute to the functional properties of *A. rugosa* as well [16]. The acacetin, 5,7-dihydroxy-4'-methoxy flavone, is a flavonoid found in *A. rugosa*. It alleviates UV damage to skin cells by scavenging ROS induced by UV radiation and mediating mitogen-activated kinase (MAPK)-related signaling to mitigate photoaging [17,18].

Owing to the usage of *A. rugosa*, varied strategies have testified to enhance the bioactive compound content of *A. rugosa*. Yeast extract or silver nitrate treatment during *A. rugosa* cultivation changed the amount of RA and other phenylpropanoid compounds [19]. Also, various illumination conditions, L-phenylalanine (the precursor of phenylpropanoid), or casein hydrolysate supplementation, changed the RA content in *A. rugosa* cultures [16,20]. Some in vitro cultivation systems were also introduced to generate *A. rugosa* that altered its metabolites and characteristics, especially in volatile compounds [21,22]. Multivariable environmental factors could alter plant characteristics during growth, and different cultivation methods may incur metabolic changes accompanying primary and secondary metabolite changes [23].

Electrical conductivity (EC) is a material's ability to develop electrical current, and EC is a fundamental hydroponic environmental factor for vertical farming because EC value connotes the concentrations of nutrients in the nutrient solution. Compared to conventional farming that does not use nutrient solutions, hydroponics improves nutrition uptake efficiency for plants and helps resist nutrient depletion. It helps control the plants' nutrient state and introduces aberrant experimental conditions not easily enticed in conventional farming systems. The modulation of nutrient solutions manages extensive plant characteristics, including growth rate, yield, chemical profiling, and sensory properties in diverse plant species [24]. Thus, we hypothesized that the EC condition could alter *A. rugosa* characteristics and qualities, but a hydroponic culture system has rarely been tested on *A. rugosa*.

This study aimed to test how various EC conditions during hydroponic cultivation in a vertical farm changed the growth parameters and functional properties of *A. rugosa*. As well as the EC conditions, we introduced two different drying methods to examine how they affect *A. rugosa* characteristics during industrial product preparation. We also verified the photoprotective effects of *A. rugosa* samples in dermal cell lines to investigate its use as a functional agent.

2. Materials and Methods

2.1. Plant Materials

We purchased *A. rugosa* seeds from Danong Seed Co. Ltd. (Seoul, Republic of Korea) and cultivated *A. rugosa* using a vertical farming system installed in the Smart U-FARM facility of Korea Institute of Science and Technology (KIST; Gangneung, Republic of Korea). We sowed *A. rugosa* seeds in moist rock-wool cubes (25 × 25 × 40 mm, W × L × H, respectively, Grodan Co., Roermond, The Netherlands) and grew them for two weeks. We used fluorescent lamps (TL5 14 W/865, Philips, Amsterdam, The Netherlands) installed in upper plants for 25 cm were used for illumination, and controlled the light intensity to $200 \pm 11 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. We utilized a 14:10 h light/dark cycle and maintained room temperatures and relative humidity at 18–26 °C and 50–80%, respectively.

Two weeks after sowing, we transplanted *A. rugosa* to a deep-flow technique (DFT) hydroponic system (103.7 plants/m²). Four weeks after sowing, we transplanted 504 plants (31.1 plants/m²) to a nutrient film technique (NFT) hydroponic system at four weeks. We used the modified Otsuka House's nutrient solution containing 808, 648, 492, 161, and 172 ppm of KNO₃, Ca(NO₃)₂, MgSO₄, NH₄H₂PO₄, and KH₂PO₄, respectively as macronutrients (Yara Korea, Gyeonggi, Republic of Korea) and 2.80, 0.32, 0.77, 0.04, 0.02, and 0.02 ppm of Fe, B, Mn, Cu, Zn, and Mo, respectively, as micronutrients (DOFagro, Gyeonggi, Republic of Korea) with 1.5 dS·m⁻¹ of EC. We refreshed the nutrient solution weekly to maintain mineral balance and pH (5.5–6.5) of nutrient solution. We cultivated

the plants under $200 \pm 15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity using fluorescent lamps (FL20EX-D 18W, Shin Kwang, Mungyeong, Republic of Korea) installed 30 cm above the plants. We adjusted the light/dark cycle to 14:10 h, and maintained the CO_2 concentration at 800 ± 35 ppm during the daytime. We maintained the temperature and relative humidity at $18\text{--}26^\circ\text{C}$ and 50–80%, respectively.

At week six, we divided the *A. rugosa* plants into three groups, each of which received different nutrient solutions (1.5, 2.0, and 4.0 for EC). The ratio of each nutrient in solutions was same as that of modified Otsuka House's nutrient solution described above, but the concentration of the total nutrients was adjusted to 1.5, 2.0, and $4.0 \text{ dS}\cdot\text{m}^{-1}$, respectively. We grew the plants for three additional weeks by refreshing each nutrient solution once a week, and we harvested their shoots and roots separately at weeks six, eight, and nine (Figure 1).

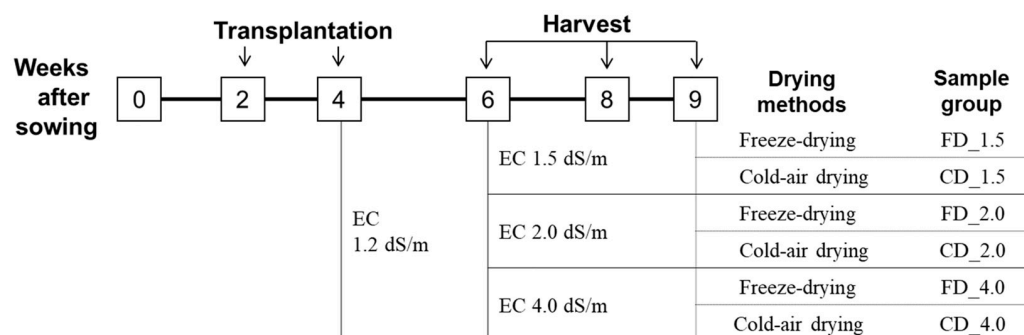


Figure 1. The preparation steps of *A. rugosa* samples. We applied the three EC conditions (1.5 , 2.0 , and $4.0 \text{ dS}\cdot\text{m}^{-1}$) for nutrient solution during cultivation and dried them by the freeze-drying (FD) or cold-air-drying (CD) methods.

