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Relationship between Phenotypes and Chemical Profiling of *Boesenbergia rotunda* Collected from Different Habitats of Northern Thailand

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Abstract: Fingerroot [*Boesenbergia rotunda* (Linn.) Mansf] is known to consist of compounds such as panduratin A and pinostrobin with inhibitory property against SARS-CoV-2 infection at both the pre-entry and post-infection phases. Consequently, demand for good phenotype selection has increased in the post-pandemic era. However, the availability of important active ingredients may be influenced by genetic variables and agronomic aspects. Thus, the purpose of this study was to investigate the relationship between characteristics and genotype, and to preliminarily compare the phytochemical profiles of the landraces collected in Northern Thailand. Five local landraces and two wild types were gathered for ex situ collection for morphological assessments, genetic evaluation, and bioactive ingredients (mainly antioxidative potentials and amounts of the active flavonoids). The morphological data were able to distinguish the plant samples to those of wild, cultivated, and adaptive types, which was confirmed by their distinctive genetic variations. However, there was no correlation between the physical attributes and the amount of their bioactive constituents. It was also observed that the adaptation of plants to environmental conditions had a pronounced impact on secondary metabolite biosynthesis, and that such adaptations were likely influenced by genetic differentiation. The findings from this study could potentially be used to improve the cultivation, selection, and breeding of this plant species for desired traits such as increased bioactive compound content, or for conservation and restoration efforts of the landraces and wild types.

Keywords: antioxidant; chemical composition; panduratin A; pinostrobin; Zingiberaceae

1. Introduction

The genus Bosenbergia has a wide distribution across tropical regions and is known for its use in spices and herbal medicine in Southeast Asia, India, Sri Lanka, and Southern China [1–3]. There are over 80 recognized species within this genus, and Thailand is considered to be one of the richest habitats with over 20 species present [3,4]. Among those,

fingerroot (*Bosenbergia rotunda*), or locally known as Krachai kaw in Thai, has recently attracted attention for its potential use as a preventative medicine [1–3]. Like its siblings in the Zingiberacea family, Krachai kaw is a small herbaceous plant with short, fleshy, or slender rhizomes in the shape of fingers growing from the mother rhizomes [2,4,5]. The rhizomes are the nutrient accumulation parts, and therefore are the rich source of active ingredients, comprising various aromatic compounds [2,6]. Propagation of *B. rotunda* can be done vegetatively; however, the hybridization could also happen naturally by cross-pollination or a breeding program, which provide variations in genetic diversity [7,8]. Although the species richness of *B. rotunda* in northern Thailand is diverse, there is limited documentation regarding the diverse genetic pools and their relation to the morphological characteristics. The phytoconstituents from the rhizomes of *B. rotunda* are generally classified into two major groups: flavanones (e.g., alpinetin, pinostrobin, and pinocembrin) and chalcones (e.g., boesenbergin, cardamonin, panduratin A, and 4-hydroxypanduratin A) [9]. They contribute vastly to many different pharmaceutical activities, including antibacterial, anti-allergic, anti-tumor, anti-mutation, anti-fungal, anti-anxiety, and anti-inflammatories [10–14]. More importantly, the recent report advised that panduratin A had a potential inhibition activity against SARS-CoV-2 (COVID-19) and prevented viral replication at both the pre-entry and post-infection phases, which is more potent than the hydroxychloroquine drug used for COVID-19 treatment [7]. Meanwhile, pinostrobin can inhibit intestinal smooth muscle contractions and pose antiviral, antioxidant, anti-leukaemic, anti-inflammatory, anti-aromatase, anti-microbial, anti-ulcer, and anti-tumor properties [15,16]. Nonetheless, the contents of these bioactive compounds in *B. rotunda* collected from various cultivation areas are variable, and the information on the phenotypic relationship with the chemical profiling is also lacking.

The demand for high-quality *B. rotunda* has been increasing in both the food and pharmaceutical industries, especially in the post-COVID era where there is a growing interest in natural remedies. This trend has led to a need for selecting good phenotypes for commercial cultivation. It is essential to evaluate the morphological traits of the plant to determine the level of bioactive compounds present in it, which could be useful for developing a plant breeding program. From this rationale, the aims of this study were to investigate the morphological differences among locally collected plant accessions and study their relationship with their phytochemical profiling. By identifying the traits that correlate with high bioactive compound levels, plant breeders can select good phenotypes with desirable characteristics and breed new cultivars with increased concentrations of such active compounds. This can ultimately lead to the production of commercial *B. rotunda* type with improved medicinal and nutritional properties, meeting the increasing demand for high-quality plants in the market.

2. Materials and Methods

2.1. Reagent and Chemicals

The genomic DNA Extraction Kit for plant material was from RBC Bioscience (New Taipei City, Taipei). Polymorphic DNA primer sequences were supplied by Integrated DNA Technologies (Coralville, IA, USA). Taq DNA polymerase 2x Master Mix RED and High Range DNA Ladder were purchased from Ampliqon PCR Enzymes & Reagents (Stenhuggervej, Odense M, Denmark). Novel Juice (DNA Staining Reagent) was supplied by Bio-Helix (New Taipei City, Taiwan). Standard pinostrobin and panduratin A were obtained from Chem Faces (Wuhan, Hubei). Gallic acid monohydrate, 2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt, (+)—Catechin hydrate (sum of enantiomers, HPLC), and 2,2-Diphenyl-1-picrylhydrazyl were from Sigma-Aldrich (St. Louis, MO, USA). Folin–Ciocalteu's phenol reagent was supplied by Merckmillipore (Billerica, MA, USA). Sodium hydrogen carbonate, sodium nitrile, aluminium chloride (Hexahydrate), sodium hydroxide (Micropearls), Potassium dihydrogen orthophosphate anhydrous, potassium persulphate, acetonitrile and glacial acetic acid (HPLC grade sol-

vents). and Formic acid and Methanol (LCMS grade solvents) were purchased from RCI Labscan Limited, Bangkok, Thailand.

2.2. Plant Materials

Seedling (rhizomes) of the *B. rotunda* were collected from their natural habitats and cultivation areas in Chiang Mai, Lamphun, and Lampang. They were five landraces and two wild types as described by the ending letters C and W in Table 1. At the Faculty of Agriculture, Chiang Mai University, Chiang Mai (CMU), Thailand, the rhizomes were cultivated in 8 × 13 inch.^{2} pots with a substrate comprising of loam soil and animal manure in a 2:1 ratio. For each line, 20 seedling were cloned and were arranged randomly in four blocks and each block contained five replications. They were maintained in a greenhouse under 50% shading with daily irrigation and 200 g/plant of manual organic fertilizer (1:20 farmyard manure-to-humus ratio) application at 65 days after cultivation.

Table 1. Physical characteristics of *B. rotunda* aboveground organs at 17 weeks.

