Seasonal Variations of Metabolome and Tyrosinase Inhibitory Activity of Lespedeza maximowiczii during Growth Periods

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ABSTRACT: Lespedeza species are useful for pasture and energy crops as well as medical plants. We determined the metabolites discriminated from the each growth period (3, 4, 6, 8, 15, and 18 months) after germination in leaves and stems of Lespedeza maximowiczii by a metabolomics technique. Specifically, levels of sugars and luteolin-dominated derivatives were significantly elevated in samples harvested in November. This may be related to the cold tolerance mechanism against the low temperatures of the winter season. The concentrations of secondary metabolites, isoflavones and flavanones, as well as tyrosinase inhibitory activity were the highest in the 6 month samples, which were harvested in September, during the fall season. The tyrosinase inhibitory activity in leaves was higher than that in stems irrespective of the growth period. This study suggests that mass spectrometry-based metabolite profiling could be used as a tool to examine quantitative or qualitative metabolite changes related to seasonal variations and to understand the correlation between activity and metabolites.

KEYWORDS: Lespedeza maximowiczii, growth period, metabolite profiling, tyrosinase inhibitory activity

INTRODUCTION

Species of the genus Lespedeza, deciduous shrubs in the family Fabaceae that are native to East Asia, are known for their use in traditional herbal medicine. It was also introduced as an energy crop to the United States for energy production. Moreover, it is being considered as a pasture crop and for erosion control because of its strong competitive ability and tolerance of drought and acidity. Studies of Lespedeza species in this genus exhibit antioxidant and anti-diabetic activities, anti-melanogenesis, and inhibition of bacterial neuraminidase activity. In particular, Lespedeza maximowiczii exhibits inhibitory activity against advanced glycation end products as well as estrogenic activity. Recently, our team studied the tyrosinase inhibitory activity of two compounds, ucinanone B and desmodianone H, isolated from L. maximowiczii, as well as haginin A (isolated from the Lespedeza cryptobotrya), a potent inhibitor of hyperpigmentation induced by ultraviolet radiation.

Recently, interest of metabolomics is increasing in plant research fields. Plant metabolomics is a useful analytical tool used to detect changes in plant metabolite levels in response to environmental changes. Gas chromatography-mass spectrometry (GC-MS) is frequently practicable toward the overall primary metabolism. On the other hand, liquid chromatography-mass spectrometry (LC-MS) is more suitable for the large group of plant secondary metabolites. Primary metabolites such as carbohydrates, proteins, lipids, and nucleic acids are essential for the survival and reproduction of plants during growth and development, while the production of secondary metabolites such as alkaloids, flavonoids, and phenolic compounds is dependent on a combination of environmental factors such as temperature, rainfall, and the length and strength of sunlight exposure. Secondary metabolites act as a defense system against hostile environmental conditions and pathogen attack. The production of secondary phytoconstituents and their concentrations also varies among plant parts and growth periods.

However, no mass spectrometry (MS)-based metabolite profiling of L. maximowiczii in the context of its growth period has been reported. In addition, seasonal variation of the bioactive constituents from L. maximowiczii is still limited. The aim of this study was to examine the significant variations in metabolite during the growth period after germination, from both the leaves and stems of L. maximowiczii, via metabolite profiling using ultraperformance liquid chromatography (UPLC)-quadrupole-time-of-flight (Q-TOF)-MS and gas chromatography (GC)-TOF-MS. The data were analyzed via multivariate analysis, including principal component analysis (PCA), partial least-squares discriminant analysis (PLS-DA), and orthogonal projection to latent structures discriminate analysis (OPLS-DA). Furthermore, we investigated changes in the tyrosinase inhibitory activity of the leaves and stems of L. maximowiczii with growth period and explained the correlation between bioactivity and secondary metabolites.