2.2. Growth Parameters and Yield

We measured the height of the epigeal portion of the plant with a ruler and manually counted the number of leaves. We measured the sample weights with an electronic scale (W-200, CAS Corp., Yangju, Republic of Korea). We determined the dry weight of the *A. rugosa* samples after drying the plants at $29 \pm 1^\circ\text{C}$ in a cold air dehumidifier (CT-24, COOLTOP, Cheonan, Republic of Korea) for 10 days.

2.3. Preparation of Dried Samples and Their Extracts

We randomly divided the aerial parts of the *A. rugosa* plants harvested nine weeks after sowing (WAS) into two groups and dried them using two different methods. We dried the first group using a freeze-dryer (PVTFD 300R, IlshinBioBase, Dongducheon, Republic of Korea) for four days and the second group using a cold air dryer (CT-24, COOLTOP, Republic of Korea) at $29 \pm 1^\circ\text{C}$ for 10 days. We prepared six *A. rugosa* sample groups with different EC conditions and drying methods (Figure 1). We pulverized the dried *A. rugosa* plants and mixed one gram of *A. rugosa* with 20 mL of ethanol and extracted the solution three times at 20°C for 6 h. We then evaporated the mixture with a rotary evaporator.

2.4. Antioxidative Properties

We examined the total phenolic acid contents (TPC) in the *A. rugosa* extract using the Folin–Ciocalteu method [25], and determined the total flavonoid content (TFC) by referring to Meda, et al. [26]. We measured the DPPH and ABTS radical scavenging activity of *A. rugosa* extract following the method of Son, et al. [27] and determined the ferric-reducing antioxidant power (FRAP) using the methods of Benzie and Strain [28]. We used Trolox as a standard for the DPPH, ABTS, and FRAP assays, and compared it to the antioxidative effects of *A. rugosa* extracts.

2.5. High-Performance Liquid Chromatography (HPLC) Analysis

We determined the major phenolic compounds in *A. rugosa* extract using an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) and the Shiseido Capcell Pak C18 MGII (250 × 4.6 mm, 5 µm) column for separation. We used water containing 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) for the mobile phase, and their gradient condition was as follows: 0–5 min, 20:80; 10 min, 50:50; 20–22 min, 0:100; 25 min, 20:80 (A:B). The flow rate was 1 mL·min^{−1}, and we detected the chromatogram at 330 nm. We quantified the RA, tilianin, and acacetin content using their respective calibration curves. We obtained the standard compounds from Sigma-Aldrich (St. Louis, MO, USA).

2.6. Cell Culture

We obtained Hs68 human dermal fibroblast cells and HaCaT human keratinocytes from the American Type Culture Collection (Manassas, VA, USA). We used Dulbecco's modified eagle medium (DMEM; Hyclone, Logan, UT, USA) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin-streptomycin solution (Hyclone, Logan, UT, USA) for cell culture. The cells were incubated under 5% CO₂ at 37 °C in a humidified incubator and subcultured for every two to three days.

2.7. Cell Viability

We seeded Hs68 or HaCaT cells in a 96-well plate for 5 × 10³ cells/well and incubated them for 24 h. We eliminated the media and washed the cells with phosphate-buffered saline (PBS). Then, we placed serum-free DMEM containing different concentrations of *A. rugosa* extract (5, 20, and 40 µg/mL) into wells and incubated them for 1 h. After removing the media, we washed the cells with PBS again, and put 100 µL of PBS solution into wells. We divided cells into two groups based on UV-B irradiation. We exposed the UV-B irradiated group to 20 mJ·cm^{−1} of UV-B (VL-6 LM; Vilber Lourmat, Marne-la-Vallée, France).

After removing PBS, we put cell culture media with various concentrations of *A. rugosa* extract into wells, and incubated the cells for 24 h. We determined the cell viability by MTT assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, St. Louis, MO, USA). We added 0.5 mg·mL^{−1} MTT solution to wells and eliminated the media after 4 h. We dissolved the reactants with dimethyl sulfoxide and determined the absorbance at 550 nm (Bio-Tek Instruments, Winooski, VT, USA).

2.8. ROS Reduction in UV-B Irradiated Cells

We seeded Hs68 or HaCaT cells in a 96-well plate (5 × 10³ cells/well) and incubated them for 24 h. After removing the media, we rinsed the cells with PBS once and treated serum-free media containing different concentrations of *A. rugosa* extract (5, 20, and 40 µg/mL) for 1 h. We washed cells with PBS again after removing the media, and put 100 µL of PBS into each well. We irradiated the UV-B for 20 mJ·cm^{−1} (VL-6 LM; Vilber Lourmat, France) and eliminated the PBS solution. Then, we placed 20 µM of 2', 7'-dichlorofluorescein diacetate (DCFDA) in serum-free DMEM into the respective wells. We determined the cells' fluorescence intensity after 30 min at 495 nm for excitation and 530 nm for emission (Bio-Tek Instruments, Winooski, VT, USA).

2.9. Statistical Analysis

We analyzed statistical differences with one-way analysis of variance (ANOVA) using SPSS Ver. 25 (IBM Corp; Armonk, NY, USA). We calculated the means ± standard deviations (SD). When the F-ratio was significant, we conducted Duncan's multiple range test for post hoc analysis. Differences were considered significant at $p < 0.05$. We analyzed Pearson's correlations within variables and visualization with OriginPro 2023 (Origin-Lab; Northampton, MA, USA). We conducted principal component analysis (PCA) and visualization using SPSS Ver. 25 (IBM Corp; Armonk, NY, USA) and GraphPad Prism 9 (GraphPad Software; San Diego, CA, USA).