Sample	Cultivated Area/GPS Coordinates	Habitat	Survivor Rate (%)	Leaf		Stems		Above Ground Part
				Width (cm)	Length (cm)	Width (cm)	Height (cm)	
MMC	Mae Taeng, Chiang Mai/19.05992, 98.83022	cultivation type	100	8.03 ± 1.68 ^{a}	25.43 ± 5.00 ^{a}	1.45 ± 0.40 ^{a}	51.75 ± 10.17 ^{a}	
MLW	Mae Tha, Lamphun/18.63397, 99.04039	wild type	50	5.57 ± 1.63 ^{b}	15.89 ± 5.25 ^{bc}	1.08 ± 0.41 ^{cd}	30.46 ± 10.45 ^{c}	
DM1C	Chiang Dao, Chiang Mai/19.30270, 98.96640	cultivation type	95	7.28 ± 1.95 ^{a}	17.71 ± 4.81 ^{b}	1.29 ± 0.32 ^{ab}	33.97 ± 11.35 ^{bc}	
DM2C	Chiang Dao, Chiang Mai/19.51182, 99.09041	cultivation type	80	7.25 ± 1.65 ^{a}	23.23 ± 4.57 ^{b}	1.33 ± 0.32 ^{ab}	44.43 ± 10.02 ^{c}	
SM1C	San Pa Tong1, Chiang Mai/18.62599, 98.88333	cultivation type	50	5.74 ± 1.82 ^{b}	17.28 ± 5.53 ^{b}	0.96 ± 0.27 ^{bc}	33.78 ± 12.78 ^{c}	

Table 1. *Cont.*

Sample	Cultivated Area/GPS Coordinates	Habitat	Survivor Rate (%)	Leaf		Stems		Above Ground Part
				Width (cm)	Length (cm)	Width (cm)	Height (cm)	
SM2C	San Pa Tong2, Chiang Mai/18.62599, 98.88367	cultivation type	100	7.82 ± 2.04 [a]	19.17 ± 5.52 [a]	1.44 ± 0.45 [a]	36.59 ± 12.72 [ab]	
MPW	Mae Moh, Lampang/18.42797, 99.82055	wild type	45	4.30 ± 1.02 [b]	12.42 ± 3.01 [c]	0.67 ± 0.28 [d]	21.40 ± 3.60 [d]	

MMC = Mae Taeng, Chiang Mai, cultivated type; MLW = Mae Tha, Lamphun, wild type; DM1C = Chiang Dao1, Chiang Mai, cultivated type; DM2C = Chiang Dao2, Chiang Mai, cultivated type; SM1C = San Pa Tong1, Chiang Mai, cultivated type; SM2C = San Pa Tong2, Chiang Mai, cultivated type; MPW = Mae Moh, Lampang, wild type. Data expressed as mean ± standard deviation. [a–d] Values in the same column with different alphabets are statistically distinct ($p > 0.05$).

2.3. Phenotypic Characterization

The morphological data were recorded for the above- and underground parts of *B. rotunda* Plant growths were measured once a week after planting and continuing until 17 weeks (before the plant entered its dormancy period), by recording leaf width (cm), leaf length (cm), stem width (cm), and stem height (cm). The rhizomes were harvested at 18 weeks after cultivation when the aboveground organs turned yellow and collapsed, when the rhizomes have reached their maturity [17]. The rhizomes were carefully washed and weighed (g) before and after the fingers were separated from the mother rhizomes. For the rhizomes, width (cm) and length (cm) were also measured. Afterward, these underground parts were freeze-dried and thereafter ground to powder for further analysis of active ingredients.

2.4. Genetic Variation Analysis of B. rotunda

2.4.1. DNA Extraction

Total genomic DNA was extracted using the technique outlined in the manual for the genomic DNA extraction kit. The 50 mg of the freeze-dried powder samples were pulverized using liquid nitrogen in an Eppendorf tube, with step-wise additions of the buffers provided in the kit. The purified DNA was obtained by centrifuging at 20,627× *g* for 30 s. The quality and quantity of DNA were then analyzed using a Nano Drop Spectrophotometers, Thermo Scientific/NanoDrop 2000C (AG-BIO/PERDO-CHE, Bangkok, Thailand). The DNA samples were stored at −20 °C prior to random amplified polymorphic DNA (RAPD) analysis.

2.4.2. RAPD-PCR

RAPD-PCR was performed in accordance with the Ampliqon PCR enzyme & Reagents manual's instructions. For RAPD analysis of the genomic DNA, 10 primers were chosen [18,19] (Table S1) and PCR reactions were operated following the procedure described in Supplementary Table S2. All reactions were carried out with a K960 Thermal cycler (Heal Force) using the following program: 1 cycle, 95 °C, 3 min; 40 cycles, 95 °C, 30 s; 37 °C, 1 min; 72 °C, 30 s; 1 cycle, 72 °C, 5 min. Consequently, the sample was separated in a 1% agarose gel in 1× TBE buffer. The PCR products were stained with Novel juice DNA staining reagent and run at 135 V for 22 min. The gels were accordingly visualized using the LED transilluminator, TT-BLT-470; Hercuvan (Cambridge, CB24 6WZ, UK).

2.5. Phytochemical Analyses

2.5.1. Rhizome Extraction of *B. rotunda*

Three grams of *B. rotunda* powder was dissolved in 30 mL of 80% (*v*/*v*) ethanol and macerated for 24 h at room temperature before being separated with Whatman No. 1 filter paper as described by Sangta et al. [20]. The residue was extracted three times, and the filtrates were combined. The solution was evaporated to dryness in the rotary evaporator resulting in the crude ethanol extract.

2.5.2. Total Phenolic Content

The crude extract (30 µL) was combined with 60 µL of Folin–Ciocalteu reagent and neutralized with 210 µL of 6% *w*/*v* $NaCO_3$ solution, which was added, mixed, and left in the dark for 90 min as described by Sangta et al. [20]. A UV-Vis spectrophotometer was used to measure the absorbance at a wavelength of 725 nm (SPECTROstar, BMG LABTECH, Offenburg, Germany). Gallic acid calibration curve was constructed at different concentrations of gallic acid (0–1000 mg/mL). The total phenolic content was evaluated in milligrams of gallic acid equivalents per dried sample.

2.5.3. Total Flavonoid Content

The total flavonoid content was determined using a modified Sunanta et al. [21] technique. The ethanol extract (25 µL) was reacted with 125 µL of distilled water and 7.5 µL of a 5% $NaNO_2$ solution. Before adding 15 µL of 10% $AlCl_3 \cdot 6H_2O$ solution, the mixture was allowed to react at room temperature for 5 min. After 6 min of incubation time, 50 µL of 1 M NaOH solution and 27.5 µL of distilled water were added. The UV-Vis spectrophotometer was used to measure the absorbance at 510 nm (SPECTROstar, BMG LABTECH, Offenburg, Germany). The catechin calibration curve was developed at concentrations of 0 to 1000 mg/mL. The total flavonoid content was reported in milligrams of catechin equivalents per dried sample.

