Metabolic Profiling of Cultivated Bush Tea (*Athrixia phylicoides* DC.) in Response to Different Pruning Types

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Abstract. Bush tea (*Athrixia phylicoides* DC.) is a popular medicinal South African indigenous plant and it has been used for many decades as a health beverage and medicine. The objective of the study was to profile metabolites for assessment of quality of bush tea (*A. phylicoides* DC.) subjected to different pruning levels. Treatments consisted of untreated control, top-branch pruning, middle pruning, and basal pruning arranged in a randomized complete block design (RCBD) using 10 single trees as replications. The liquid chromatography quadrupole time-of-flight mass spectrometry (LC–QTOF–MS) was carried out to annotate the bush tea metabolites present in bush tea. Orthogonal partial least square-discriminatory analysis (OPLS-DA) from ¹H nuclear magnetic resonance (NMR) revealed a separation between the basal, middle, top pruning, and the unpruned bush tea plants. The pruned (top) and unpruned tea plants, exhibited higher levels of metabolites than the basal and middle pruned. Pruning bush tea showed a significant effect on accumulation of secondary metabolites and thus could enhance bush tea quality. The study successfully annotated 28 metabolites (compounds), which elucidated canonical differences in pruning treatment of bush tea, as validated through multivariate analysis. Top pruning (apically pruned) resulted in improved metabolite accumulation than other treatment and can be recommended in bush tea cultivation. Future studies to enhance vegetative enhancement after pruning will be evaluated.

Bush tea (*A. phylicoides* DC.) is a South African indigenous and traditional herbal tea rich in secondary metabolites, which have therapeutic effects (Padayachee, 2011), pharmacological properties (McGaw et al., 2007), and different phenolic and flavonoid compounds (Maudu et al., 2010). These secondary metabolites are compounds synthesized from primary metabolites as the plant interacts with its environment for adaptation or defense (Ramakrishna and Ravishankar, 2011). The compounds are used by the plants against herbivores and pathogen attack and also environmental stress. Thus, concentrations of different metabolites are highly dependent on the growing conditions and the type of stresses the plant is subjected to viz., pathogen attack, ultraviolet irradiation, high light, wounding, nutrient deficiencies, temperature, and herbicides (Ramakrishna and Ravishankar, 2011).

In tea cultivation, cultural practices such as mineral nutrition (Maedza, 2015), irrigation (Bandara, 2012), harvesting methods (Mphangwe, 2012), and processing (Hlahla et al., 2010) have been reported to influence the chemical composition of tea (Njogu et al., 2014; Venkatesh et al., 2007). Moreover, agronomic practices such as nutrient application was found to enhance accumulation of carbohydrates for plant growth and to increase photosynthetic rates (Haukioja et al., 1998), which results in the biosynthesis of carbon-based secondary metabolites, such as flavonoids, phenolic acids, and tannins, known as total polyphenols (Chabeli et al., 2008). Pruning is also one of the most important cultural practices that has been reported to improve productivity and quality of tea (*Camellia sinensis*) (Yilmaz et al., 2004).

Although several studies have investigated phytochemicals in bush tea, there is lack of literature on the effect of pruning on accumulation of metabolites in bush tea. Herbal tea extracts have gained popularity and their chemical analysis has also increased, nonetheless, there are no studies which reported the comprehensive chemical characterization of bush tea using the NMR and liquid chromatography linked to mass spectrometry (LC–MS) techniques to quantify

differences in metabolic profiles of bush tea. Therefore, the objective of this study was to profile metabolites for assessment of quality of bush tea subjected to different pruning types.

Materials and Methods

Experimental site. The trial was conducted at the Agricultural Research Council, Vegetable and Ornamental Plant Institute, situated ≈25 km north of Central Pretoria on the Moloto/KwaMhlanga Road (R573), GPS coordinates 25°59′S; 28°35′E. The farm covers ≈4000 ha, of which only 650 ha is under irrigation. The bush tea was grown under 40% shade net and drip irrigation at frequency of 5 mm per 2 h throughout the study.