3. Results

3.1. Effect of EC Condition on Growth Parameters and *A. rugosa* Yield

Figure 2 presents photographs of harvested *A. rugosa* samples. We measured six growth parameters (plant height, number of leaves, and fresh and dry weights of the aerial and root parts) to estimate the effects of EC on the yield and growth rate of *A. rugosa* (Figure 3). There were no significant differences in yield and growth rate at week six within the groups. At weeks eight and nine, different ECs caused changes in all of the growth parameters and *A. rugosa* plant yields. At week nine, plants grown under EC 4.0 had a significantly higher weight (49.20 ± 1.92 cm) in the aerial (52.25 ± 9.16 g/plant and 6.70 ± 1.27 g/plant of fresh and dry weight; 105.40 ± 18.66 leaves/plant) and root parts (19.95 ± 4.65 g/plant and 1.60 ± 0.49 g/plant of fresh and dry weight) than the other groups ($p < 0.05$). On the contrary, plant height (45.80 ± 1.79 cm), number of leaves (84.40 ± 5.73 leaves/plant), and fresh and dry weight of aerial (27.35 ± 4.06 g/plant and 4.57 ± 0.74 g/plant) and root (8.28 ± 1.21 g/plant and 0.93 ± 0.10 g/plant) parts for plants grown under the EC 1.5 conditions were significantly low compared with other conditions at week nine ($p < 0.05$). These results indicated that EC 1.5 nutrient solution may not have provided adequate nutrients to *A. rugosa* during weeks six to nine in vertical farming, while EC 4.0 nutrient solution promoted *A. rugosa* growth.

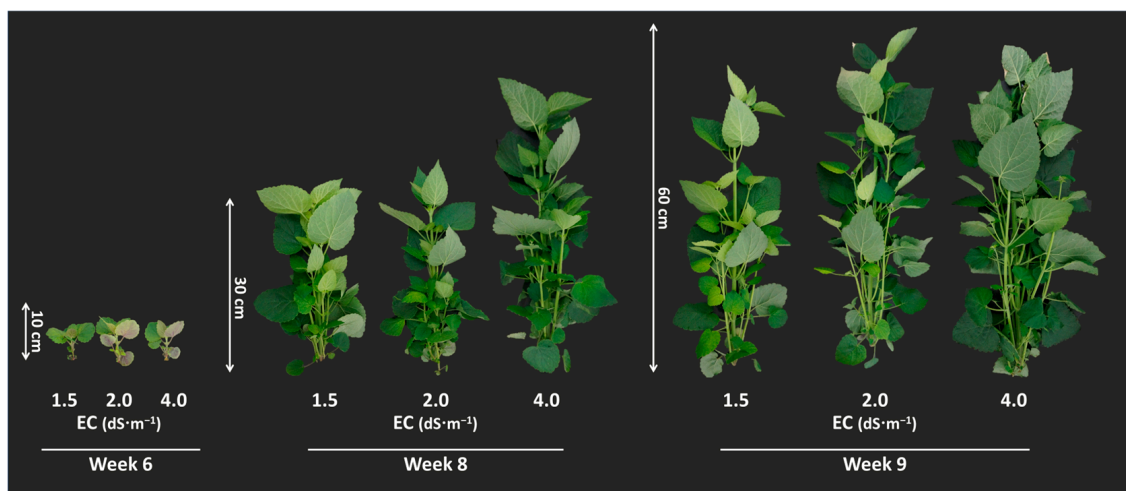


Figure 2. Photographs of *A. rugosa* plants collected at weeks six, eight, and nine.

3.2. EC Conditions Varied the *A. rugosa* Samples' Antioxidative Properties

In company with an implication of EC conditions to *A. rugosa* during cultivation, we additionally tested the effects of drying methods after harvest. We used two types of drying methods (cold- and freeze-drying) on *A. rugosa* samples harvested at week nine. First, we analyzed TPC and TFC in each sample group owing to their strong influence on antioxidative activities (Figure 4A,B). *A. rugosa* cultivated under EC 2.0 showed significantly higher TPC and TFC than other sample groups ($p < 0.05$). Likewise, the *A. rugosa* grown under EC 2.0 nutrient solution showed the highest DPPH, ABTS, and FRAP activities regardless of the drying method (Figure 4C–E; $p < 0.05$). Among the freeze-dried samples, *A. rugosa* cultivated with EC 4.0 nutrient solution had the lowest ABTS, FRAP, TPC, and TFC compared with those grown in EC 1.5 and EC 2.0 conditions. Compared to the EC conditions, the drying methods did not dramatically change the TPC, TFC, and antioxidative activities.