2.5.4. Antioxidant Potential

DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity was operated in accordance with Sangta, Wongkaew, Tangpao, Withee, Haituk, Arjin, Sringarm, Hongsibsong, Sutan and Pusadee [20]. A 25 µL of the ethanol extracts was incubated with 250 µL of 0.2 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (SPECTROstar, BMG LABTECH, Offenburg, Germany). DPPH radical scavenging activity was calculated using the following equation;

$$\text{DPPH scavenging activity (\%)} = [(\text{A control} - \text{A sample})/\text{A control}] \times 100 \quad (1)$$

where A control is a DPPH absorbance and A sample is the sample absorbance.

$ABTS^{\bullet+}$ Radical Cation Activity

The ABTS [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] assay was determined using the method described by Sangta et al. [20]. The working solution was prepared by combining the two stock solutions, 7.0 mM ABTS solution and 2.45 mM $K_2S_2O_8$ solution. The solutions were mixed in equal amounts and left to react in the dark at room temperature for 24 h. The ABTS solution was arranged by diluting 1.0 mL of ABTS with 60 mL of 80% ethanol to achieve an absorbance of 0.70 ± 0.02 units at 734 nm. A 10 µL of the ethanol fingerroot extracts and 200 µL of ABTS working solution were added in each well, shaken and incubated for 30 min at room temperature. The absorbance was accordingly measured at 734 nm. The ABTS scavenging activity was estimated using the following equation;

$$\text{ABTS radical scavenging activity (\%)} = [(\text{A control} - \text{A sample})]/(\text{A control})] \times 100 \quad (2)$$

where A control is the absorbance of ABTS radical mixed with 80% methanol; A sample is the absorbance of ABTS radical reacted with sample extract/standard.

2.5.5. Analyses of Pinostrobin and Panduratin A by High-Performance Liquid Chromatography (HPLC)

The crude extract was dissolved in 95% methanol to a final concentration of 0.1 mg/mL and analyzed for the pinostrobin and panduratin A using the HPLC (Shimadzu, Kyoto, Japan) with an automatic injection (SIL-20ACHT), diode array detection (CTO-20AC), pump (LC-20AD), and automatic control. Ultra-Aqueous C18 (250 4.6 mm, 5 m) was utilized for reverse-phase column chromatography (RESTEK, Bellefonte, PA, USA). The mobile phase was prepared according to Ruttanapattanakul et al. [22]. For the detection, gradient elution was done using two solvents, A [1% (*v*/*v*) acetic acid in water] and B (100% acetonitrile). Sample (10 µL) was injected with an initial flow rate of 1.0 mL/min and detection at 285 nm. Gradient elution mode was used conditions was according Jirakiattikul et al. [23] at a volume ratio of 80% A in 5 min, then reduced to 65% A in 5 min, remained at 0% A for 4 min, and then raised to 40% A in 5 min before returning to 80% A in 6 min. The overall run time per a sample was 25 min. The pinostobin and panduratin A calibration curve was developed at concentrations of 50 to 1000 mg/mL. The pinostobin and panduratin A content was reported in milligrams equivalents per dried sample.

2.5.6. Non-Volatile Metabolite Profiling Using Liquid Chromatography-Mass Spectrometer (LC-MS)

The identification of the soluble metabolites in the *B. rotunda* samples was investigated according with Sunanta, Chung, Kunasakdakul, Ruksiriwanich, Jantrawut, Hongsibsong, Sommano and Nutrition [21] using an Agilent 6545 QTOF-LC/MS system consisting of an Agilent 1260 LC with a 6545 UHD accurate-mass QTOF mass spectrometer. The crude extract of 0.01 mg was redissolved with 1.0 mL of LCMS-grade methanol (*v*/*v*) and filtered through a 0.25 m nylon syringe membrane filter. Separation of the chemical compounds was performed using the Agilent Poroshell 120 EC-C18 column (2.1 mm × 100 mm, 2.7 µm) at 25 °C. The mobile phase consisted of 0.1% (*v*/*v*) formic acid dissolved in water (phase A) and 0.1% (*v*/*v*) formic acid dissolved in methanol (phase B). The gradient elution was run as follows: 5 percent phase B for 1 min, increasing to 95 percent for 10 min, and then maintained for 19 min. The total run time was 30 min. The applied flow rate was 0.4 mL/min and the injection volume was 10 µL. MS and MS/MS analyses were done using a 6545 Agilent Ultra-High-Definition Accurate-Mass QTOF-MS linked to the LC, outfitted with an Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface in positive ionization mode, and Mass Hunter software was utilized for data elaboration (Agilent Technologies, Santa Clara, CA, USA). The correct mass and molecular formula prediction were screened against the database for hypothetical compounds (Agilent METLIN PCDL, Santa Clara, CA, USA).

2.5.7. Volatile Metabolite Profiling Using Pyrolysis Gas Chromatography Mass Spectrometry (Py-GC-MS)

Py-GC is a method of chemical analysis in which organic material is heated and thermochemically decomposed without participation of oxygen to produce smaller molecules that are separated by gas chromatography and detected using mass spectrometry [24,25]. The identification of volatile organic compounds in the *B. rotunda* powder was by the adapted method from Fakhrudin et al. [26] using an Agilent 7890B GC instrument. The powder samples 0.1 mg were placed in a pyrofoil (255 °C pyrolysis temperatures). The determination of volatile organic compounds was done using the GC (JCI-77pyrolysis probe, Japan Analytical Industry Co., Ltd., Tokyo, Japan). The column for gas chromatography was Rxi®-1 ms (30 m, 0.25 mm ID, and 0.25 µm df) produced by Restek (Cat. Number #13323; Bellefonte, PA, USA) with the FID detector set to 330 °C. The column conditions are as follows: Column oven temperature: 80 °C; injector temperature: 260 °C; and carrier gas Helium, constant flow rate 2.0 mL/min. The column temperature program: Initial

temperature of 80 °C for 1 min and then increased 20 °C/min until the final temperature of 320 °C (maintained for 3 min). GC/MS data were processed with the ChemStation software (Agilent Technologies, Santa Clara, CA, USA) and the NIST 05 mass spectra library (Agilent, Santa Clara, CA, USA)

2.6. Statistical Analysis

The analyses of physical and chemical properties were carried out at least in biological and technical triplicates. Data was analyzed using one-way analysis of variance and Duncan's test. Differences in values were considered significantly different when the *p*-value was <0.05 at the significant level of 95%. All statistical analysis was performed using IBM SPSS program v. 20. Physiological and physicochemical data among the samples were analyzed using principal component analysis (PCA). The RAPD data were displayed on dendrogram using the NTSYSpc2.2 program (Applied Biostatistics Inc., New York, NY, USA). The expression-based heat maps of metabolite compounds obtained from LC-MS and GC-MS data were performed using Heatmapper.ca (Creative Commons Attribution-ShareAlike 2.0 Generic).