Treatments and experimental design. Treatments consisted of untreated control, top-branch pruning, middle pruning, and basal pruning arranged in a RCBD using 10 single plants as replications. The untreated control involved leaving individual bush tea plants intact with no pruning from the start to end of the study, top branch (apical) pruning involved pruning of all top branches and stems of individual bush tea plants at the top up to 15 cm length, middle pruning involved pruning of all branches and stems of individual plants right in the middle of individual plants, whereas basal pruning involved pruning individual plants at the base just above the soil surface. Treatments were imposed once per the duration of the study, with all plant variables collected 90 d after starting the treatments.

Leaf area index (LAI). Leaf area index was measured nondestructively, using a LAI 2200 plant canopy analyzer (LI-COR Bioscience, Lincoln, NE). The instrument uses measurements made above and below the canopy to calculate light interception at five zenith angles, from which LAI is computed using a model of radiative transfer in vegetative canopies.

Stomatal conductance (g_s). Stomatal conductance was measured using SC-1 leaf porometer (Decagon Devices, Pullman, WA). Stomatal conductance was described as a function of the density, size, and degree of opening of stomata. The measurements were taken on the abaxial (bottom) side of a fully expanded mature leaf, during midday when the environmental factors were at their peak.

Chlorophyll content. Leaf chlorophyll content was measured with a chlorophyll content meter (SPAD 502 plus; Konica Minolta, Chiyoda, Tokyo, Japan).

Sample preparation. Two-year mature leaf and twig samples were harvested from bush tea plants. Harvested leaves were air-dried at room temperature in the shade. Samples were then ground to a powder using a benchtop grinder and stored in glass vials below –50 °C until extraction.

¹H-NMR data acquisition. NMR spectral data were obtained using a 600 MHz ¹H NMR spectrometer (Varian, Inc., Palo Alto, CA), with 40 scans recorded. The sample preparation, data acquisition, analysis, mining, and processing were carried out as described by Maree and Viljoen (2012). The

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powdered bush tea samples of 10 mg were weighed in 2-mL Eppendorf tubes for extraction and analysis. A solvent of 1.0 mL of solvent, which was made by a ratio of 1:1 methanol D4 and deuterium oxide (pH 6.0), was added. The mixture was vortexed at room temperature for 1 min and ultrasonicated for 15 min to break the cell membranes, and centrifuged for 5 min. About 0.5 mL of the supernatant was transferred into NMR tubes for analysis.

The phasing and baseline corrections were performed using MestreNova software (10.0; Mestrelab Research, Santiago de Compostela, Spain) with consistent settings for all sample spectra. The chemical shift ranges 4.70–4.90 and 3.23–3.36 representing water and methanol, respectively, were excluded from further analysis (Mediani et al., 2012). SIMCA-P (software 14.0; Umetrics, Umeå, Sweden) with Pareto scaling method was used to perform the multivariate data analysis using the principal component analysis (PCA) and OPLS-DA. Contribution plots and scatter plots from PCA were constructed to identify and evaluate the groupings, trends, and strong outliers.

Liquid Chromatography Linked to Mass Spectrometry Analysis

Extraction of bush tea samples for LC–MS. Ten milligrams of each sample (five

replications for each) were weighed in a 2-mL Eppendorf tube. One milliliter of solvent (0.1% formic acid in methanol) was added to dissolve the samples and vortexed for 1 min then placed in a sonicator waterbath for 30 min. Samples were then centrifuged for 5 min at 10,000 rpm on a benchtop centrifuge. About 700 µL of the supernatant was pipetted into high performance liquid chromatography (HPLC) vials and metal caps with rubber septa secured with a crimper.

LC–MS analysis. The supernatant was injected into the LC–MS instrument from Bremen, Germany. Metabolites were separated using a gradient of H₂O with 0.1% formic acid (solvent A) and acetonitrile (solvent B), using a Dionex UltiMate 3000 ultra performance liquid chromatography (UPLC) at a flow rate 0.3 mL·min^{−1} on a Waters BEH C18, 2.1 × 100-mm column (Ireland). Mass spectrometry was obtained on a TOF Bruker Impact II (Germany) using electron spray ionization running in positive mode, which scans between 50 and 1600 m/z, with nebulizer at 1.8 bar and dry gas as 8 L/min.

Liquid chromatography–MS data were analyzed using the Compass data analysis tool version 4.3.110 and converted into buckets after peak integration and Pareto scaling. Principal component analysis was performed using the Profile Analysis software version 2.1 (Bruker®, Bremen, Germany) on buckets imported from the data analysis tool on a minimum of three samples

for each tea. Peaks were identified according to actual mass, MS/MS, and retention time. Accurate mass and MS/MS spectral data were compared with the Kyoto Encyclopedia of Genes and Genomes, Chemical Entities of Biological Interest, Chemspider, and Pubchem online databases.