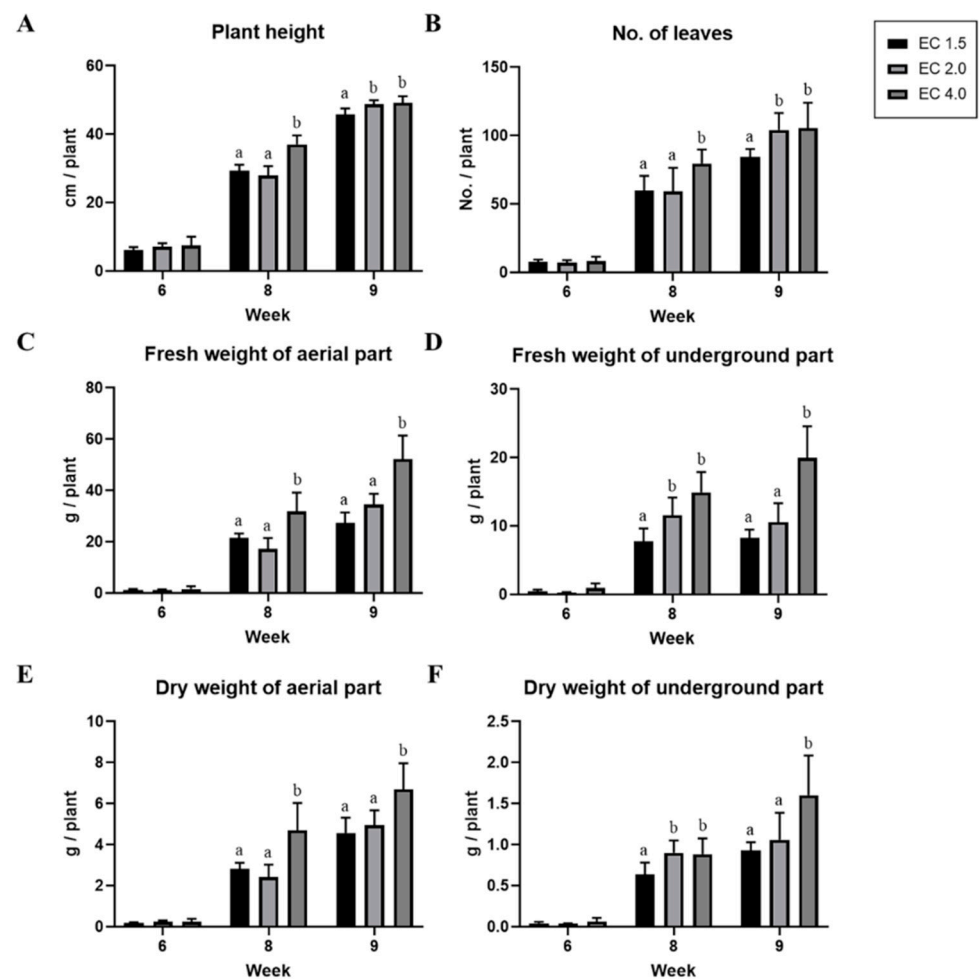


Figure 3. Growth parameters of *A. rugosa* plants cultivated under different nutrient solution concentrations. We measured plant height (A), the number of leaves (B), the fresh weight of the aerial (C) and underground parts (D), and the dry weight of the aerial (E) and root parts (F). Growth parameters were measured at weeks six, eight, and nine. Results are represented by the mean $\pm$ SD. Different letters indicate statistical differences within samples collected at the same week ($p < 0.05$, $N = 5$).

3.3. The Drying Method Changed RA, Tiliandin, and Acacetin Content in Dried *A. rugosa*

We also verified three kinds of typical active phenolic compounds (RA, tiliandin, and acacetin) in *A. rugosa* with HPLC to verify the differences in the secondary metabolite profiles of the samples (Table 1). The drying method considerably influenced the ratios of the major secondary metabolites. The tiliandin and acacetin content were higher in the cold-air-dried samples, while the RA content was higher in the freeze-dried samples when the EC conditions were the same ($p < 0.05$). The FD_1.5 group showed the highest RA content (4.50 ± 0.61 mg/g dry basis) and CD_4.0 showed the lowest RA content (0.09 ± 0.01 mg/g dry basis). The tiliandin content was elevated in cold-air-dried samples compared to the freeze-dried samples with identical EC conditions. The tiliandin content of CD_2.0 (9.10 ± 1.22 mg/g dry basis) was 1.9 times higher than FD_2.0 ($p < 0.05$). Acacetin content was significantly higher in cold-air-dried conditions. CD_2.0 and CD_4.0 had 3.4 and 7.3 times higher acacetin content than FD_2.0 and FD_4.0, respectively ($p < 0.05$). These results indicated that the drying method may cause changes in each chemical compound's content.

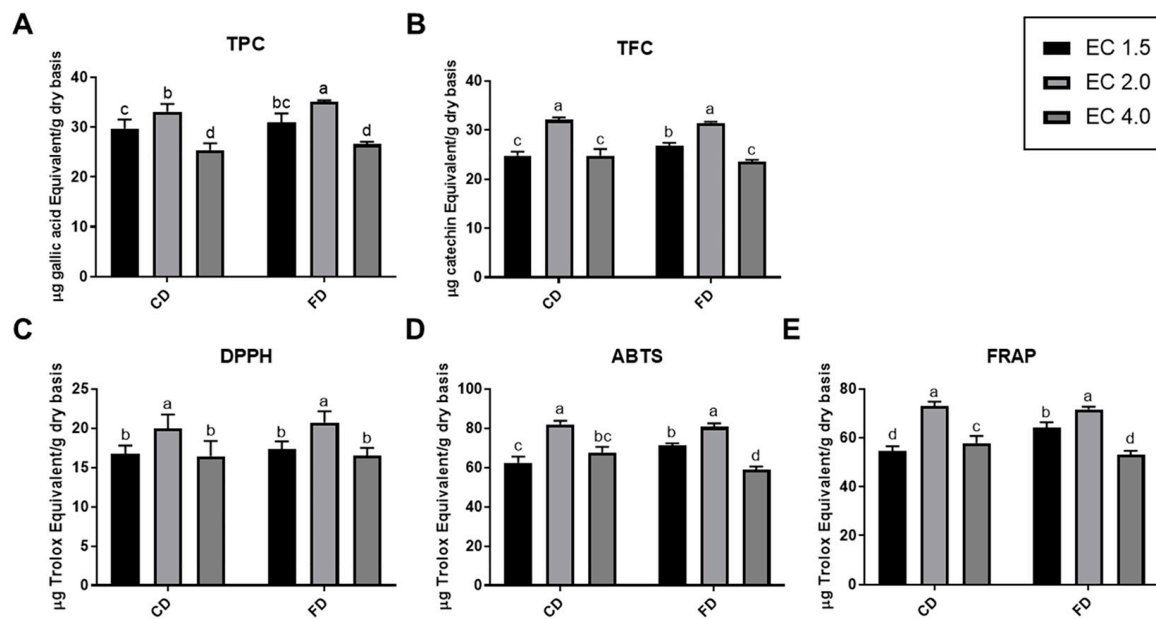


Figure 4. The antioxidative properties of *A. rugosa* samples. TPC (A), TFC (B), DPPH (C), ABTS (D), and FRAP (E) were determined. Results are represented by the mean $\pm$ SD. Different letters indicate statistical differences in $p < 0.05$ ($N = 4$). TPC, total phenolic acids contents; TFC, total flavonoid contents.

Table 1. Concentration of rosmarinic acid (RA), tilianin, and acacetin in dried *A. rugosa* samples. The samples were harvested at week 9 and dried with cold-air-drying or freeze-drying methods.

Sample	Rosmarinic Acid (mg/g, DW)	Tilianin (mg/g, DW)	Acacetin (mg/g, DW)
CD_1.5 ⁽¹⁾	3.20 $\pm$ 0.83 b ⁽²⁾	3.83 $\pm$ 1.23 a	0.02 $\pm$ 0.05 a
CD_2.0	0.61 $\pm$ 0.23 a	9.10 $\pm$ 1.22 b	0.98 $\pm$ 0.46 b
CD_4.0	0.09 $\pm$ 0.01 a	4.01 $\pm$ 3.13 a	0.87 $\pm$ 0.72 b
FD_1.5	4.50 $\pm$ 0.61 c	3.30 $\pm$ 2.10 a	0.20 $\pm$ 0.10 a
FD_2.0	4.18 $\pm$ 1.22 c	4.86 $\pm$ 2.57 a	0.29 $\pm$ 0.21 a
FD_4.0	3.00 $\pm$ 0.37 b	3.17 $\pm$ 2.37 a	0.12 $\pm$ 0.06 a

⁽¹⁾ CD_1.5, *A. rugosa* grown under EC 1.5 and cold-air-dried; CD_2.0, *A. rugosa* grown under EC 2.0 and cold-air-dried; CD_4.0, *A. rugosa* grown under EC 4.0 and cold-air-dried; FD_1.5, *A. rugosa* grown under EC 1.5 and freeze-dried; FD_2.0, *A. rugosa* grown under EC 2.0 and freeze-dried; FD_4.0, *A. rugosa* grown under EC 4.0 and freeze-dried. ⁽²⁾ Different letters indicate statistical differences within each column ($p < 0.05$, $N = 6$).