3. Results and Discussion

3.1. Morphology

For morphological characteristics of the aerial parts, leaf growth as determined by the expanding of width and length of the MMC sample was noticed after one week after cultivation, while such characteristics of the DM1C, DM2C, SM2C, and MLW samples occurred at 3 weeks after cultivation. The leaf structure of the SM1C and MPW innitiated at 5 weeks after cultivation. All samples exhibited constant leaf growth at 9 weeks after cultivation before entering the dormant phase after 17 weeks (Figure 1a,b) In terms of stem growth, the MMC sample had stem growth as detemonstrated by expanding of the width and height was detected one week after cultivation. The stem growth of DM1C, DM2C, SM1C, SM2C, and MLW samples occurred at 3 weeks after cultivation, while this growth in MPW occurred at 5 weeks. The height of MLW samples was subtle after 11 weeks after cultivation while all plants stopped to expand after 15 weeks (Figure 1c,d) and thereafter entering the dormant period at 17 weeks. The morphological data of the aboveground parts are shown in Table 1. Overall, it appeared that MMC, and SM2C had the highest survivor rate (100%). MMC illustrated the biggest structure, viz., 8.03 cm of leaf width, 25.43 cm of leaf length, and 51.75 cm of stem height. However, the stem width of all samples was not significantly different (~1.06 cm). The morphology of the *B. rotunda* rhizome is shown in Table 2. It was found that the rhizome part of the DM1C and SM2C had the highest weight (~14.79 g), where as MMC had the highest finger weight (47.07 g). Structurelly, the rhizome length and finger length of all samples were not significantly different (~15.5 cm of rhizome length and~ 6 cm of finger length).

The distribution of the morphological data of all samples was depicted using multivariate PCA. For physical characteristics of the aboveground organs (Figure 2a,b), the PCA space accounted for 87.06% in PC1 and 8.71% in PC2. Based on the observation, the data were divided into two main clusters with the first cluster containing the samples of the cultivated type (MMC, DM1C, and DM2C) illustrating the larger structures of the leaf and stem compared to other samples as colored in blue. The second cluster contained those belonging to the wild types with smaller forms as indicated in pink. It was interesting that some of the cultivated type fell within these color regions (viz., those from the MLW, SM1C, and SM2C). The habitat information advised that these cultivated samples were previously from the wild seeding that had begun to adapt to the environment. The relationship of the physical characteristics of the underground organs were demonstrated in Figure 2c,d. The PCA space distributes 38.39% in PC1 and 33.00% in PC2. Based on the observation, data were clustered into two groups, the first cluster contained samples of the cultivated type (MMC, DM1C and DM2C) with larger underground structures as compared to other samples. The second cluster contains samples of the wild type (MLW and MPW), while

SM1C, SM2C are those of the adapting type. All in all, the results showed that the physical characteristics of the aboveground organs corresponded to the physical characteristics of the rhizome. This finding is in line with Casper and Jackson [27], who advised that, although it was commonly assumed, shoot and root competition influenced plant growth characteristics. In addition, plant habitat often exerts its greatest influence on the characteristic of the aerial parts by altering leaf/stem ratios along with other morphological modifications [28]. During environmental stress, the expression of a variety of genes is induced which greatly influences plant growth and productivity [29]. Li et al. [30] also added that the environmental variables acted in a combinatorial fashion, best-explaining outlier genetic variation, and indicating environmentally dependent local adaptation for both leading- and trailing-edge populations. In the Zingiberaceae family, it is also found that plants exhibits adaptive evolution under different environmental conditions according to their phylogenetic relations [31]. Soil moisture is one of the most important constraints in cultivation of plants within the ginger family [32,33]. The moisture affects the accumulation of dry materials and the transportation of nitrogen in plants [34]. Contradictory, Osakabe, Osakabe, Shinozaki, and Tran [34] also pointed out that water stress gave the adverse impacts on plant physiology, especially the photosynthetic capacity. Plants have evolved complex physiological and biochemical adaptations to adjust and adapt to a variety of environmental stresses, thereby inducing the biosynthesis of the secondary metabolites. Beside this, plant genetics also contribute to phenotypic variation [35].

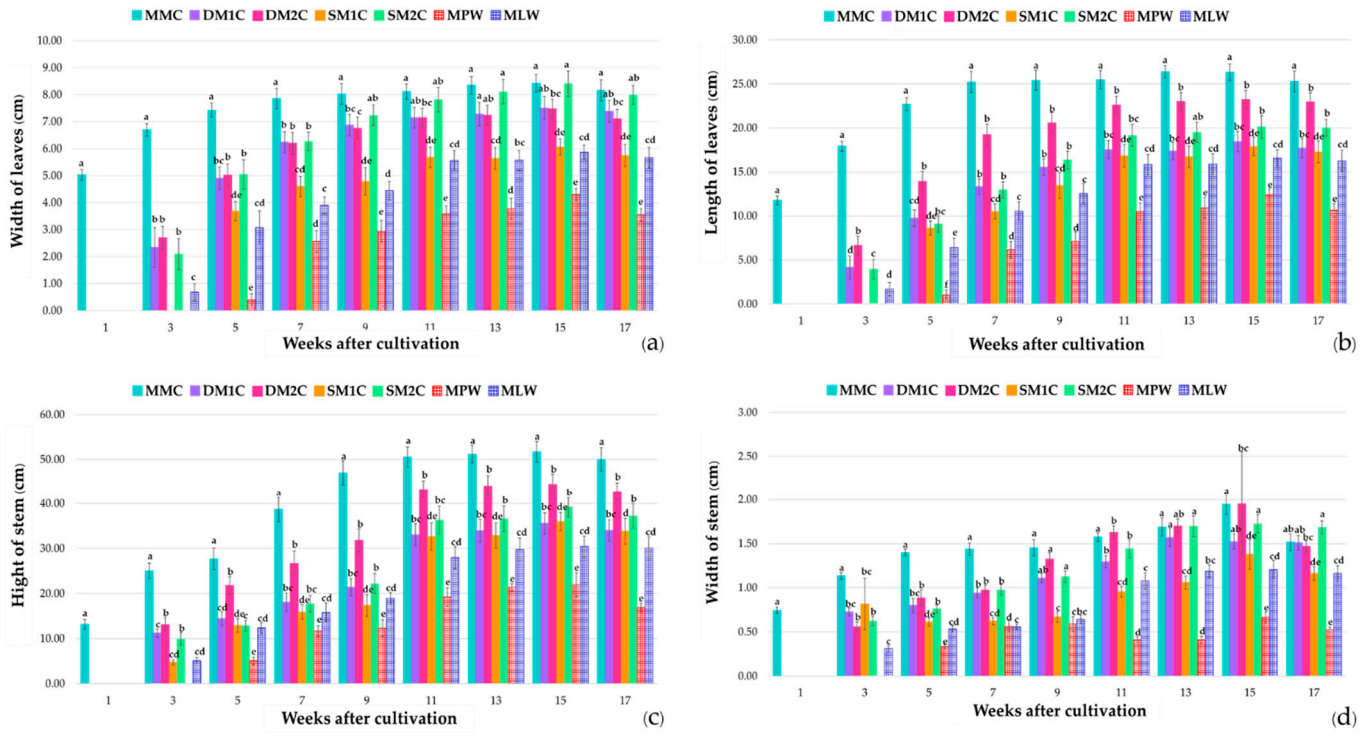


Figure 1. Morphological characteristics of *B. rotunda* after cultivated for 17 weeks. (**a**) leaf width (**b**) leaf length (**c**) stem height and (**d**) stem width. Data are means of the replicate samples. Bars represent standard deviation and different alphabet letters denote significant differences (95%) among the samples of each week ($p > 0.05$). MMC = Mae Taeng, Chiang Mai, cultivated type; MLW = Mae Tha, Lamphun, wild type; DM1C = Chiang Dao1, Chiang Mai, cultivated type; DM2C = Chiang Dao2, Chiang Mai, cultivated type; SM1C = San Pa Tong1, Chiang Mai, cultivated type; SM2C = San Pa Tong2, Chiang Mai, cultivated type; MPW = Mae Moh, Lampang, wild type. Data expressed as mean ± standard deviation.

