Polyphenol Production in *Peucedanum japonicum* Thunb. varies with Soil Type and Growth Stage

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In Japan, several plant species have high commercial value because of their functional properties. In this study, we aimed to investigate the effects of soil type (red, dark-red, and gray soil) and growth stage (vegetative and reproductive) on the growth and polyphenol production (chlorogenic acid, rutin, hesperidin, and diosmin) of *P. japonicum*. Plants grown in gray soil had the heaviest dry weight, followed by those grown in dark-red soil. Flowering plants grown in gray soil had a polyphenol concentration lower than those grown in the other two soil types. However, differences in the concentration of polyphenols were even larger between the growth stages. During the flowering period, the concentration of polyphenols sharply increased in the stems. Additionally, the flowers contained relatively similar amounts of polyphenols to stems and leaves, accounting for approximately 1/4–1/2 of the net amount in the plant.

**Key Words:** flowering, *Peucedanum japonicum* Thunb., polyphenol.

**Introduction**

*Peucedanum japonicum* Thunb. [PJT, family Umbelliferae; Japanese name: Botan boufu, Okinawa local name: Sakuna or Chom(e)igusa] is found in the warm to subtropical regions of East Asia and is native to Japan, where it is particularly common in the south-west region. Okinawa Prefecture has a subtropical climate (the prefectural capital is located at latitude 26°12'45''N and longitude 127°40'52''E) and a unique food culture, which is rich in seaweed, greenish-yellow vegetables, medicinal plants, and fat, but low in sodium, compared with that in other regions of Japan (Maeda et al., 2006). PJT, which is often eaten as an accompaniment to sashimi (raw fish) in Okinawa, is considered effective against paralysis and hypertension and is an antipyretic in cold (Sho, 2001). Because such functionality is known, people in Okinawa often call this plant a Chom(e)igusa, which can be translated as “long life grass” in English. Consequently, the Department of Agriculture, Forestry and Fisheries in Okinawa Prefecture has created a list of 28 traditional vegetables with unique characteristics (Okinawa Prefectural Government, 2006), including PJT that contains high levels of phenolic compounds and exhibits strong 2,2-diphenyl-1-picrylhydrazyl activity, which scavenges free radicals (Hisamoto et al., 2003; Maeda et al., 2006; Morioka et al., 2004). Polyphenols (e.g., chlorogenic acid and rutin) have been isolated from the aerial parts of PJT (Hisamoto et al., 2003), and diosmin and hesperidin, which are commonly found in citrus, have recently been detected in the leaves (Norimatsu and Mori, 2012). Several studies have investigated the function of these compounds and showed that chlorogenic acid has antioxidant activities, *in vivo* anti-edema, anti-obesity, and anti-diabetic effects (Lee et al., 2004; Norimatsu and Mori, 2012; Okabe et al., 2011). Because of its health benefits, PJT is cultivated commercially and its production has markedly increased in Okinawa Prefecture in response to the high demand (Okinawa General Bureau, 2015). The cultivated land of Okinawa Prefecture mainly comprises red soil (Kunigami maji, 31.4%), dark-red soil (Shimajiri maji,
40.9%), and gray soil (Jahagaru soil, 17.9%) (Kuba, 1993). On the main Okinawa Island, these soils are mainly distributed in the north (red soil), south (dark-red soil), and middle to south (gray soil) area. Red soil is low in organic matter and is acidic; dark-red soil ranges from acidic to alkaline, while gray soil is alkaline (Miyamaru, 2013). Thus, the soil type greatly differs from region to region in Okinawa Prefecture. Differences in the soil environment are known to affect plant growth and secondary metabolism (Heimler et al., 2017). The latter is also affected by the growth stage of the plant. For example, Song et al. (2014) reported that the concentrations of several flavones (and some polyphenols) in soybean leaves were altered by growth stage. Therefore, the quantity of polyphenol compounds in PJT may vary with soil type and growth stage. For functional plants such as PJT, the content of particular chemicals is important. Hence, it is important to identify the factors that affect the chemical profile of PJT during the cultivation period. In this study, we aimed to examine the effects of soil type and growth stage on the yield of polyphenol compounds in PJT by conducting a pot experiment.