MATERIALS AND METHODS

Chemicals and Reagents. Methanol, acetonitrile, and water were purchased from Fisher Scientific (Pittsburgh, PA). Formic acid, N-
methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), methoxyamine hydrochloride, pyridine, kojic acid, mushroom tyrosinase (1000 units/mL), L-tyrosine, and commercial standard compounds were obtained from Sigma Chemical Co. (St. Louis, MO). Disodium hydrogen phosphate 12-water and sodium dihydrogen phosphate dehydrate from Sigma Chemical Co. (St. Louis, MO). Disodium hydrogen chloride, pyridine, kojic acid, mushroom tyrosinase (1000 units/mL), was used as a reference lock mass (\([z] m/z 554.2615, [+] m/z 556.2771, 10 \mu L/min\) to ensure accuracy and reproducibility, by independent LockSpray interference. LC-MS data were analyzed using a LTQ XL linear ion trap mass spectrometer/mass spectrometer from Thermo Fischer Scientific (San Jose, CA) coupled with an RSL autosampler, RS column compartment, RS diode array detector, and Dionex UltiMate 3000 RS pump (Dionex Corp., Sunnyvale, CA). The separation was performed on a Thermo Scientific SynchroC18 UHPLC column with a particle size of 1.7 \(\mu m\). The mobile phases were 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B). The solvent gradient system was as follows: 5% B for 1 min, increased to 100% B for 9 min, held at 100% for 1 min, decreased to 5% B over 2 min, and finally held at 5% B for 1 min. Five microliters of a sample extract solution was injected, and the flow rate was maintained at 0.3 mL/min. The waters Q-TOF Primer (Micromass MS Technologies, Manchester, U.K.) was operated in quadrupole mode for the MS data, and the TOF data were collected in the range of m/z 100–1000 in both negative (−) and positive (+) ion modes. The operating parameters were as follows: ion source temperature, 100 °C; desolvation gas flow, 650 L/h at 200 °C; capillary voltage, 2.3 kV; and cone voltage, ≤35 V. The V mode of the mass spectrometer was used, and data were collected in the centroid mode with a scan accumulation time of 0.2 s. Leucine enkephalin was used as a reference lock mass (\([-z] m/z 554.2615, [+] m/z 556.2771, 10 \mu L/min\) to ensure accuracy and reproducibility, by independent LockSpray interference. LC-MS data were analyzed using an LTQ XL linear ion trap mass spectrometer/mass spectrometer from Thermo Fischer Scientific (San Jose, CA) coupled with an RSL autosampler, RS column compartment, RS diode array detector, and Dionex UltiMate 3000 RS pump (Dionex Corp., Sunnyvale, CA). The separation was performed on a Thermo Scientific SynchroC18 UHPLC column with a particle size of 1.7 \(\mu m\). The mobile phases were 0.1% (v/v) formic acid in water (A) and acetonitrile (B). The gradient system was as follows: from 10 to 100% solvent B over 18 min and then re-equilibrated to initial conditions over 4 min. The flow rate was 0.3 mL/min, and the injection volume was 10 \(\mu L\). Mass spectra were obtained using ion traps in both negative (−) and positive (+) modes within a range of m/z 100–1000. A photodiode array was set for detection at 200–600 nm and managed using 3DField. The operating parameters were as follows: source voltage, ±5 kV; capillary temperature, 350 °C; and capillary voltage, 39 V. Tandem MS analysis was conducted via scan-type turbo data-dependent scanning (DDS) under the same conditions used for MS scanning.

**Tyrosinase Inhibitory Activity.** The analysis of tyrosinase inhibitory activity was performed as previously described with slight modifications. The reaction mixture, consisting of 125 \(\mu L\) of 0.1 M sodium phosphate buffer (pH 6.5), 5 \(\mu L\) of the methanol extract of *L. maximowiczii* dissolved in 100% methanol, 40 \(\mu L\) of 1.5 M L-tyrosine, and 30 \(\mu L\) of mushroom tyrosinase (1000 units/mL), was added to a 96-well plate. After incubation at 37 °C for 20 min, the absorbance was measured at 490 nm every 0 and 20 min with a spectrophotometer (Spectronic Genesys 6, Thermo Electron, Madison, WI). The enzyme activity was expressed as the following formula:
% inhibition rate = \left( \frac{C_{20\text{ min}} - C_{0\text{ min}}}{S_{20\text{ min}} - S_{0\text{ min}}} \right) \times 100

where $C_{20\text{ min}}$ and $C_{0\text{ min}}$ are the absorbance of the control after 20 and 0 min, respectively, whereas $S_{20\text{ min}}$ and $S_{0\text{ min}}$ are the absorbance of the sample after 20 and 0 min, respectively.