The LC–QTOF–MS data were preprocessed using XCMS for relative quantitation and statistical analysis, and were subjected to parametric analysis of variance (ANOVA). The XCMS uses sample class information during preprocessing to help decide which groups of peaks are significant. The unsupervised PCA and the supervised partial least squares–discriminant analysis, the most well-known classification procedures in chemometrics, were carried out using MetaboAnalyst. The quality of the model was described by R²X and Q² values.

Statistical analysis. Data were subjected to one-way ANOVA, carried out using STATISTICA (StaSoft, Inc., Tulsa, OK) package (2011). Mean separation was carried out using Tukey's honest significant difference at $P < 0.05$.

Results and Discussion

Information on the influence of pruning on accumulation of primary and secondary metabolites in herbal teas is limited to allow a balanced discussion of some of the variables collected. The present study is critical in tea industry because periodic harvesting of shoots generally imposes significant perturbation of carbon-based secondary metabolites, which are important in general tea plant growth and development. Metabolites accumulate more when plants were subjected to stresses for adaptation (Ramakrishna and Ravishankar, 2011).

Physiological measurements. Basal-pruned bush tea plants exhibited low (0.2 m²·m^{−2}) LAI

Table 1. Leaf area index (LAI), chlorophyll, and stomatal conductance (g_s) of bush tea in response to different pruning types.

Pruning	LAI (m ² ·m ^{−2})	Chlorophyll (µmol·m ^{−2})	g_s (m ² ·s ^{−1})
Top	0.5 ± 0.1 b	38.2 ± 3.0 b	12.1 ± 1.1 b
Medium	0.7 ± 0.2 a	35.2 ± 3.2 b	10.3 ± 1.2 bc
Basal	0.2 ± 0.0 c	29.4 ± 3.2 c	9.1 ± 0.8 c
Unpruned	0.7 ± 0.2 a	64.5 ± 20.4 a	18.2 ± 3.1 a

Means in the same column with different letters indicate significance ($P < 0.05$) using Turkey's honest significant multiple range test. Values are means ± SE.

Fig. 1. Orthogonal partial least square discriminatory analysis results of bush tea samples from nuclear magnetic resonance spectra, with ellipse representing Hotelling within 95% confidence.

Fig. 2. Partial least squares–discriminant analysis from liquid chromatography linked to mass spectrometry data of samples from 40 *A. phylicoides* tea samples.

Fig. 3. Dendrogram for the clustering of *A. phylicoides* tea pruning treatments.

while unpruned and middle pruned showed high LAI ($0.7 \text{ m}^2\text{m}^{-2}$). Chlorophyll content significantly increased in the unpruned bush tea plants with $64.5 \mu\text{mol}\cdot\text{m}^{-2}$ and was low ($29.4 \mu\text{mol}\cdot\text{m}^{-2}$) in the basal-pruned bush tea plants. Current findings are consistent with a study by Mahanta and Baruah (2006), who

reported that unpruned black tea exhibited higher chlorophyll content than pruned black tea (*C. sinensis*). Moreover, the unpruned bush tea exhibited high g_s ($18.2 \text{ m}^2\cdot\text{s}^{-1}$) whereas basal pruning resulted in low g_s ($9.1 \text{ m}^2\cdot\text{s}^{-1}$) (Table 1).

¹H-NMR profiling data. The untargeted metabolites profiling using ^1H NMR were

subjected to a supervised method, the OPLS-DA, which revealed distinct groupings between the basal, middle, top pruning, and the unpruned control (Fig. 1). The basal-pruned treatments (green) clustered to the bottom left of the ellipse whereas the top-pruned treatments (yellow) grouped to the top left. On the

Table 2. Response of concentrations of identified bush tea compounds to different pruning types (LC-QTOF-MS).