**Materials and Methods**

**Plant cultivation**

The pot experiment was conducted from August 22 to November 27, 2014 in an open field at the Department of Agriculture, University of the Ryukyus, Okinawa Prefecture. PJT seedlings with three expanded leaves were transplanted on August 22 into 1/5000-a Wagner pots filled with air-dried soil. A total of 15 pots were used for each soil type (red, dark-red, and gray soil). The soils were collected from the main Okinawa Island (Fig. 1). On a weekly basis, the plants were irrigated, and 500 mL of modified Hoagland’s nutrient solution (Hoagland and Arnon, 1950), containing 6 mmol·L⁻¹ Ca(NO₃)₂, 4 mmol·L⁻¹ KNO₃, 2 mmol·L⁻¹ KH₂PO₄, 2 mmol·L⁻¹ MgSO₄, 25 μmol·L⁻¹ H₃BO₃, 10 μmol·L⁻¹ MnSO₄, 2 μmol·L⁻¹ ZnSO₄, 0.5 μmol·L⁻¹ CuSO₄, 0.5 μmol·L⁻¹ (NH₄)₆MoO₄·4H₂O, and 0.1 mmol·L⁻¹ Fe(III)-EDTA (C₁₀H₁₆FeN₂O₄), was applied as a fertilizer. The pH and electrical conductivity (EC) of the solution were 6.17 and 1.48 mS·cm⁻¹, respectively.

**Sampling and plant biomass**

Sampling was conducted at 10 weeks (October 31, 2014; T1) and 14 weeks (November 27, 2014; T2) after transplanting. At each sampling, the plants were separated into leaf blades, stems, and flowers (in the case of flowering plants). At the first sampling, all plants were in the vegetative growth stage, whereas at the second sampling, both non-flowering (T2A) and flowering (T2B) plants were observed, which were treated separately in all analyses. The samples were lyophilized for 72 h before measuring the dry weight. The lyophilized plant materials were then ground to a powder with a vibrating sampling mill (Model T1-100; Heiko Company, Fukushima, Japan) and used for chemical analysis.

**Soil analysis**

The soil pH and EC of each soil type were measured in soil that was diluted 2.5- and 5-fold with distilled water, respectively. The mineral content (Mg, P, K, and Ca) in soil was diluted 5-fold with distilled water was extracted by a laboratory shaker (SA-300; Yamato Scientific, Tokyo, Japan) overnight and measured using a Coupled Plasma Spectrometer (ICPS-2000; Shimazu, Kyoto, Japan). The total N of each soil type was measured using a Sumigraph (NC-90A; Shimazu) equipped with a gas chromatograph (GS-8A; Shimazu). Table 1 presents the chemical properties of each soil type. Each measurement was replicated three (N concentration) or five times.

**Determination of polyphenols**

The percentage of polyphenol content of each powdered plant sample was determined as described by Sakakibara et al. (2003). Briefly, 50 mg of powder was extracted with 2 mL of 90% methanol, containing 0.5% acetic acid. The solution was allowed to stand in a sonicator (US-5KS; SND, Nagano, Japan) for 1 min, and the supernatant was recovered by centrifugation at 3000 rpm for 10 min, and this was repeated three times. The extracts were then dried with a centrifugal concentrator (CVE-2000; Tokyo Rikakiki, Tokyo, Japan). The residues were dissolved in 0.5 mL dimethyl sulfoxide and filtered through a 0.2-μm pore size polytetrafluoroethylene membrane filter (13JH20AN; Toyoo Roshi Kaisha, Tokyo, Japan) before high-performance liquid chromatography (HPLC) analysis. HPLC was conducted using HPLC system (CBM-20A; Shimazu) equipped with a chromatography data station software (CBM-20A; Shimazu), an auto sampler (SIC-20A; Shimazu).
Shimazu), a column oven (CTO-20A0; Shimazu), and a diode array detection system (SPDM-20A; Shimazu) that could monitor all wavelengths from 200 nm to 600 nm. A Cosmosil 5C18-AR-II column (2 × 150 mm; Nacalai Tesque, Kyoto, Japan) was used at 35°C. Gradient elution was performed with solution A (50 mM sodium phosphate [pH 3.3] and 10% (v/v) methanol) and solution B (70% (v/v) methanol) delivered at a flow rate of 1.0 mL·min⁻¹, starting with 100% solution A, followed by 70% solution A for 15 min, 65% solution A for 30 min, 35% solution A for 10 min, and finally 0% solution A for 7 min. The injection volume for the extract was 10 μL. An example of sample analysis is shown in Figure 2. The detection of chlorogenic acid, rutin, hesperidin, and diosmin was performed at 320 nm, 260 nm, 280 nm, and 270 nm, respectively.