3.4. EC Conditions and Drying Methods Affect *A. rugosa*'s Photoprotective Effects

We tested *A. rugosa*'s photoprotective effects in two cell lines (Hs68 and HaCaT). First, we confirmed that none of the samples demonstrated toxic effects in the ranges of 5–40 $\mu\text{g/mL}$ in either cell line (Figure 5A,B). UV-B irradiation in both cell lines decreased cell viability (by about 20–30%), and some treatment groups (such as 40 $\mu\text{g/mL}$ for CD_2.0) showed a slight increase in cell viability in the HaCaT cell line against UV-B (Figure 5C). However, treatment of *A. rugosa* extracts did not change cell viability in the UV-B irradiated Hs68 cells (Figure 5D).

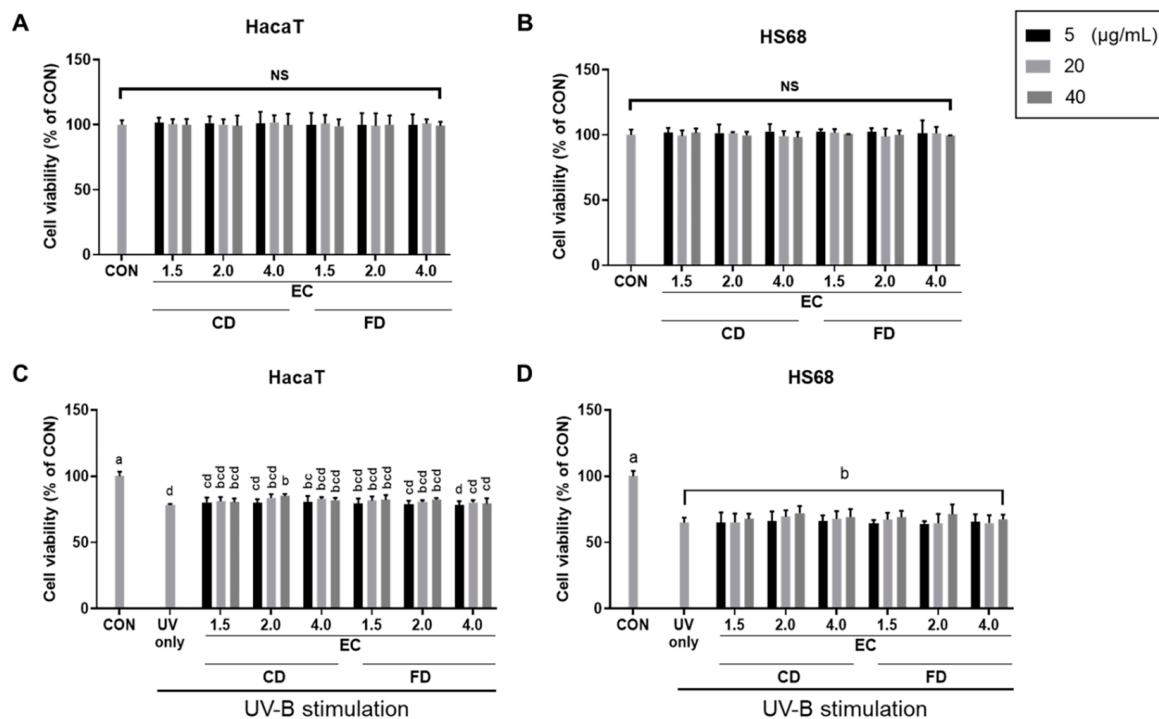


Figure 5. The cell viabilities of HaCaT and Hs68 cell lines after treatment with *A. rugosa* extracts. We first determined the cell toxicities of *A. rugosa* extracts in HaCaT and Hs68 cells (A,B). We also determined cell viability after treatment with *A. rugosa* extracts in UV-B irradiated HaCaT and Hs68 cells (C,D). The results are represented as the mean $\pm$ SD. Different letters indicate statistical differences in $p < 0.05$ ($N = 4$). NS, not significant.

We also examined if the *A. rugosa* extracts could reduce ROS due to UV-B because the importance of ROS in the skin cells' photodamage mechanism (Tables 2 and 3). We found that the ROS contents multiplied 34 and 30 times after UV-B irradiation in the HaCaT and Hs68 cell lines, respectively. However, *A. rugosa* extracts effectively removed ROS in both cell lines. The CD_2.0 extract demonstrated the strongest potency for scavenging ROS in both two cell lines, and FD_2.0 showed the second-highest ROS scavenging activities among the sample groups. CD_4.0 and FD_4.0 showed the weakest photoprotective effects.

Table 2. The reactive oxygen species (ROS) content in UV-B irradiated HaCaT cells after *A. rugosa* extract treatment.

Sample Condition		Sample Concentration ($\mu\text{g/mL}$)	ROS Content (Fold)
Drying Method	EC		
Control cell (UV-B only)		-	34.29 ± 1.95^a
Cold air drying	1.5	5	32.39 ± 2.20^{abc}
		20	31.38 ± 1.62^{abcde}
		40	28.55 ± 1.74^{efg}
	2.0	5	31.83 ± 0.83^{abcd}
		20	28.48 ± 1.60^{efg}
		40	26.68 ± 1.78^g
	4.0	5	32.34 ± 1.17^{abc}
		20	31.28 ± 2.07^{bcde}
		40	29.85 ± 2.06^{cdef}

Table 2. Cont.

Sample Condition		Sample Concentration ($\mu\text{g/mL}$)	ROS Content (Fold)
Drying Method	EC		
Freeze drying	1.5	5	33.52 ± 1.46^{ab}
		20	31.42 ± 2.36^{abcde}
		40	28.53 ± 2.02^{efg}
	2.0	5	32.57 ± 1.85^{abc}
		20	29.89 ± 1.49^{cdef}
		40	27.57 ± 2.02^{fg}
	4.0	5	33.50 ± 2.06^{ab}
		20	31.84 ± 1.55^{abcd}
		40	29.11 ± 1.49^{defg}

Results are represented as the mean $\pm$ SD. Different letters indicate statistical differences in $p < 0.05$ ($N = 4$).