Pruning type	Control (unpruned)				Basal				Middle				Top			
	Mass	RT (min)	Concn (peak area)	Concn (peak area)	Mass	RT	Concn (peak area)	Concn (peak area)	Mass	RT	Concn (peak area)	Concn (peak area)	Mass	RT	Concn (peak area)	Concn (peak area)
Chlorogenate	354.095	4.61	1,891.7	243.5	354.0951	4.61	354.0951	4.61	354.0951	4.61	354.0951	4.61	354.0951	4.61	1,297.1	1,297.1
Coumarin	146.037	5.16	—	—	146.037	5.16	146.037	—	146.037	5.16	146.037	—	146.037	5.16	177.8 a	177.8 a
6-cis-docosamide	337.334	11.93	—	68.4	337.334	11.93	68.4	—	337.334	11.93	337.334	—	337.334	11.93	—	—
Naringenin 7-O-beta-D-glucoside	434.121	8.70	—	—	434.121	8.70	—	—	434.121	8.70	434.121	—	434.121	8.70	—	—
5-p-coumaroylquinic acid	338.108	4.21	—	—	338.108	4.21	—	—	338.108	4.21	338.108	—	338.108	4.21	48.0	48.0
(6'Z,8'E,10E,10'E,12E,12'E,14 + E,14'E)-3,4,4',5'-tetrahydro-5',9-dihydro-beta-beta-caroten-9-ol	550.418	11.15	—	—	550.418	11.15	—	—	550.418	11.15	550.418	—	550.418	11.15	1,981.5	1,981.5
Integrastatin A	332.053	7.25	116.49	270.0	332.053	7.25	270.0	—	332.053	7.25	332.053	—	332.053	7.25	117.98	117.98
luteolin 7-O-(6-O-malonyl-beta-D-glucoside)	534.101	6.29	—	—	534.101	6.29	—	—	534.101	6.29	534.101	—	534.101	6.29	—	—
1,3-dicaffeoylquinic acid	516.127	5.18	—	—	516.127	5.18	—	—	516.127	5.18	516.127	—	516.127	5.18	139.0	139.0
Magnoshimin	414.204	8.85	168.0	109.7	414.204	8.85	109.7	—	414.204	8.85	414.204	—	414.204	8.85	59.0	59.0
Okanin	288.063	6.55	1,299.0	100.4	288.063	6.55	100.4	—	288.063	6.55	288.063	—	288.063	6.55	380.1	380.1
(2S)-5-hydroxy-7-methoxy-6,8-dimethylflavanone	298.121	9.00	—	—	298.121	9.00	—	—	298.121	9.00	298.121	—	298.121	9.00	52.6	52.6
(9Z,12Z,15Z)-Octadecatrienoic acid	278.225	10.18	—	—	278.225	10.18	—	—	278.225	10.18	278.225	—	278.225	10.18	11,935.5	11,935.5

RT = retention time.

right side of the ellipse the middle-pruned treatments clustered to the bottom whereas the unpruned (control) grouped to the top right. The discriminating peaks in the bush tea samples were from the aromatic region 6–8.5 ppm. The unpruned bush tea plant showed more intense peaks in the phenolic region from 4 to 7 ppm chemical shifts compared with other pruning treatments. However, top-pruned bush tea had more intense peaks throughout the ¹H NMR.

The unpruned bush tea plant exhibited more peaks in the aromatic region from 6 to 8.5 chemical shifts than the basal-pruned plants, which did not exhibit any peak under the aromatic region. Similar trends have been observed with the phenol region (4–7 ppm chemical shifts). The basal-pruned plants showed more intense peaks under the sugar region (chemical shifts) and aldehyde region (9.5–9.6 ppm). However, top-pruned bush tea plants exhibited more intense peaks throughout the ¹H NMR spectra regions from 0.04 to 9.96 ppm compared with other pruning levels. The control or unpruned plants showed more peaks in the aromatic (7.3 ppm), alcohol and amine (0.5–5 ppm), and aniline (3–5 ppm) regions more than all the pruned bush tea plants.

The supervised comparison of bush tea pruned at different levels revealed a distinct grouping among the treatments (Fig. 2). The unpruned (green) treatment clustered to the middle left, whereas top-pruned (light blue) clustered to the top center. The basal-pruned (red) treatment clustered to the bottom center of the graph, whereas the middle-pruned (dark blue) was scattered through the middle center to the right side of the graph.

R^2X is the proportion of variance in the data explained by the model and it designates the goodness of fit test which in the study was 0.126. R^2Y value of the model was 0.198.