Results

Soil analysis

The gray soil had a higher pH and EC than the red and dark-red soil (Table 1). The content of N and other minerals, except P, was also higher in the gray soil than in the other two soils.

Dry weight accumulation

The dry weights of T1, T2A, and T2B in each soil type are presented in Table 2. No significant differences were identified in the total dry weight of T1 among the three soil types. However, T2A grown in gray soil had a significantly greater stem dry weight, whereas T2B grown in dark-red and gray soil had a significantly greater leaf and stem dry weight. The plants grown in gray soil had significantly lower flower dry weight than those grown in the other two soils. The total dry weight of T2A in gray soil was significantly higher than that of the plants grown in dark-red soil. Furthermore, the total dry weight of T2B in dark-red soil was significantly higher than that of the plants in red soil.

Polyphenol concentration in leaves

The concentration of polyphenols in the leaves of T1, T2A, and T2B in each soil type is presented in Table 3. No significant differences were identified in the concentration of chlorogenic acid, hesperidin, or diosmin in T1 among the three soil types; however, the concentration of rutin was significantly higher in T1 grown in dark-red soil than in red soil. Similarly, no significant differences were identified in the polyphenol concentrations of T2A among the three soil types. However, the concentration of polyphenols, except diosmin, was higher in T2B grown in dark-red soil than in the other two soil types. Additionally, T2B showed a significantly higher concentration of polyphenols than T2A in all three soil types, except for rutin in red and gray soil and hesperidin in gray soil. The concentration of total polyphenols in the leaves was higher in T1 than in T2A.

Table 1. Characteristics of red, dark-red, and gray soil.

| Soil type | pH (H₂O) | EC (mS·cm⁻¹) | Mg (mg/100 g) | P (mg/100 g) | K (mg/100 g) | Ca (mg/100 g) | N (% w/w) |
|-----------|----------|--------------|---------------|-------------|-------------|-------------|---------|
| Red       | 5.55 ± 0.13 a | 6.6 ± 0.2 a | 0.54 ± 0.04 a | 0.041 ± 0.005 b | 1.13 ± 0.03 a | 2.15 ± 0.13 a | 0.093 ± 0.0053 a |
| Dark-red  | 5.21 ± 0.14 a | 7.7 ± 0.1 b | 0.83 ± 0.01 b | 0.010 ± 0.002 a | 1.18 ± 0.03 a | 2.26 ± 0.06 a | 0.088 ± 0.0005 a |
| Gray      | 6.84 ± 0.07 b | 16.8 ± 0.3 c | 1.19 ± 0.04 c | 0.013 ± 0.006 a | 1.64 ± 0.03 b | 7.49 ± 0.25 b | 0.123 ± 0.0005 b |

Values are mean and standard error of three (N concentration) or five replicates. Different letters denote significant differences in pH, EC, and mineral concentration (Mg, P, Ca, K, and N) among soil types according to Tukey’s test (P < 0.05).
Table 2. Dry weight of leaves, stems, flowers, and all parts of *Peucedanum japonicum* grown in red, dark-red, or gray soil at the vegetative stage (T1) or the reproductive stage (T2A, non-flowering; T2B, flowering).

| Sampling Stage | Soil type | Leaf (g/plant) | Stem (g/plant) | Flower (g/plant) | Total (g/plant) |
|----------------|-----------|----------------|----------------|------------------|-----------------|
| T1             | Red       | 7.30±0.9 a     | 4.86±0.8 a     | 2.2±1.3 a        |                 |
|                | Dark-red  | 6.60±1.2 a     | 4.46±1.0 a     | 11.1±2.1 a       |                 |
|                | Gray      | 8.24±0.9 a     | 5.30±1.1 a     | 13.5±1.8 a       |                 |
| T2A            | Red       | 6.11±1.5 a     | 3.96±1.4 a     | 10.1±2.6 ab      |                 |
|                | Dark-red  | 5.15±1.2 a     | 3.60±0.3 a     | 8.8±1.5 a        |                 |
|                | Gray      | 7.79±1.3 a     | 6.90±1.6 b     | 14.7±2.7 b       |                 |
| T2B            | Red       | 3.60±0.4 a     | 4.12±1.2 a     | 4.62±1.8 b       | 12.3±3.2 a      |
|                | Dark-red  | 6.22±1.4 b     | 8.33±1.3 b     | 3.72±0.9 b       | 18.3±1.0 b      |
|                | Gray      | 7.55±1.4 b     | 8.71±2.2 b     | 1.33±0.9 a       | 17.6±2.3 b      |