Table 3. The ROS content in UV-B irradiated Hs68 cells after *A. rugosa* extract treatment.

Sample Condition		Sample Concentration ($\mu\text{g/mL}$)	ROS Content (Fold)
Drying Method	EC		
Control cell (UV-B only)		-	30.22 ± 1.44^a
Cold air drying	1.5	5	29.69 ± 1.75^{abc}
		20	28.44 ± 1.98^{abcde}
		40	28.81 ± 2.67^{abcd}
	2.0	5	28.54 ± 0.98^{abcde}
		20	26.37 ± 1.46^{ef}
		40	25.67 ± 2.07^f
	4.0	5	30.49 ± 0.31^a
		20	28.99 ± 1.39^{abcd}
		40	28.96 ± 0.83^{abcd}
Freeze drying	1.5	5	29.13 ± 0.79^{abcd}
		20	28.56 ± 0.34^{abcde}
		40	27.80 ± 0.76^{bcdef}
	2.0	5	30.35 ± 1.26^a
		20	27.45 ± 0.79^{cdef}
		40	26.96 ± 0.74^{def}
	4.0	5	29.81 ± 0.56^{ab}
		20	28.39 ± 1.18^{abcde}
		40	28.53 ± 1.78^{abcde}

Results are represented as the mean $\pm$ SD. Different letters indicate statistical differences in $p < 0.05$ ($N = 4$).

3.5. Correlations between *A. rugosa* Characteristics and PCA Plots

The modulations of EC conditions and drying methods resulted in changes in *A. rugosa* traits. We employed additional statistical analysis to elucidate the relationship between variables. We used antioxidative properties (TPC, TFC, DPPH, ABTS, and FRAP), major metabolites (RA, tilianin, and acacetin), and the in vitro photoprotective effects as variables. We excluded the growth parameters, as they did not embrace the drying processes. First, we conducted Pearson's correlation analysis (Figure 6). The five variables (TPC, TFC, DPPH, ABTS, and FRAP) related to antioxidative activities showed highly positive correlations and were negatively related to ROS amount in the HaCaT and Hs68 cell lines, which indicates elevated in vitro photoprotective activities. Among the major *A. rugosa* metabolites, we found that RA amounts were not correlated with antioxidative properties and in vitro photoprotective effects. Instead, tilianin showed the highest negative correlation with ROS content in the HaCaT ($r = -0.81$) and Hs68 ($r = -0.86$) cell lines.

Tilianin was positively correlated with the antioxidative variables. Acacetin was positively correlated with antioxidative properties, but its correlation values were lower than tilianin. It signified that tilianin is the major active compound of *A. rugosa* against oxidative stress and photodamage. RA content was negatively correlated with tilianin or acacetin contents in *A. rugosa*. The content of each major metabolite was shifted by drying methods, which explains the high antioxidative and photoprotective effects of cold-air-dried *A. rugosa* samples.

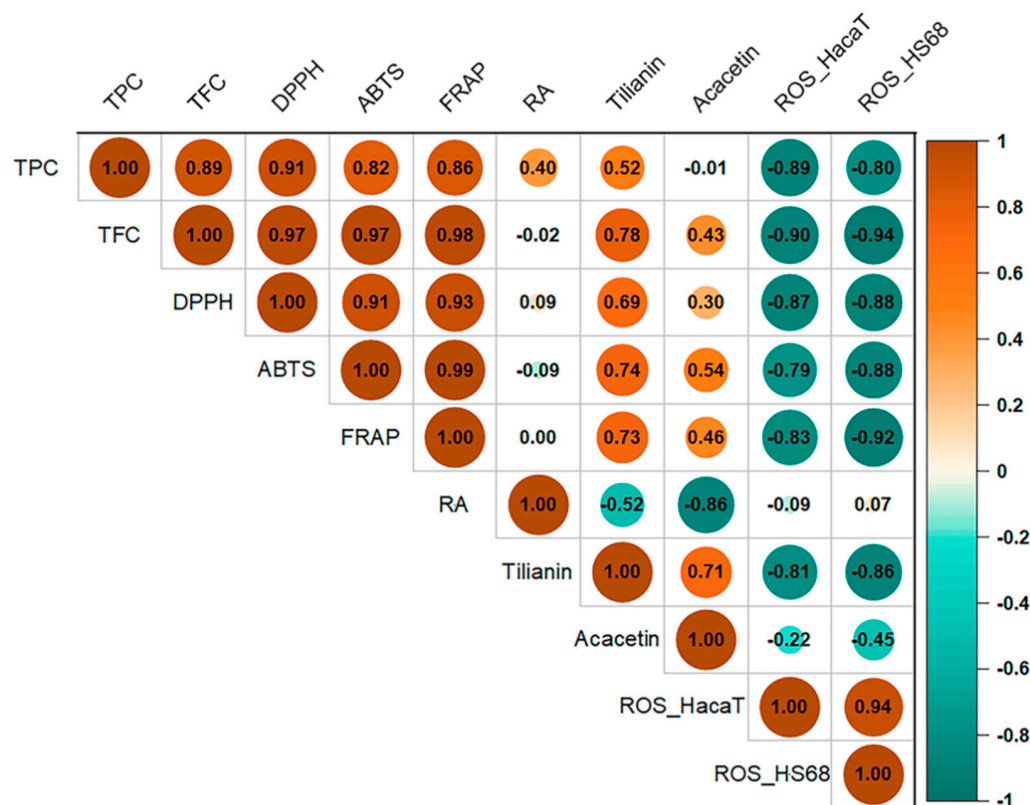


Figure 6. Correlation plot of the characteristics of *A. rugosa* samples. The Pearson's correlation was conducted between variables related to the antioxidative properties (TPC, TFC, DPPH, ABTS, and FRAP), major metabolites (RA, tilianin, and acacetin), and photoprotective effects (ROS_HaCaT and ROS_Hs68) of *A. rugosa*. ROS_HaCaT, reactive oxygen species amounts in UV-B irradiated HaCaT cell with treatment of *A. rugosa* extracts (40 µg/mL); ROS_Hs68, reactive oxygen species amounts in UV-B irradiated Hs68 cell with *A. rugosa* extract treatment (40 µg/mL).