**Data Processing and Multivariate Analysis.** GC-TOF-MS data files were converted to CDF format (*.cdf) using the Leco ChromaTOF software program (version 4.44, Leco Corp.). Data processing for the UPLC-Q-TOF-MS analysis was conducted using MassLynx software, and raw data files were converted into netCDF (*.cdf) format using Waters DataBridge version 4.1. After conversion, the MS data were processed using the metAlign software package (http://www.metalign.nl) to obtain a data matrix containing retention times, accurate masses, and normalized peak intensities. The resulting data were exported to Excel (Microsoft, Redmond, WA), and multivariate statistical analysis was performed using SIMCA-P+ (version 12.0, Umetrics, Umeå, Sweden). Unsupervised PCA and supervised PLS-DA models were used to compare the production of different metabolites among growth periods in the leaves and stems of *L. maximowiczii*, respectively. The OPLS-DA was also performed to compare metabolite differences at 6 months of the growth period between leaves and stems of *L. maximowiczii*. Metabolites with variable importance as determined by a projection (VIP) value of >0.7 and a $p$ value of <0.05 were selected. To visualize differences in metabolites among samples, heat map analyses were performed using Mev software program version 4.8 (http://www.tm4.org). Significant differences were tested by one-way analysis of variance (ANOVA) using the STATISCA program (version 7.0, StaSoft Inc., Tulsa, OK). Differences in tyrosinase inhibitory activity were analyzed by one-way ANOVA and Duncan’s multiple-range test using PASW Statistics 18 (SPSS Inc., Chicago, IL). Pairwise correlations between secondary metabolites and tyrosinase inhibitory activity were calculated by Pearson’s correlation coefficient test using PASW Statistics 18, and correlation maps were generated by the MEV software program.

**RESULTS**

The Differentiated Metabolites in the Leaves and Stems of *L. maximowiczii* during Different Growth Periods Using GC-TOF-MS Analysis. Differences between the growth periods of the leaves and stems of *L. maximowiczii* were clearly visible in the principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) score plots, respectively (panels a and c of Figure 1, respectively). In particular, both leaves and stems harvested 6 months after...
germination were significantly distinguished from those harvested during other months. Significantly different metabolites were selected as variables by PLS1 and PLS2 in the PLS-DA score plot, based on variable importance in the projection (VIP) value (>0.7) and p value (<0.05). As shown in the Supporting Information (Table S1), 18 and 26 metabolites in the leaves and stems, respectively, were selected as variables, which changed with growth period. Metabolites in leaves and stems were signiﬁcantly distinguished from those harvested during other months.

Figure 2. Heat maps of different metabolites from the leaves (a and c) and stems (b and d) of *L. maximowiczii* during different growth periods, from GC-TOF-MS (a and b) and UPLC-Q-TOF-MS (c and d) analysis. Growth periods after germination are shown in each column, with colors representing relative abundance, and relative intensities indicated by the heat scale. The numbering of metabolites is identical to that in Tables S1 and S2.
stems, including amino acids, organic acids, sugars and sugar alcohols, fatty acids, and catechins, were identified on the basis of their retention times and mass fragmentation, using the NIST® library, and compared to the commercial standards.

Among discriminated variables according to the growth period in leaves, most primary metabolites, except for mannitol (19), maltose (22), palmitic acid (23), stearic acid (24), and nonidentified metabolite (27), were produced in relatively smaller quantities 6 months after germination than during other months (Figure 2a). Four metabolites, including fructose (16), glucose (17), galactose (18), and sucrose (21), were detected in the largest quantities in L. maximowiczii leaves harvested in November (at 8 months).

However, the following metabolites were detected in the largest quantities in stems 6 months after germination (Figure 2b): amino acids such as valine (2), isoleucine (3), serine (4), threonine (5), aspartic acid (6), and tyrosine (9); sugars such as xylitol (15), myo-inositol (20), and maltose (22); malonic acid (10); palmitic acid (23); and two nonidentified metabolites (26 and 27). The levels of sugars such as fructose (16), glucose (17), and sucrose (21) were higher in stems harvested in November (at 8 months) than in stems harvested during other months.

The Discriminated Metabolites in Leaves and Stems of L. maximowiczii during Different Growth Periods Using UPLC-Q-TOF-MS Analysis. In the UPLC-Q-TOF-MS analysis, the leaves and stems of L. maximowiczii samples were observed to produce different patterns in the PCA and PLS-DA score plots, according to growth period (3, 4, 6, 8, 15, and 18 months). In terms of overall secondary metabolites, leaves harvested 6 months after germination were significantly different from those harvested during other months (Figure 1b). As seen in Figure 1d, the stem samples collected 6 months after germination are clearly separated by PCA (29.1%) from those collected at other times. To reveal the differences in secondary metabolites produced in the leaves and stems during different growth periods, both a VIP value of >0.7 and a p value of <0.05 of variables obtained from PLS1 and PLS2 were applied. As shown in the Supporting Information (Table S2), these metabolites were tentatively identified according to retention time, molecular weight, elemental composition, error and i-Fit, from the UPLC-Q-TOF-MS and UV spectral data, MS/MS fragmentation patterns obtained via UHPLC-ESI-MS/MS, and comparisons with reference compounds.