Values are mean and standard deviation of plant dry weight of leaves, stems, flowers, and whole plants. Number of replicates was five at T1 in all soil types; five, three, and four at T2A in red, dark-red, and gray soil, respectively; and three, six, and four at T2B in red, dark-red, and gray soil, respectively. Different letters denote significant differences in dry weight of each part among different growth stages and soil types according to Tukey-Kramer test ($P<0.05$).

Table 3. Polyphenol concentration in the leaves of *Peucedanum japonicum* grown in red, dark-red, or gray soil at the vegetative stage (T1) or the reproductive stage (T2A, non-flowering; T2B, flowering).

| Sampling stage | Soil type | Chlorogenic acid (mg/100 g DW) | Rutin (mg/100 g DW) | Hesperidin (mg/100 g DW) | Diosmin (mg/100 g DW) | Total (mg/100 g DW) |
|----------------|-----------|-------------------------------|--------------------|-------------------------|-----------------------|--------------------|
| T1             | Red       | 231±68 B a                    | 99±9 B a           | 79±9 A a                | 19.9±0.3 A a          | 429±72 B a        |
|                | Dark-red  | 183±32 A a                    | 151±7 B b          | 83±13 A a               | 21.3±0.6 A a          | 439±47 B a        |
|                | Gray      | 259±57 B a                    | 126±19 B ab        | 90±13 A a               | 19.7±0.9 A a          | 494±84 B a        |
| T2A            | Red       | 46±26 A a                     | 38±7 A a           | 112±13 A a              | 20.0±0.4 A a          | 216±38 A a        |
|                | Dark-red  | 69±30 A a                     | 48±11 A a          | 120±15 A a              | 18.3±0.2 A a          | 255±54 A a        |
|                | Gray      | 42±22 A a                     | 48±18 A a          | 117±16 A a              | 19.9±0.9 A a          | 227±28 A a        |
| T2B            | Red       | 306±24 B b                    | 72±36 AB a         | 224±11 B b              | 27.3±0.9 B ab         | 630±10 B b        |
|                | Dark-red  | 434±29 B c                    | 229±17 C b         | 350±28 B c              | 33.0±1.7 B b          | 1047±63 C c       |
|                | Gray      | 127±21 B a                    | 84±10 AB a         | 104±7 A a               | 26.1±1.2 B a          | 341±19 AB a       |

Values are mean and standard error of polyphenol concentration. Number of replicates was five at T1 in all soil types; five, three, and four at T2A in red, dark-red, and gray soil, respectively; and three, six, and four at T2B in red, dark-red, and gray soil, respectively. Different capital and small letters denote significant differences in polyphenol concentration among different growth stages and soil types according to Tukey-Kramer test ($P<0.05$).

**Polyphenol concentration in stems**

The concentrations of polyphenols in the stems of T1, T2A, and T2B in each soil type are presented in Table 4. No significant differences were identified in the concentration of polyphenols in T1 (except diosmin) or T2A grown in different soil types. In T2B, the concentrations of chlorogenic acid and rutin were significantly higher in dark-red soil, hesperidin in red soil, and diosmin in red and dark-red soil. In all three soil types, the concentrations of all polyphenols, except rutin, were significantly higher in T2B than in T2A.