The same tendencies were observed in the scoring PCA plot within the variables (Figure 7A). Tilianin and acacetin had a negative relationship with RA content, and tilianin had a strong relationship with factors related to antioxidative activities. Moreover, high antioxidative properties accompanied decreased ROS in UV-B irradiated cell lines. In a loading plot (Figure 7B), the EC 2.0 condition had a stronger relationship with antioxidative and photoprotective activities than other EC conditions, and cold-air-dried samples contained higher levels of tilianin but lower RA compared to the lyophilized groups.

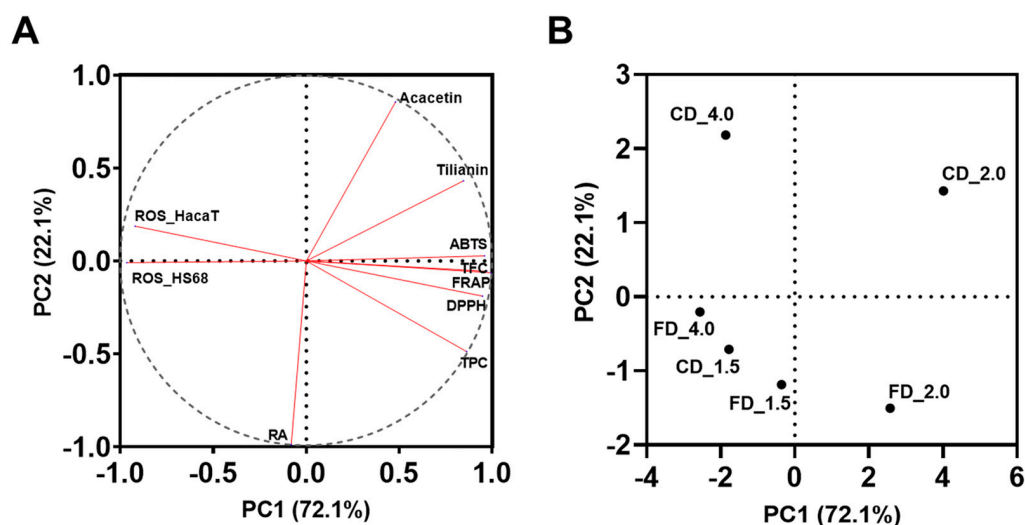


Figure 7. Principal component analysis (PCA) plot of the variables and sample groups. The PCA was analyzed using the variables (A) and the loading plot was visualized (B).

4. Discussion

The relationship between EC and plant characteristics has been verified in many types of crops. For example, in tomatoes, EC converted the growth parameters during the vegetative growth stage, and high EC promoted leaf formation [29]. Tomatoes' sugar and acid content and sensory properties were also affected by the nutrient solutions' EC values [30]. In addition, the EC condition and its application timing changed the amounts of lycopene, a typical secondary metabolite of tomatoes [31]. EC 2.0 and 4.0 treatment promoted all of *A. rugosa* growth parameters compared with those of EC 1.5 in the present study. Although a nutrient solution rich in minerals provides nutrients to plants, sometimes excessive EC can accumulate Na^+ , change the osmotic potential, and may cause ion toxicities [32]. Salt stress impedes crop growth and makes plants produce compatible metabolites with dissipating energies, leading to a severe yield reduction [33]. Lam, et al. [34] also showed a reduced growth rate and yield for *A. rugosa* when the EC condition exceeds 4.0, which may be causative of salt stress. Therefore, we concluded that EC 2.0 to 4.0 do not cause osmotic stress and they would be the optimal conditions to obtain lavish *A. rugosa*.

Although *A. rugosa* growth was promoted with an EC 4.0 nutrient solution, it did not increase the contents of active compounds in *A. rugosa*. The TPC and TFC of *A. rugosa* cultivated with EC 4.0 were low compared to those of the EC 2.0 group. In addition, low TPC and TFC may lead to low DPPH, ABTS, and FRAP activities of the EC 4.0 group. We also found that typical active compounds (RA, tilianin, and acacetin) of *A. rugosa* were lowered in the EC 4.0 condition. Phenolic compounds are secondary metabolites derived from central or primary metabolic processes in plants, and the metabolite profile of the plant is closely related to its nutritional characteristics [35]. Plants thrive under plentiful nutrients and optimum environments, but secondary metabolites are produced against stressful factors as a defensive reaction to harsh environments [36]. That is, biotic stresses by herbivores, bacteria, fungi, and viruses and abiotic stresses such as temperature, salinity, drought, flooding, excessive radiation, and nutrient deficiency trigger the synthesis of secondary metabolites in flora [37]. Likewise, a high EC nutrient solution increases the growth parameters and yield in green perilla; however, it decreases perillaldehyde and RA content [38]. Yuhang, et al. [39] also found that fertilizers assist *Prunella vulgaris* L. growth, but reduce the secondary metabolites (RA, ursolic acid, and oleanolic acid). Well-nourished environments increase the growth and yield of crops but may lower the accumulation of secondary metabolites. Nutrient deficiency may accelerate secondary

metabolite production, and nitrogen deprivation stimulates flavonol accumulation in the vegetative tissues of *Arabidopsis thaliana* and tomatoes [40].

RA, tilianin, and acacetin biosynthesis are conducted through a phenylpropanoid pathway starting from L-phenylalanin, and various genes, such as phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-coumarate coenzyme A ligase (4CL), are involved [41]. Previous studies reported that environmental factors modulate the expression of these genes in *A. rugosa*. That is, the treatment of specific wavelength light and methyl jasmonate changed the accumulation rates of each phenylpropanoid [3,16]. Besides the phenylpropanoid pathway, the shikimic acid pathway is a central mechanism providing aromatic compounds as versatile precursors for many secondary metabolites involving phenylpropanoids in plants [42]. The nutrition state of plants manipulates the expression levels of genes associated with the shikimic acid pathway, and deprivation of nutrients increases the signaling [43]. We surmise that the relatively low flavonoid content of the EC 4.0 group compared to EC 2.0 is due to the superior nutrition condition of *A. rugosa* therefore.