Table 2. Physical properties of *B. rotunda* underground organs at 18 weeks.

Sample	Mother Rhizomes			Fingers			Rhizome
	Weight (g)	Width (cm)	Length (cm)	Weight (g)	Width (cm)	Length (cm)	
MMC	8.24 ± 2.66 ^{ab}	14.49 ± 0.56 ^{a}	17.61 ± 2.70 ^{ns}	47.07 ± 31.69 ^{a}	12.69 ± 0.79 ^{a}	5.86 ± 0.97 ^{ns}	
MLW	4.36 ± 2.84 ^{b}	12.53 ± 0.28 ^{b}	13.90 ± 1.03 ^{ns}	20.79 ± 1.75 ^{ab}	11.44 ± 3.24 ^{ab}	6.50 ± 1.59 ^{ns}	
DM1C	14.16 ± 5.61 ^{a}	14.07 ± 0.89 ^{a}	17.87 ± 2.60 ^{ns}	37.53 ± 25.23 ^{ab}	13.47 ± 0.39 ^{a}	6.49 ± 2.32 ^{ns}	
DM2C	3.23 ± 1.38 ^{b}	12.13 ± 0.62 ^{bc}	16.98 ± 3.43 ^{ns}	23.51 ± 2.45 ^{ab}	12.89 ± 2.49 ^{a}	6.92 ± 1.04 ^{ns}	
SM1C	2.84 ± 2.25 ^{b}	10.06 ± 0.62 ^{d}	13.71 ± 2.94 ^{ns}	19.30 ± 3.11 ^{ab}	12.73 ± 0.89 ^{a}	5.94 ± 1.08 ^{ns}	
SM2C	15.42 ± 7.63 ^{a}	13.26 ± 0.39 ^{ab}	17.84 ± 1.33 ^{ns}	34.83 ± 24.39 ^{ab}	13.96 ± 0.60 ^{a}	6.83 ± 0.68 ^{ns}	
MPW	2.99 ± 1.05 ^{b}	11.18 ± 1.14 ^{cd}	16.97 ± 4.45 ^{ns}	4.87 ± 1.87 ^{b}	9.31 ± 2.06 ^{b}	5.32 ± 5.10 ^{ns}	

MMC = Mae Taeng, Chiang Mai, cultivated type; MLW = Mae Tha, Lamphun, wild type; DM1C = Chiang Dao1, Chiang Mai, cultivated type; DM2C = Chiang Dao2, Chiang Mai, cultivated type; SM1C = San Pa Tong1, Chiang Mai, cultivated type; SM2C = San Pa Tong2, Chiang Mai, cultivated type; MPW = Mae Moh, Lampang, wild type. Data expressed as mean ± standard deviation. $^{a–d}$ Values in the same column with different alphabets are statistically distinct ($p > 0.05$). ^{ns} not significant.

3.2. Genetic Variation of B. rotunda

Usually, different habitats and environmental factors have always had an impact on the observable traits. In addition, it is possible to mistakenly identify two distinct sympatric species with the same personal characteristics as one species [36]. The *Boesenbergia* is one of the consistently difficult genera within this family taxonomically, due to the heterogeneity of the physical characteristic at both the intra- and interspecific levels. As a result, molecular markers are necessary to clearly identify the correct species [19,37]. The RAPD-PCR fingerprints were generated from DNA extract from the fingerroot tissue of *B. rotunda* samples with 10 oligonucleotide primers, as shown in Table 1. The results indicated that there were 74 fragments based on the RAPD (Figure 3a), which were used to create a dendrogram, as shown in Figure 3b. The dendrogram categorized the samples into three groups. DM1C was projected away from the others, while the first cluster consisted of samples of cultivated-type (MMC and DM2C) and wild-type MLW having more than

70% similarity. The second cluster contained samples of wild type (MPW, SM1C, and SM2C) which exhibited more than 68% similarity. From the results, we then are able to assume the genetic linkage between the samples collected in the wild and the cultivated types. Though, when considering their morphological characteristics, they were clearly distinctive, while the SM1C and SM2C were within the midrange. Ebrahimi et al. [38] explained that variables in a gene pool could be as a result of plant adaptation to their natural habitat. However, the genetic similarity of the cultivated *B. rotunda* (Krachai Ban) and the wild type (Krachai Pah) with the identical morphological characteristics of having a small rhizome, green color on both sides of lamina and midrib, had been found [39]. The genome assembly of *B. rotunda* (2n = 36) of the vegetatively propagated species illustrated an unusually high heterozygosity of 3.01%, advising that the cultivated species may have undergone whole-genome duplication events or hybrid origin [40].