**Polyphenol concentration in T2B**

The concentrations of polyphenols in the leaves, stems, and flowers of T2B in each soil type are presented in Table 5. The concentrations of total polyphenols were significantly higher in flowers than in leaves and stems in all soil types. In particular, the chlorogenic acid and diosmin concentrations in flowers were markedly higher than those in the leaves and stems of plants grown in all soil types.
### Table 4. Polyphenol concentration in the stems of *Peucedanum japonicum* grown in red, dark-red, or gray soil at the vegetative stage (T1) or the reproductive stage (T2A, non-flowering; T2B, flowering).

| Sampling stage | Soil type | Polyphenols (mg/100 g DW) | Polyphenols (mg/100 g DW) |
|----------------|-----------|---------------------------|---------------------------|
|                |           | Chlorogenic acid           | Rutin                     | Hesperidin                 | Diosmin                    | Total                      |
| T1             | Red       | 37±8 a                    | 27±6 a                    | 111±6 a                    | 12±7±0.4 A ab              | 188±17 A a                 |
|                | Dark-red  | 23±5 A                    | 41±6 AB a                 | 72±32 A a                  | 13.9±6.2 AB b             | 149±9 A a                  |
|                | Gray      | 34±8 A                    | 29±6 B a                  | 108±20 A a                 | 10.3±0.5 A a              | 181±30 A a                 |
| T2A            | Red       | 8±3 a                     | 10±4 A a                  | 89±16 A a                  | 12.1±0.7 A a              | 119±17 A a                 |
|                | Dark-red  | 14±9 A                    | 16±7 A a                  | 81±47 A a                  | 12.1±7.0 A a              | 123±17 A a                 |
|                | Gray      | 6±1 a                     | 7±3 A a                   | 81±7 A a                   | 10.4±0.3 A a              | 105±5 A a                  |
| T2B            | Red       | 179±17 B ab               | 20±7 A a                  | 389±41 B b                 | 18.9±0.8 B b             | 607±27 B c                 |
|                | Dark-red  | 206±6 B b                 | 49±6 B b                  | 242±99 B a                 | 18.4±7.5 B b             | 516±18 B b                 |
|                | Gray      | 141±17 B a                | 24±3 AB a                 | 181±10 B a                 | 13.4±0.5 B a             | 360±19 B a                 |

Values are mean and standard error of polyphenol concentration. Number of replicates was five at T1 in all soil types; five, three, and four at T2A in red, dark-red, and gray soil, respectively; and three, six, and four at T2B in red, dark-red, and gray soil, respectively. Different capital and small letters denote significant differences in polyphenol concentration among different growth stages and soil types according to Tukey-Kramer test ($P<0.05$).

### Table 5. Polyphenol concentration in the leaves, stems, and flowers of flowering (T2B) *Peucedanum japonicum* grown in red, dark-red, or gray soil.

| Plant part | Soil type | Polyphenols (mg/100 g DW) | Polyphenols (mg/100 g DW) |
|------------|-----------|---------------------------|---------------------------|
| Leaf       | Red       | 306±24 A b                | 72±36 A a                 | 224±11 A b                 | 27.3±0.9 B ab             | 630±10 A b                 |
|            | Dark-red  | 434±29 A c                | 229±17 B b                | 350±28 B c                 | 33.0±1.7 A b             | 1047±43 B c                |
|            | Gray      | 127±21 A a                | 84±10 B a                 | 104±7 A a                  | 26.1±1.2 A a             | 341±19 A a                 |
| Stem       | Red       | 179±17 A ab               | 20±7 A a                  | 389±41 B b                 | 18.9±0.8 B a             | 607±27 A c                 |
|            | Dark-red  | 206±6 A b                 | 49±6 B b                  | 242±99 A a                 | 18.4±7.5 B a             | 516±18 B b                 |
|            | Gray      | 141±17 A a                | 24±3 AB a                 | 181±10 B a                 | 13.4±0.5 B a             | 360±19 B a                 |
| Flower     | Red       | 783±66 B a                | 75±26 A b                 | 186±39 A a                 | 74.4±3.0 c               | 1118±108 B a               |
|            | Dark-red  | 1342±139 B b              | 38±5 A ab                 | 316±19 AB b                | 144.7±20.6 B a           | 1840±168 C b               |
|            | Gray      | 1388±71 B b               | 18±1 B a                  | 242±29 B ab                | 79.4±12.8 B a           | 1727±96 B ab               |

Values are mean and standard error of polyphenol concentration in leaves, stems, and flowers. Number of replicates was three, six, and four in red, dark-red, and gray soil, respectively. Different capital and small letters denote significant differences in polyphenol concentration among different plant parts and soil types according to Tukey-Kramer test ($P<0.05$).

### Discussion

This study investigated changes in the concentrations of several polyphenol compounds in PJT grown in different soil types and at different growth stages. Our results showed that both polyphenol concentration and plant dry weight were affected by soil type.