A total of 19 metabolites, including p-coumaric acid (29), luteolin glucosyl arabinoside or arabinosyl glucoside (30), apigenin glucosyl arabinoside or arabinosyl glucoside (31), isoferulitn glucoside (32), hydroxyleuteolin (33), kaempferol diharmnoside (34), quercetin glucoside (35), isovitexin glucoside (36), luteolin glucoside (37), isovitexin (38), daidzein (39), naringenin (42), genistein (43), kaempferol (44), desmodianone H (48), uncinnanine B (49), and two nonidentified metabolites (45 and 46), were present in the largest quantities during November (at 8 months) in L. maximowiczii leaves.

As shown in Figure 2d, eight metabolites were present in large quantities in stems collected 6 months after germination: isovitexin glucoside (36), isovitexin (38), daidzein (39), quercetin (41), desmodianone H (48), uncinnanine B (49), and two nonidentified metabolites (45 and 46).

Correlation between Tyrosinase Inhibitory Activity and Secondary Metabolites in the Leaves and Stems of L. maximowiczii. Both the leaves and stems of L. maximowiczii collected 6 months after germination showed the highest levels of tyrosinase inhibitory activity, while those harvested 8 months after germination showed the lowest activity (Figure 3a,b). The tyrosinase inhibitory activity in leaves (54.6%) at 6 months was significantly higher than that in stems at the same harvest time (20.3%).

To visualize the correlations between tyrosinase inhibitory activity and secondary metabolites in the leaves and stems of L. maximowiczii, correlation maps are presented (Figure 3c,d). The Pearson’s correlation coefficients (r) of all secondary metabolites were calculated and represented as positive correlations when $0 < r < 1$ and negative correlations when $-1 < r < 0$. The results indicate that nine secondary metabolites were positively correlated with tyrosinase inhibitory activity in the leaves: kaempferol diharmnoside (34), isovitexin glucoside (36), isovitexin (38), daidzein (39), hydroxyleuteolin (40), naringenin (42), genistein (43), desmodianone H (48), and uncinnanine B (49). Meanwhile, seven secondary metabolites were strongly positively correlated with tyrosinase inhibitory activity in the stems: isovitexin (38), daidzein (39), quercetin (41), desmodianone H (48), uncinnanine B (49), and two nonidentified metabolites (45 and 46). Of the secondary metabolites that were positively correlated with tyrosinase inhibition activity, four metabolites were present in the largest quantities in both leaves and stems 6 months after germination: isovitexin (38), daidzein (39), quercetin (41), desmodianone H (48), uncinnanine B (49), and uncinnanine B (49).

Differences in the Tyrosinase Inhibitory Activity and Metabolite Composition According to Plant Parts. The result that tyrosinase inhibitory activity in leaves of L. maximowiczii was higher than that in stems irrespective of the growth period was considered on the basis of difference in metabolite composition between them. Therefore, we applied PCA and PLS-DA from LC-MS data sets to identify metabolite differences between the plant parts, leaves and stems. The PLS-DA models demonstrated the differences between samples: $R^2_{Y(cum)} = 0.647, R^2_{Y(cum)} = 0.992, Q^2_{Y(cum)} = 0.877$, and $p < 0.05$. Leaves and stems were clearly separated by PLS1 (Supporting Information, Figure 1Sa). Eight metabolites were
Tyrosinase inhibitory activity was also significantly affected by secondary metabolite changes. Six months after germination, many primary metabolites were present in smaller quantities in the leaves, while most metabolites were present in larger quantities in the stems than in other months (Figure 2a,b). In particular, amino acids were more abundant in stems than in leaves and were present in high concentrations 6 months after germination. Amino acids are the major metabolic carbon and nitrogen sources for plant metabolic engineering during growth and development.23 Our data also showed that primary metabolites such as sugars, organic acids, and fatty acids were present in larger quantities in stems than in leaves. Among them, the level of sucrose, an important product from photosynthesis, was elevated in both leaves and stems harvested at 3 and 4 months, which are the summer quarter, from June to July. In general, the rate of photosynthesis during the summer season is higher than in other seasons because more sunlight and higher temperatures elevate the rate of photosynthesis. In plants, primary metabolites are essential for growth, development, respiration, and photosynthesis.22 As is shown in Table 1, the L. maximowiczii plants grew to 70 cm within 6 months of germination. The rate of vertical growth was also the highest 6 months after germination. These data indicate that the morphological changes observed are largely related to changes in metabolite production during growth.