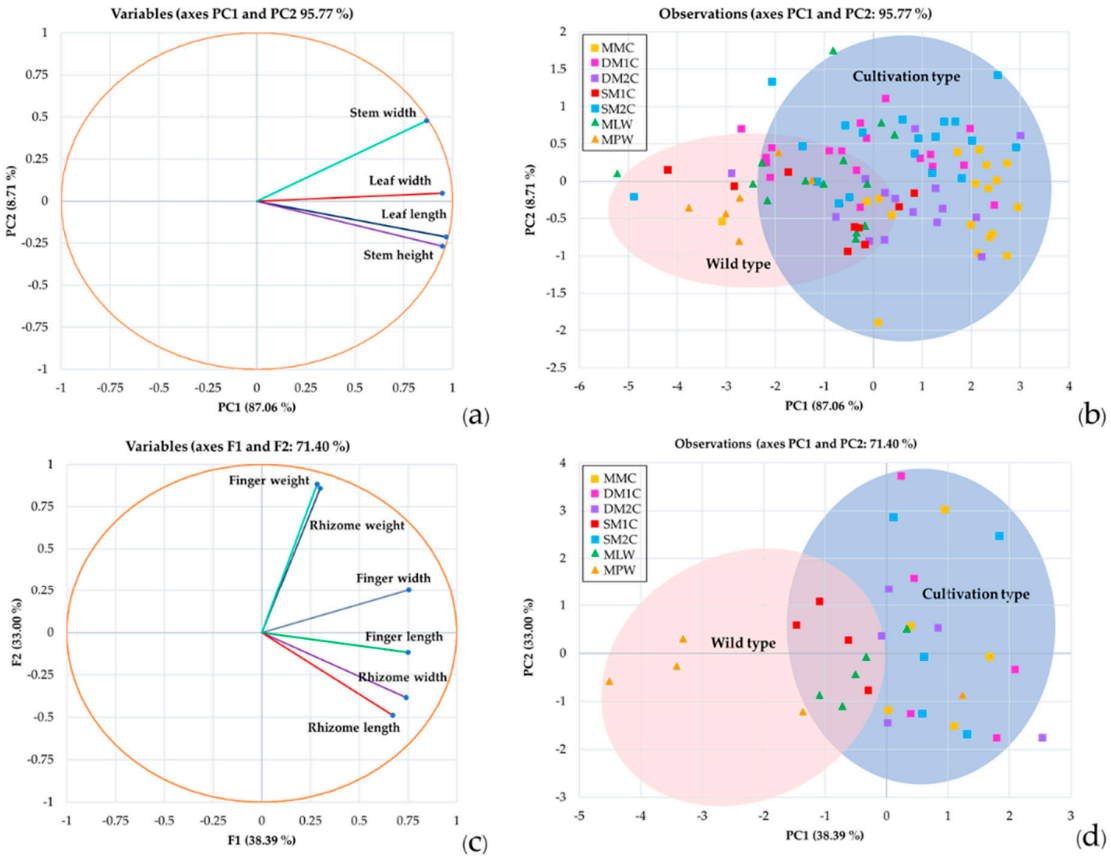
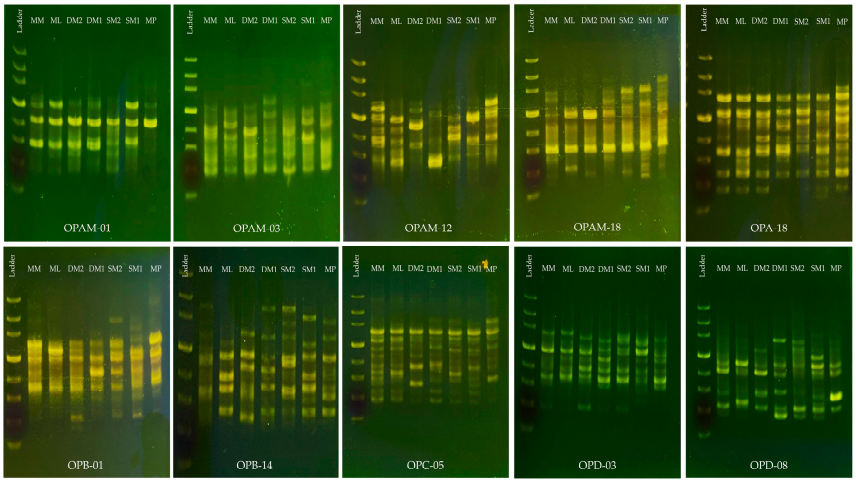


Figure 2. The principal component (PC) analysis of the *B. rotunda* sample based on their physical characteristics. (**a**) Variables of aboveground organ; (**b**) observations of aboveground organ; (**c**) variables of underground organ; and (**d**) observations of underground organ. MMC = Mae Taeng, Chiang Mai, cultivated type; MLW = Mae Tha, Lamphun, wild type; DM1C = Chiang Dao1, Chiang Mai, cultivated type; DM2C = Chiang Dao2, Chiang Mai, cultivated type; SM1C = San Pa Tong1, Chiang Mai, cultivated type; SM2C = San Pa Tong2, Chiang Mai, cultivated type; MPW = Mae Moh, Lampang, wild type.

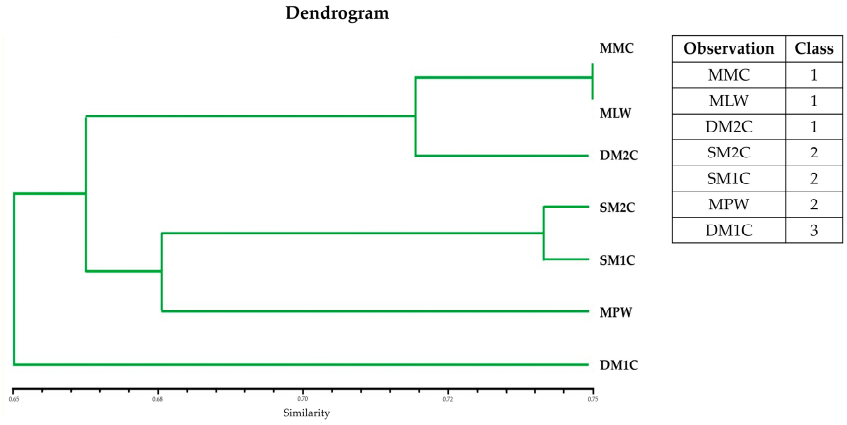
3.3. Phytochemical Compositions of B. rotunda

The phytoconstituent analysis of *B. rotunda* rhizomes from different sources are shown in Table 3. The highest contents of pinostrobin and panduratin A were found in the SM2C (43.27 ± 5.15 and 7.49 ± 1.11 mg/g dried sample), followed by the cultivated types DM1C and MLW. The MMC, DM2C and MPW were those that illustrated the lower contents of these phytochemicals. The highest contents of total phenolic constituents were found in the DM2C (23.79 ± 1.15 mg/g dried sample), followed by MPW, MMC, MLW, and SM2C.

Nonetheless, the highest contents of total flavonoid constituents were found in the MPW (95.12 ± 31.00 mg/g dried sample), followed by the cultivated types MMC, DM2C, SM1C, DM1C, and SM2C. The antioxidant potential as illustrated by DPPH was rather low in all samples (~25.00%), while high potency as determined by the ABTS (>92.00%) was shown. The relationships of these phytochemical compositions among the *B. rotunda* samples were evaluated on PCA panels. The PCA score plots depicted > 70% of the data within PC1 and PC2 (Figure 4). The biplot analysis showed a relationship between the contents of pinostrobin and panduratin A and the DM1C, SM2C, and MLW sources. MMC, DM2C, and MPW were projected closely to the antioxidant activities as well as the total phenolic and flavonoid levels. However, there was no correlation between the SM1C and the bioactive potency. The projection patterns from the PCA were divergent from the previous results of the morphological traits. We can then assume that the morphological characteristics may not be able to describe the amount of the bioactive ingredients contained in the rhizome.