The dendrogram presented in Fig. 3 demonstrated biochemical differences in bush tea with respect to pruning treatments. There were two main clusters with the first group represented by basal and unpruned treatments. In the fourth samples, eight were basal and five were unpruned dominated in the first cluster was dominated, whereas top pruning was four and only one middle pruned sampled grouped with the latter. In the second cluster, there were four main subclusters with middle-pruned treatment dominating (60%), whereas the unpruned treatment had 50%. However, the top-pruned bush tea samples grouped together in the fourth subcluster denote large proportion of chemical compositions.

There were significant differences among the pruning treatments regarding compound concentration or metabolite accumulation (Tables 2 and 3). Tables 2 and 3, denote the exact masses and concentration based on LC-MS peak areas and variation of compounds attributed to pruning techniques. This aspect has not been investigated in commercial cultivation of bush tea in spite of the fact that these compounds have been reported to have health attributes (Malongane et al.,

Table 3. Response of concentrations of identified bush tea compounds to different pruning types (LC-QTOF-MS).

Pruning type	Control (unpruned)				Basal				Middle				Top			
	Mass	RT (min)	Concn (peak area)	Concn	Mass	RT (min)	Concn (peak area)	Concn	Mass	RT (min)	Concn (peak area)	Concn	Mass	RT (min)	Concn (peak area)	Concn
2"-deamino-2"-hydroxy-6"-dehydroparomamine	322.138	6.48	206.7	354.1	322.138	6.48	354.1	588.5	322.138	6.48	588.5	464.9	322.138	6.48	464.9	464.9
O-butanoylcarnitine	231.147	3.09	937.6	1,108.4	231.147	3.09	1,108.4	914.5	231.147	3.09	914.5	967.1	231.147	3.09	967.1	967.1
Myricitrin	464.095	6.90	257.2	1,072.4	464.095	6.90	1,072.4	552.3	464.095	6.90	552.3	895.9	464.095	6.90	895.9	895.9
Gorlic acid	278.225	10.18	1,834.4	12,213.2	278.225	10.18	12,213.2	5,403.2	278.225	10.18	5,403.2	6,010.8	278.225	10.18	6,010.8	6,010.8
Tetracenomycin X	484.101	8.18	194.7	—	484.101	8.18	—	—	484.101	8.18	—	77.9	484.101	8.18	77.9	77.9
Sakuranin	448.137	6.36	—	54.04	448.137	6.36	54.04	—	448.137	6.36	—	—	448.137	6.36	—	—
D-tryptophan	204.093	4.13	471.1	220.0	204.093	4.13	220.0	366.2	204.093	4.13	366.2	1,407.8	204.093	4.13	1,407.8	1,407.8
Linoleamide	279.256	3.83	3,501.4	4,069.6	279.256	3.83	4,069.6	7,037.5	279.256	3.83	7,037.5	4,766.5	279.256	3.83	4,766.5	4,766.5
Laricitrin 7-monoglucoside	494.106	8.26	0.0	239.5	494.106	8.26	239.5	—	494.106	8.26	—	—	494.106	8.26	—	—
L-beta-phenylalanine	165.079	3.33	61.8	848.6	165.079	3.33	848.6	629.9	165.079	3.33	629.9	—	165.079	3.33	—	—
L-Proline	115.063	8.73	351.7	—	115.063	—	—	—	115.063	—	—	—	115.063	—	—	—
Pheophorbide A	592.269	7.83	396.1	1,481.9	592.269	7.83	1,481.9	1,157.2	592.269	7.83	1,157.2	1,147.3	592.269	7.83	1,147.3	1,147.3
Stearidonic acid	276.209	9.94	—	—	276.209	9.94	—	—	276.209	9.94	—	29.3	276.209	9.94	29.3	29.3
Gibberellin A14 aldehyde	332.199	5.49	116.5	270.0	332.199	5.49	270.0	—	332.199	5.49	—	117.9	332.199	5.49	117.9	117.9
N-(2-hydroxyheptadecanoyl)-1-O-beta-D-glucosyl-15-methylhexadecaspheing-4-enine	301.049	5.52	—	601.0	301.049	5.52	601.0	168.3	301.049	5.52	168.3	64.2	301.049	5.52	64.2	64.2

RT = retention time.