The three soil types used in this study had different nutrient concentrations and characteristics. N is known to increase plant dry weight, whereas EC values are known to be involved in plant growth. The relatively high values of the soluble cations Ca, Mg, and K contribute to the high values of EC in gray soil. Gray soil is a heavy clay soil with excess moisture as well as higher N and EC values than red and dark-red soils and is considered the most fertile for plant cultivation in Okinawa Prefecture (Miyamaru, 2013; Onaga and Gibo, 1984). Similarly, in the present study, the dry weight of T2A in gray soil was the highest and that of T2B in gray soil was not the highest but still relatively higher in gray soil (Table 2). However, the presence of N and other minerals in the soil has been reported to suppress poly-

![Fig. 3. Net amount of polyphenols (the sum of chlorogenic acid, rutin, hesperidin, and diosmin) in the leaves, stems, and flowers of flowering *Peucedanum japonicum* grown in red, dark-red, or gray soil. Values are mean and standard error of polyphenol net amount. Number of replicates was five at T1 in all soil types; five, three, and four at T2A in red, dark-red, and gray soil, respectively; and three, six, and four at T2B in red, dark-red, and gray soil, respectively. Different capital and small letters denote significant differences in polyphenol concentration among different plant parts and soil types according to Tukey-Kramer test ($P<0.05$).](image-url)
phenol production in plants (Li et al., 2007; Ruan et al., 2009; Zheng et al., 2008). Heimler et al. (2017) pointed out that the polyphenol content of plants varies with environmental factors, among which the relationship with N is particularly strong. Thus, despite the relatively high dry weight production, we observed that plants grown in gray soil exhibited a lower polyphenol yield than those grown in the other two soils, possibly because of the soil nutrient richness and/or physical condition.

The concentration of polyphenols sharply increased in the stems during flowering (Table 4). Chen et al. (2016) reported that during the in vitro induction of somatic embryogenesis, changes in the growing phase of PJT promotes synthesis of the secondary metabolite chlorogenic acid, similarly as observed in the present study. Additionally, Verma and Kasera (2007) reported that the concentration of phenols reaches a peak at the flowering stage in Boerhavia diffusa and Sida cordifolia. Previous studies reported that the sink demand for nutrients often controls leaf photosynthesis and other metabolic processes (Suwa et al., 2006) and that an increased demand for polyphenols in the reproductive parts induces polyphenol synthesis in the leaves. The accumulation of polyphenols in the stems after flowering (Fig. 3) suggested that polyphenols, similarly to other chemical compounds that are synthesized in the leaves, were translocated to other parts of the plant, such as the growing parts or fruits, via the phloem (del Baño et al., 2003). In particular, the chlorogenic acid and diosmin concentrations greatly increased in the flowers in all three soils (Table 5), revealing that these two polyphenols may be needed by the sink parts. This coincides with Sugawara and Igarashi (2013) that the accumulation of polyphenols (including chlorogenic acid) in reproductive parts suggests protection against damage caused by ultraviolet rays or oxidative stress.

In the present study, the leaves of T1 contained higher concentrations of polyphenols than those of T2A (Table 3). Verma and Kasera (2007) reported that maximum accumulation of alkaloids and phenols occurred in summer in Asparagus racemosus, Boerhavia diffusa,
and Sida cordifolia. Thus, the difference we observed may be caused by seasonal factors, since the first sampling was done close to summer, whereas the second sampling was done close to winter. Therefore, the accumulation of polyphenols could be influenced by seasonal changes, but the effect of the growth stage is stronger.

PJT leaves are usually the only plant part that is harvested and used either raw as food or processed as supplements. However, our findings showed that the polyphenol concentration increases after flowering in the stems (Table 4), and that the polyphenol concentration in the flowers is also relatively high (Table 5). This suggests that additional plant organs can be used for developing commercial products.

Overall, our results showed that suitable soil conditions can increase the yield of polyphenols in PJT. Additionally, the current cultivation practices that include the growing of PJT exclusively for their leaves can be changed, since the stems after flowering and the flowers contain similar levels of polyphenols to those of polyphenols in leaves. These modifications of cultivation practices, especially utilization of stems and flowers after flowering, do not require additional equipment, labor, or financial inputs, and therefore, immediate application is possible.

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