(a)



Observation	Class
MMC	1
MLW	1
DM2C	1
SM2C	2
SM1C	2
MPW	2
DM1C	3

(b)

Figure 3. Genetic variation analysis of *B. rotunda* samples obtained from different habitats using the RAPD-PCR technique. (**a**) The random amplified polymorphic DNA (RAPD)-PCR fingerprints using 10 random primers: OPAM-01 = TCACGTACGG, OPAM-03 = CTTCCCTGTG, OPAM-12 = TCTCACCGTC, OPAM-18 = ACGGGACTCT, OPA-18 = AGGTGACCGT, OPB-01 = GTTTCGCTCC, OPB-14 = TCCGCTCTGG, OPC-05 = GATGACCGCC, OPD-03 = GTCGCCGTCA, and OPD-08 = GTGTGCCCCA; (**b**) The dendrogram generated from the similarity matrix of RAPD data. MMC = Mae Taeng, Chiang Mai, cultivated type; MLW = Mae Tha, Lamphun, wild type; DM1C = Chiang Dao1, Chiang Mai, cultivated type; DM2C = Chiang Dao2, Chiang Mai, cultivated type; SM1C = San Pa Tong1, Chiang Mai, cultivated type; SM2C = San Pa Tong2, Chiang Mai, cultivated type; MPW = Mae Moh, Lampang, wild type.

Table 3. Phytoconstituents of *B. rotunda* from different sources.

Sample	Pinostrobin ($mg/g_{dried\ sample}$)	Panduratin A ($mg/g_{dried\ sample}$)	Total Phenolic ($mg_{gallic}/g_{dried\ sample}$)	Total Flavonoids ($mg_{catechin}/g_{dried\ sample}$)	DPPH (%)	ABTS (%)
MMC	27.09 ± 6.97^{bc}	4.90 ± 1.16^{bc}	20.80 ± 2.64^{ab}	73.71 ± 17.04^{b}	30.76 ± 5.88^{a}	92.25 ± 0.52^{b}
MLW	30.05 ± 7.55^{bc}	5.83 ± 0.58^{ab}	19.69 ± 0.34^{bc}	50.00 ± 10.82^{c}	24.99 ± 2.33^{ab}	92.59 ± 1.27^{ab}
DM1C	35.38 ± 10.43^{ab}	6.06 ± 1.50^{ab}	16.97 ± 2.12^{c}	57.46 ± 13.36^{bc}	24.57 ± 2.73^{ab}	93.28 ± 0.43^{ab}
DM2C	27.56 ± 8.30^{bc}	5.04 ± 1.49^{bc}	23.79 ± 1.15^{a}	65.62 ± 2.75^{bc}	27.71 ± 3.47^{a}	93.82 ± 0.39^{a}
SM1C	14.41 ± 2.43^{c}	3.08 ± 0.81^{c}	16.52 ± 1.99^{c}	63.23 ± 3.78^{bc}	14.07 ± 5.45^{b}	93.08 ± 1.44^{ab}
SM2C	43.27 ± 5.15^{a}	7.49 ± 1.11^{a}	19.22 ± 1.92^{bc}	54.99 ± 7.45^{bc}	26.44 ± 4.60^{a}	92.69 ± 1.39^{ab}
MPW	17.27 ± 6.24^{c}	3.10 ± 0.69^{c}	22.88 ± 2.07^{ab}	95.12 ± 31.00^{a}	23.65 ± 2.68^{ab}	93.96 ± 0.53^{ab}

MMC = Mae Taeng, Chiang Mai; MLW = Mae Tha, Lamphun; DM1C = Chiang Dao1, Chiang Mai; DM2C = Chiang Dao2, Chiang Mai; SM1C = San Pa Tong1, Chiang Mai; SM2C = San Pa Tong2, Chiang Mai; MPW = Mae Moh, Lampang. Data are expressed as mean ± standard deviation. The $^{a–c}$ values in the same column with different alphabets are statistically distinct ($p > 0.05$).

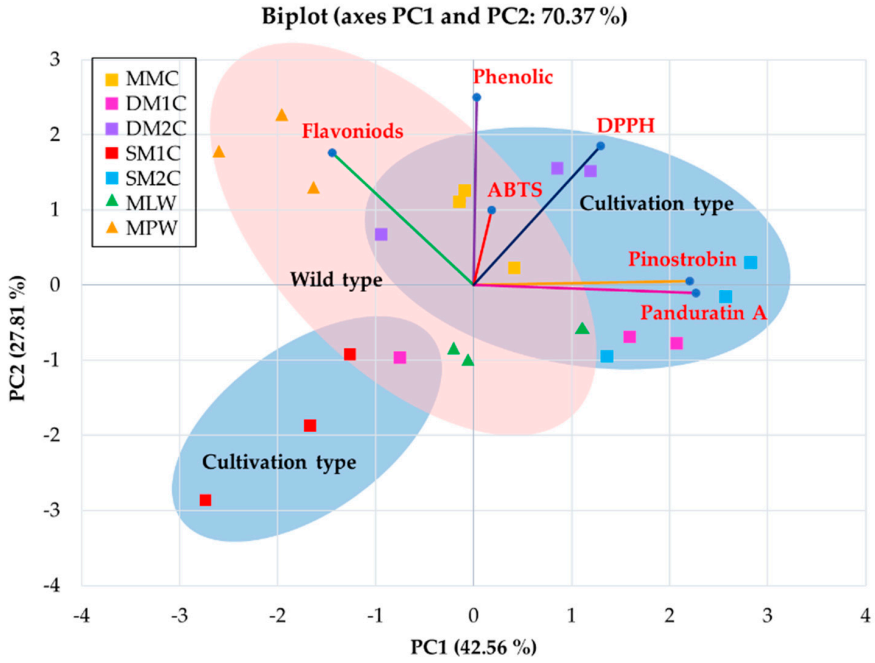


Figure 4. The principal component (PC) analysis of the *B. rotunda* sample based on their phytochemical compositions. MMC = Mae Taeng, Chiang Mai, cultivated type; MLW = Mae Tha, Lamphun, wild type; DM1C = Chiang Dao1, Chiang Mai, cultivated type; DM2C = Chiang Dao2, Chiang Mai, cultivated type; SM1C = San Pa Tong1, Chiang Mai, cultivated type; SM2C = San Pa Tong2, Chiang Mai, cultivated type; MPW = Mae Moh, Lampang, wild type.

B. rotunda is a potential source of bioactive compounds flavonoids including alpinetin, boesenbergin, cardamonin, geraniol, krachaizin, panduratin, pinostrobin, pinocembrin, rotundaflavone, and silybin that showed many interesting pharmacological activities, such as antifungal, antibacterial, antioxidant, etc. Interestingly [41,42], polyphenols including caffeic acid, coumaric acid, chlorogenic acid, hesperidin, kaempferol, naringen, and quercetin [8], flavones, and cyclohexenyl chalcone derivatives. Several of these secondary metabolites have shown promising antiviral and anticancer activities [43,44]. Cardamonin and pinostrobin chalcone were found to be the most potential natural compounds against breast cancer cell line MDA-MB-231 and colon cancer (HT-29, HL-60) cell line [45,46]. Pinostrobin (5-hydroxy-7-methoxy flavanone) is a naturally occurring dietary bioflavonoid [47–49]. Many pharmacological activities are attributed to pinostrobin, viz., anti-oxidative [50], anti-inflammatory activity [51], anti-cancer [52], anti-viral activity [53], anti-Alzheimer [54], gastroprotective activity, quinone reductase-inducer activity, antioxidant activity, anti-microbial activity, anti-nociceptive activity, and anti-fungal activity [55].