2017). Most found peak area responses occurred in the top-pruned bush tea and exhibited 22 out of 28 compounds. Compounds included coumarin, 6-cis-docosenamide, 5-p-coumaroylquinic acid, (6'Z,8'E,10E,10'E,12E,12'E,14 + E,14'E)-3,4,4',5'-tetrahydro-5',9-dihydro-beta-betacaroten-9-ol, integrastatin A, 1, 3-dicaffeoylquinic acid, magnoshinin, okanin, (2S)-5-hydroxy-7-methoxy-6,8-dimethylflavanone, (9Z,12Z,15Z)-octadecatrienoic acid, 2"-deamino-2"-hydroxy-6"-dehydroparomamine, O-butanoylcarnitine, myricitrin, gorlic acid, tetracenomycin X, D-tryptophan, linoleamide, L-beta-phenylalanine, pheophorbide A, stearidonic acid, gibberellin A14 aldehyde, N-(2-hydroxyheptadecanoyl)-1-O-beta-D-glucosyl-15-methylhexadecaspheing-4-enine. In addition, middle-pruning yielded 14 out of 28 compounds and they were chlorogenate, coumarin, naringenin 7-O-beta-D-glucoside, luteolin 7-O-(6-O-malonyl-beta-D-glucoside), okanin, 2"-deamino-2"-hydroxy-6"-dehydroparomamine, O-butanoylcarnitine, myricitrin, gorlic acid, D-tryptophan, linoleamide, L-beta-phenylalanine, pheophorbide A, N-(2-hydroxyheptadecanoyl)-1-O-beta-D-glucosyl-15-methylhexadecaspheing-4-enine. Basal-pruned bush tea plants exhibited 17 out of 28 compounds and they were chlorogenate, 6-cis-docosenamide, integrastatin A, magnoshinin, okanin, 2"-deamino-2"-hydroxy-6"-dehydroparomamine, O-butanoylcarnitine, myricitrin, gorlic acid, sakuranin, D-tryptophan, linoleamide, laricitrin 7-monoglucoside, L-beta-phenylalanine, pheophorbide A, gibberellin A14 aldehyde, N-(2-hydroxyheptadecanoyl)-1-O-beta-D-glucosyl-15-methylhexadecaspheing-4-enine. Similar compounds were evident in the research reported by Malongane et al. (2018) of bush tea metabolites using untargeted approach by LC-MC. However, the compounds exhibited by the unpruned bush tea plants were 15 out of 28 and they consistently appeared in the other pruned treatments.

In this study, different pruning heights significantly enhanced accumulation of secondary metabolites that are not only important in plant stress physiology for adaptation but also important as quality indicators of herbal teas. It was observed from the spectra that control (unpruned) plants exhibited more secondary metabolites than the other plants that were middle pruned or basal pruned, with basal pruning exhibiting much less metabolites. Consistent with a finding by Maudu et al. (2010) which demonstrated that basal pruning was not viable in potted bush tea growing under 50% shade nets as the treatment revealed highly reduced total phenolics. Although unpruned bush tea exhibited higher chlorophyll content and higher g_s (Table 1), the concentrations of the secondary metabolites were not enhanced (Tables 2 and 3). This could be a consequence of pruning, possibly due to concentrations of various secondary plant products accumulated when the plants were subjected to stress (Ramakrishna and Ravishankar, 2011). Hence, secondary metabolites were improved in the pruned than the unpruned bush tea plants. Our results are consistent with a study by Yilmaz et al. (2004) who reported that pruning enhances tea quality.

The top pruning (apically pruned) showed intensive peaks throughout the spectra compared with other pruning levels. Conversely, Maudu et al. (2010) reported that top-pruned plants exhibited the lowest total polyphenols whereas the highest total polyphenol concentration was observed in unpruned plants. However, the improved metabolites accumulation by top pruning (apically pruned) bush tea in this study could be attributed to the fact that cutting of the apical bud exposed the bush tea plants to severe stress, hence, the top-pruned plant exhibited more metabolites with improved concentrations (Tables 2 and 3). This study revealed 28 metabolites (compounds) that explained significant differences in pruning treatments of bush tea.

In conclusion, pruning bush tea showed a significant effect on accumulation of secondary metabolites and thus could enhance bush tea quality. The study successfully annotated 28 metabolites (compounds) that elucidated canonical differences in pruning treatments of bush tea, as validated through multivariate analysis. Top pruning (apically pruned) resulted in improved metabolites than the control (no pruning) and can be recommended in bush tea cultivation.

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