In addition to cultivation manners, the post-harvest process also critically switches the properties of crops. Drying is a basic process that eliminates moisture from foods and other industrial raw materials to enhance shelf life and reduce storage and transportation costs [44]. Moreover, dehydration is indispensable to gaining higher yields when extracting materials. We tested two popular drying methods (freeze drying and cold air drying) to confirm if they alter the active compound composition in *A. rugosa*. Although TPC, TFC, and DPPH and ABTS radical scavenging activities were not different between the freeze-dried and cold-air-dried samples, the RA, tilianin, and acacetin content were altered by the drying method. In comparison with lyophilized *A. rugosa*, cold-air-dried samples showed lowered RA content but increased tilianin and acacetin after dehydration. The diminution of active compounds in plant samples after dehydration is a common phenomenon due to the destruction and oxidation of chemical compounds during drying. The freeze-drying method requires high energy costs and drying times, but it highly impedes changes in the material quality and characteristics and active compound content compared with conventional air drying [45]. Therefore, the freeze-drying process is preferred in pharmaceutical industries to retain the high potency of medicinal herbs. However, tilianin and acacetin were increased in *A. rugosa* by cold air drying, and Park, et al. [46] also reported that oven drying at 25 °C reduced RA but increased tilianin content in dried *A. rugosa*. It implied that RA was degraded but tilianin was generated during cold air drying, and we took note of the biosynthetic pathway of phenylpropanoid in plants. Naringenin chalcone is a precursor of tilianin that converts to acacetin by chalcone isomerase, flavone synthase, and apigenin 4'-O-methyltransferase; and further, acacetin is transformed into tilianin by glucosyltransferase [41]. The general temperature condition of cold air drying cannot restrict enzymatic reactions. This therefore contributes to the mounting up of specific compounds. For example, oven-dried wheatgrass showed higher TPC and TFC compared to lyophilized samples in a study by Das, et al. [47], and Najjaa, et al. [48] observed that the changes in chemical compound profiling of *Allium sativum* by oven-drying may be due to allinase. Hence, we surmise that cold air drying may encourage increasing acacetin and tilianin followed by the actions of related enzymes, yet it demands further investigation.

In turn, altering the content of active compounds affected the photoprotective activity of *A. rugosa* in UV-B-stimulated skin cell lines. *A. rugosa* extract has potent antioxidation, antiphotaging, and skin-whitening effects via multiple mechanisms [49,50], and here, we verified the antioxidation and photoprotective activities of *A. rugosa* samples. We observed that TPC and TFC levels are closely related to the photoprotective effects of *A. rugosa* samples, and it connotes that proper cultivation and post-harvest processing can improve *A. rugosa* potency. Meanwhile, when the TPC and TFC are similar within the *A. rugosa* sample groups, the amounts of tilianin and acacetin highly affected the photoprotective effects of *A. rugosa*. Among the major compounds in *A. rugosa* extract, acacetin has versatile and powerful antioxidant and melanogenesis inhibition properties [51]. In UV-B-induced

HaCaT cells, acacetin attenuated MMP-1 gene expression and phosphorylation of MAPK signaling [17] and mitigated photo-aging by mediating sirtuin-3 (SIRT3)/ROS/MAPKs pathway against UV-A [18]. Tiliandin is also known for its powerful antioxidative and anti-inflammatory activities [52]. Therefore, the increased amounts of tiliandin and acacetin may have enforced the photoprotective effects of the *A. rugosa* extracts in the present study. Not only do acacetin and tiliandin enhance photoprotective effects, but acacetin downregulates monoamine oxidase (MAO) A and B expression, which are the therapeutic targets of depression and anxiety (MAO-A) and Alzheimer's and Parkinson's diseases (MAO-B) [53]; and tiliandin improves mitochondrial energy metabolism and alleviates oxidative stress in myocardial ischemia/reperfusion injury (MIRI) rat [54]. Hence, the stimulating acacetin and tiliandin synthesis by adjusting EC and drying methods could produce superior *A. rugosa* for industrial use.

5. Conclusions

In the present study, we verified the growth rates and functional properties of *A. rugosa* under different EC conditions and with two drying methods (freeze drying and cold air drying). When *A. rugosa* was grown with EC 1.5, 2.0, or 4.0 nutrient solutions, all growth parameters and yields were significantly increased with the EC 4.0 nutrient solution compared to those of EC 1.5 at 8 and 9 weeks. The EC 2.0 condition also increased two growth parameters (height and the number of leaves) compared with the EC 1.5 condition. Although the EC 4.0 condition led to mounted growth rates, TPC, TFC, FRAP, and radical scavenging activities were significantly lower than in the EC 2.0 condition. The EC 4.0 sample had lower levels of three representative active compounds in *A. rugosa* (RA, tiliandin, and acacetin) compared to the EC 2.0 condition, which may have been driven from overly affluent states, because the synthesis of secondary metabolites is boosted against stressful environments in plants. The TPC, TFC, FRAP, and radical scavenging activities were not prominently changed by the drying method, but cold air drying elevated the contents of tiliandin and acacetin. As tiliandin and acacetin are potent chemicals, cold-air-dried samples showed higher photoprotective effects in HaCaT and Hs68 cell lines compared to lyophilized groups. Put together, EC 4.0 condition could elevate *A. rugosa*'s growth rate and yield, but *A. rugosa* cultivated with the EC 2.0 nutrient solution showed higher functional activities. In the post-harvest process, cold air drying augmented the additional synthesis of tiliandin and acacetin and resulted in increased photoprotective effects of *A. rugosa*. Our results reveal the impacts of EC and drying methods during *A. rugosa* product formation and propose optimized conditions depending on its industrial purposes.

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Abbreviations

HIV: human immunodeficiency virus; HO-1, heme oxygenase-1; GSH, glutathione; SOD, superoxide dismutase; UV, ultraviolet; ROS, reactive oxygen species; MMP, matrix metalloproteinase; RA, rosmarinic acid; MAPK, mitogen-activated kinase; EC, electrical conductivity; DFT, deep-flow technique; WAS, weeks after sowing; TPC, total phenolic acid contents; TFC, total flavonoid con-

tents; DMEM, Dulbecco modified eagle medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline; DCFDA, 2', 7'-dichlorofluorescein diacetate; ANOVA, analysis of variance; SD, standard deviation; PCA, principle component analysis; PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate coenzyme A ligase; SIRT3, sirtuin-3; MAO, monoamine oxidase; MIRI, myocardial ischemia/reperfusion injury.

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