Panduratin A is a natural chalcone compound [56] that exhibits a potent inhibitory effect against SARS-CoV-2 infection at both the pre-entry and post-infection phases. Also, it suppresses viral infectivity in human airway epithelial cells [7] and inhibits the growth of MCF-7 human breast cancer and HT-29 human colon adenocarcinoma cells [57].

3.4. Metabolite Profiling of B. rotunda

The results from the LC-MS illustrated 50 different metabolites of the nonvolatile organic compound and are listed in Supplementary Table S3. The heat map categorized the samples into two clusters (Figure 5a). The first cluster consisted of samples of the cultivated-type (SM1C and MMC) and wild-type MLW. The second cluster contained samples of the cultivated-type (SM2C, DM1C, and DM2C) and wild-type MPW. The results of the volatile organic compound profiling with the GC-MS are illustrated in Supplementary Table S4. The heat map categorized the samples into three groups (Figure 5b). MLW was projected away from the others, while the first cluster consisted of samples of the cultivated-type (SM2C and MMC) and wild-type MPW. The second cluster contained samples of the cultivated type (SM1C, DM1C, and DM2C). From the results, we are able to extrapolate the genetic relationship between the samples collected in the wild and cultivated types. As said, environmental factors manipulate the different genetic expression the secondary metabolites biosynthesis pathways [58–61]. Plant secondary metabolites are not only a useful array of natural products but also an important part of plant defense systems against biotic and abiotic stresses [61]. Verma and Shukla [62] explained that the content of different secondary metabolites varied from generation to generation, and it did not remain stable because several biotic and abiotic factors play an important role in their synthesis and regulation.

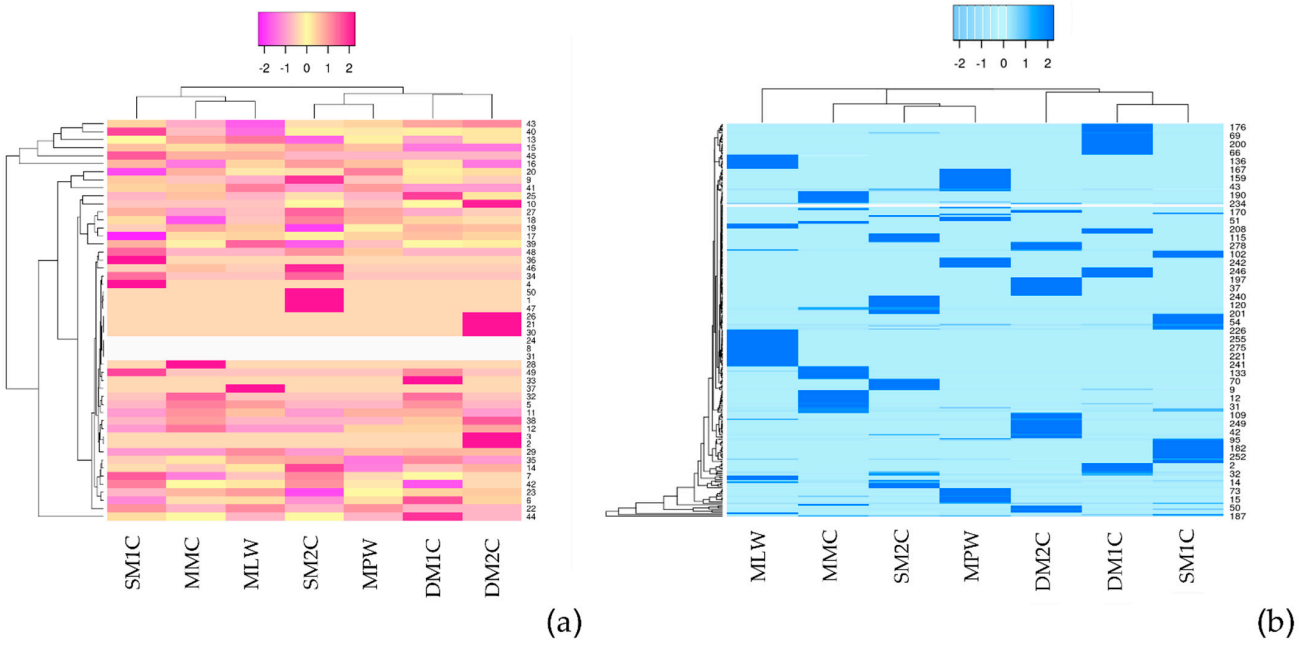


Figure 5. Heat map plots clusters of *B. rotunda* samples based on their phytochemical compositions using (**a**) LC-MS and (**b**) GC-MS techniques based on the content. MMC = Mae Taeng, Chiang Mai, cultivated type; MLW = Mae Tha, Lamphun, wild type; DM1C = Chiang Dao1, Chiang Mai, cultivated type; DM2C = Chiang Dao2, Chiang Mai, cultivated type; SM1C = San Pa Tong1, Chiang Mai, cultivated type; SM2C = San Pa Tong2, Chiang Mai, cultivated type; MPW = Mae Moh, Lampang, wild type.

4. Conclusions

This work evaluated the relationship between phenotypes and chemical profiling of *B. rotunda* collected from different habitats of Northern Thailand. Wild and domesticated,

the findings of this study suggested that the morphological characteristics of the plants were not directly correlated with the levels of significant phytochemicals present, including pinostrobin and panduratin A, but rather were influenced by various agronomic factors. The random amplification of polymorphic DNA with ten base-pair primers indicates the genetic correlation between wild and cultivated types. The results of this study could assist plant breeders in selecting lines with favorable phenotypes, such as those with the largest biomass, under optimal agronomic conditions. The potential trait of fingerroot might thereafter be employed as a source of medicinal plants for commercial production of bioactive products for the prevention of SARS-CoV-2 infection. Further research is necessary to identify the most suitable conditions for the biosynthesis of the active compounds.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13061435/s1, Supplementary Table S1. Random amplified polymorphic DNA primer sequence of genetic variation experiment. Supplementary Table S2. Reaction compounds (reaction mix and template DNA). Supplementary Table S3. Metabolite compounds by using LC-MS of *B. rotunda* rhizome extracts. Supplementary Table S4. Metabolite compounds by using GC-MS of *B. rotunda* rhizome extracts.

Author Contributions: Conceptualization, S.R.S.; methodology, S.R.S., C.L. and M.W.; validation, S.R.S. and M.W.; formal analysis, S.T.; investigation, S.T. and S.R.S.; writing—original draft preparation, S.T. and M.W.; writing—review and editing, S.R.S., S.T. and M.W.; supervision, S.R.S., P.J., M.W. and W.B.; project administration, S.T.; funding acquisition, S.R.S. and M.W. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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