We also detected variations with growth period in the production of many flavonoids, such as isoavone, flavanone, flavonol, flavone, flavone glucosides, and flavonol glucosides. Our results show a remarkable difference between the chemical contents and constituents of L. maximowiczii leaves and stems 6 months after germination and other months during the growth period (Figure 2c,d). Moreover, our data showed significant tyrosinase inhibitory activity in both leaves and stems during the same period. The activity in the leaves was significantly higher than in the stems 6 months after germination. In the correlation map (Figure 3c,d), four metabolites, isovitexin (38), daidzein (39), desmodianone H (48), and uncinanone B (49), which were strongly positively correlated with each other, were reported to be regulatory agents of melanogenesis.9,23,24 As these metabolites were present with the largest quantities in the stems 6 months after germination, they may be the most active 6 months after germination. The samples at this time were harvested in September, which is the start of the fall season in Korea. Among the secondary metabolites, the accumulation of isoavonoids and flavonanes contributed to high tyrosinase inhibitory activity in the fall (September 2013). On the other hand, a correlated decline in isoavones and flavonanes resulted in relatively low tyrosinase inhibitory activity in winter (November). For a year after germination, the tyrosinase inhibitory activity at 15 months (June 2014) was lower than after 3 months (June 2013). This was identically

**DISCUSSION**

In this study, we determined that L. maximowiczii underwent the most significant changes in metabolites in both the leaves and the stems 6 months after germination. This was determined by comprehensive MS-based metabolite profiling. Tyrosinase inhibitory activity was also significantly affected by secondary metabolite changes. Six months after germination, many primary metabolites were present in smaller quantities in the leaves, while most metabolites were present in larger quantities in the stems than in other months (Figure 2a,b). In particular, amino acids were more abundant in stems than in leaves and were present in high concentrations 6 months after germination. Amino acids are the major metabolic carbon and nitrogen sources for plant metabolic engineering during growth and development.23 Our data also showed that primary metabolites such as sugars, organic acids, and fatty acids were present in larger quantities in stems than in leaves. Among them, the level of sucrose, an important product from photosynthesis, was elevated in both leaves and stems harvested at 3 and 4 months, which are the summer quarter, from June to July. In general, the rate of photosynthesis during the summer season is higher than in other seasons because more sunlight and higher temperatures elevate the rate of photosynthesis. In plants, primary metabolites are essential for growth, development, respiration, and photosynthesis.22 As is shown in Table 1, the L. maximowiczii plants grew to 70 cm within 6 months of germination. The rate of vertical growth was also the highest 6 months after germination. These data indicate that the morphological changes observed are largely related to changes in metabolite production during growth.

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shown between 6 months (September 2013) and 18 months (September 2014). Moreover, the phenotypes of *L. maximowiczii* showed differences in the same period of the two different years. The leaves and stems within 1 year of germination were smaller and thinner than others. On the other hand, the shapes of leaves and stems harvested in the same period of the second year were larger and thicker than those of leaves and stems harvested within one year. These differences were closely associated with metabolite changes. These results indicated that differences in phytochemicals for the one-year interval were attributed to the contents of secondary metabolites, such as flavonoids, affecting tyrosinase inhibitory activity in each growth period. Also, the tyrosinase inhibitory activity in both leaves and stems harvested in September was higher than those in June. Therefore, our study suggests that harvesting *L. maximowiczii* in the fall will be useful in bioactivities because the inhibition properties of melanogenesis were the highest during this season.

In the winter season, the changes in metabolites for surviving with cold tolerance were greater than those related to tyrosinase inhibitory activity. We found the changes in the production of some sugars 8 months after germination to be particularly significant. One of the environmental factors potentially affecting plant growth is temperature. Low temperature, in particular, subjects growing plants to cold stress and induces carbohydrate changes in many plant species. Sugar metabolism plays an important role in the survival of cold stress. Sucrose is well known as one of the soluble sugars, which accumulate in plants as a reserve carbohydrate source in preparation for cold conditions. Our results showed that the levels of fructose (16), glucose (17), and sucrose (21) were significantly increased in both leaves and stems harvested in November (Figure 2), which is the beginning of the winter season to start at a low temperature (average temperature of 5 °C) in Korea. These results suggest that changes in sugars are associated with seasonal variations depending on the growth period. Various derivatives of luteolin dominated the metabolite profiles of leaves harvested in November (8 months after germination), including luteolin glucosyl arabinoside or arabinosyl glucoside (30), hydroxyluteolin (33), and luteolin glucoside (37). Phenolic compounds play an important role by replacing other compounds in cellular metabolism during the winter. Phenolic compound contents are affected by environmental factors such as temperature, UV light, rainfall, and soil conditions. Our data suggest that the accumulation of phenolic compounds in the leaves might be an adaptation to low-temperature stress during the winter. From the overall results, the relationship between metabolite changes and tyrosinase inhibitory activity in *L. maximowiczii* during the growth period is briefly summarized in Figure 4.

In addition, the variation of metabolites from *L. maximowiczii* was largely influenced by the monthly average precipitation within a year of germination. In the summer rainy season, the levels of organic acids such as succinic acid and shikimic acid were significantly decreased. In the fall dry season, amino acid contents were generally increased (Figure 2).

Moreover, we determined a significant difference in the contents of metabolites and tyrosinase inhibition activity between the plant parts, leaves and stems. Many researchers reported that gene expression varied between plant parts, in spite of being subjected to the same environmental factors. For this reason, they predicted that there are differences also in the metabolite composition of plant parts. Also, our team previously studied the fact that phytochemical production was dependent on the particular parts of the plant in *Lespedeza*.

Leaves are exposed to much higher levels of UV radiation than stems, and therefore, photosynthesis in the leaves is heavily influenced by leaf structure characteristics such as thickness and density. Thus, we assume that metabolite production in the leaves is more strongly affected by these conditions than in the stems. In particular, these tendencies were remarkably shown 6 months after germination (Supporting Information, Figure 2S). Isolavonoids such as daidzein (39), hydroxygenistein (40), genistein (43), and uncinabinone B (49) were closely associated with tyrosinase inhibitory activity, and their contents in leaves were superior to those in stems 6 months after germination in our data. This phenomenon was also observed in the different distributions of isolavonones in different parts of red clover and in red clover harvested at different growing stages. This study demonstrated the two different parts of *L. maximowiczii* plants exhibited different levels of tyrosinase inhibitory activity due to differences in bioactive metabolites. These results suggest these metabolites are the variables discriminated from the plant parts in *L. maximowiczii* and affect its activity.

In conclusion, we confirmed that the production of primary and secondary metabolites in the leaves and stems of *L. maximowiczii* changed in response to seasonal variations. Furthermore, *L. maximowiczii* was found to undergo remarkable changes in morphology and activity after germination along with seasonal variations. The tyrosinase inhibitory activity was high in the fall because secondary metabolites involved in the activity accumulated. Both leaves and stems possessed the capacity to produce melanogenesis-regulatory substances, but the leaves had tyrosinase inhibitory activity higher than that of stems. This study revealed the relationship between metabolite changes and activity in *L. maximowiczii* during the growth period. This MS-based metabolomics approach can be applied to understand correlation between bioactivities and the changes in metabolites according to seasonal variations of any other plants.

![Figure 4. Suggested mechanism of seasonal metabolite changes resulting in tyrosinase inhibitory activity.](https://example.com/figure4.png)

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**ASSOCIATED CONTENT**

© Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b03566.
PLS-DA score plot and box and whisker plots using UPLC-Q-TOF-MS from the leaves and stems of L. maximowiczii. OPLS-DA score plot and S plot using UPLC-Q-TOF-MS from the leaves and stems of L. maximowiczii, harvested at 6 months, and a list of metabolites discriminated by different growth periods using GC-TOF-MS and UPLC-Q-TOF-MS (PDF)

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**Notes**

The authors declare no competing financial interest.

## ABBREVIATIONS USED

MSTFA, N-methyl-N-(trimethylsilyl)trifluoroacetamide; PCA, principal component analysis; PLS-DA, partial least-squares discriminant analysis; OPLS-DA, orthogonal projection to latent structures discriminant analysis; VIP, variable importance in the projection; GC-TOF-MS, gas chromatography-time-of-flight-mass spectrometry; UPLC-Q-TOF-MS, ultraperformance liquid chromatography-quadrupole/time-of-flight-mass spectrometry; UHPLC-EI-SI-MS/MS, ultra-high-performance liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry; N.I., nonidentified metabolites; N.D., not detected.

## REFERENCES


