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# Evaluation of bioactive compound contents in 50 varieties of mulberry leaves originating from different regions

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**Abstract**

Bioactive ingredients found in mulberry leaves have many medical and health promoting functions including antioxidant, anti-anxiety, anti-depressant, and anti-allergy. In order to better utilise the bioactive potential of mulberry resources, we determined the levels of 1-deoxynojirimycin (DNJ), gamma-aminobutyric acid (GABA), tannins, flavonoids, and polysaccharides in 50 different varieties of mulberry leaves. The bioactive contents in mulberry leaves may vary across species or region of origin, thus we investigated six different species as well as mulberry leaves originating from Japan, Sri Lanka, Uzbekistan, and 11 different Chinese provinces or cities. We found that bioactive compounds greatly varied among the 50 mulberry leaves examined (DNJ, 32.51 ~ 154.11 mg/100 g; GABA, 122.88 ~ 427.12 mg/100 g; tannins, 0.45 ~ 1.49 g/100 g; flavonoids, 12.86 ~ 31.57 mg/g; and polysaccharides, 7.55 ~ 20.52 g/100 g). High amounts of DNJ were obtained in Shenglidaye variety, high amounts of GABA, tannins, and flavonoids were obtained in Huasang variety, and high amounts of polysaccharides were obtained in Ganluo variety. Different varieties of mulberry leaves were rich in bioactive compounds with significant variations in their levels. DNJ content in leaves depended on variety and place of origin, while GABA, tannin, and flavonoid contents in leaves depended on variety and species. Six mulberry varieties, including Huasang, Jiuwenlong, Shigu 11-6, Shenglidaye, Qinpifusang, and HuoSang were identified as good sources for further utilisation. Huasang and Shenglidaye varieties were especially rich in DNJ or GABA, and thus have high potential value. The evaluation of mulberry leaves resources can supply precious data for screening accessions containing high levels of individual DNJ, GABA, and other bioactive compounds for use in breeding programs, and food and pharma industries.



## Introduction

Mulberry leaves have commercial uses as food in many Asian countries including China, Japan, Korea, and Thailand. Mulberry leaves can be used for processing mulberry leaves tea (Vichasilp *et al.,* 2012). Mulberry leaves could be appropriate materials for functional foods or drinks because of the high content of bioactive compounds such as phenolics or 1-deoxynojirimycin (DNJ), and have a potential application as protein-rich vegetables (Yu *et al.,* 2018). In recent years, more and more Chinese consumers have started to eat mulberry leaves as a kind of vegetable. Parts of the mulberry plant have been demonstrated to have a range of therapeutic effects, from improving vision and lowering blood pressure, to reducing fever and strengthening joints (Chang *et al.,* 2011). Recent studies have begun to investigate the anti-inflammatory, antioxidant, glucose-regulating, and organ-protective properties of mulberry (Yang *et al.,* 2010; Chang *et al.,* 2011; Zhang *et al.,* 2018b; Jung *et al.,* 2019).

DNJ is the main bioactive component in mulberry leaves (Jiang *et al.,* 2014), and has been demonstrated to have a range of therapeutic effects including increasing antioxidant and anti-inflammatory capacity, as well as lowering glycemia by inhibiting a-glucosidase and glucose absorption (Cai et al., 2017; Ma et al., 2019). Additionally, DNJ has been shown to have several protective functions against obesity, including preventing hepatic lipid abnormalities, mitochondrial dysfunction, and endoplasmic reticulum stress, as well as moderating food intake (Do *et al.,* 2015; Kim *et al.,* 2017).

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Other important bioactive compounds found in mulberry leaves include gamma-aminobutyric acid (GABA), tannins, flavonoids, and polysaccharides. GABA is a non-protein amino acid, and one of the main inhibitory neurotransmitters in the mammalian nervous system. GABA has important roles in autonomic functions, sleep, analgesia, and memory, as well as in preventing cancer (Yoo *et al.,* 2011; Lin *et al.,* 2012; Yamatsu *et al.,* 2015). Tannins have been shown to inhibit allergic reactions and may help to prevent the development of allergies (Yamada *et al.,* 2012). Tannins also have antioxidant, anticancer, and antibacterial activities, and cardiovascular disease protection (Shu *et al.,* 2018). Flavonoids have been shown to have potentials in preventing diabetes and related disorders (Wang *et al.,* 2019). Many polysaccharides found in plants have been shown to have antioxidant, immunomodulatory, antitumor, anti-inflammatory, antidiabetic, and antiviral properties, and have been reported to inhibit renal fibrosis, potentially treat diabetic nephropathy, and protect against alloxan-induced pancreatic islet damage (Zhang *et al.,* 2003; Yu *et al.*, 2009; Wu, 2019).

Mulberry trees have been cultivated in many Asian countries since ancient times. More than 400 mulberry varieties among 15 different *Morus* species are found in China (Liu *et al.,* 2010), and at least 173 of those varieties contain DNJ (Song *et al.,* 2009; Hu *et al.,* 2013). Previous studies have shown that DNJ concentrations in mature mulberry leaves ranged from 0.1341 to 1.472 mg/g of dry leaves among 132 examined varieties (Hu *et al.,* 2013); however, other bioactive ingredients were not analysed. In order to compare the bioactive ingredients more comprehensively in different varieties of mulberry leaves, we measured the levels of DNJ, GABA, tannins, flavonoids, and polysaccharides in 50 varieties of mulberry plants differing in species or region of origin. Our results will provide an important reference for developing future therapeutic uses of mulberry resources.

## Materials and methods

*Sample*

Bioactive compounds were investigated in 50 mulberry leaves collected from the Chongqing Sericulture Science and Technology Research Institute (Chongqing, China) in mid-May. The 50 selected mulberry varieties originated from Japan, Sri Lanka, Uzbekistan, and 11 Chinese provinces or cities, and represented six different species from the *Morus* genus, with taxonomic classification according to Vijayan *et al.* (2011). Mulberry leaves were picked around 10:00 a.m., and leaf position and maturity were consistent. All of the leaves were collected from the basal end of branches (mature leaves). Mulberry leaves were cleaned and dried in an oven at 80 ± 1°C. All experimental concentrations reported in this paper used dry sample weight. Dry mulberry leaves samples were ground into powder, and stored at 4°C until five bioactive ingredients analyses.

*Chemicals*

Standards of DNJ, tannin, and rutin were purchased from Beijing Dewei Sodium Biotechnology Co. Ltd., and glycine, GABA, and 9-fluorenylmethyl chloroformate (FMOC-Cl) were purchased from Sigma Chemical Co. (St. Louis, MO). All reagents were of analytical grade except acetonitrile, which was of chromatographic grade.

*DNJ extraction and derivatisation*

DNJ was extracted and derivatised following previously published methods (Kim *et al.,* 2003; Wei *et al.,* 2011). Briefly, 90 mg of dried mulberry leaf powder was dissolved in 2.5 mL of 0.05 M HCl (aq), and sonicated in an ice bath for 30 min. Samples were centrifuged for 15 min at 17,000 *g* and the supernatant were collected. The samples were re-extracted and the supernatants were pooled. The supernatant was brought to a volume of 6 mL with distilled water and derivatised using FMOC-C1.

*Determination of DNJ concentration*

A HPLC system (Waters, New York, USA) with a Waters Xterra RP-19 column (150 × 4.6 mm, 5 µL, maintained at 25°C) and a Waters 2996 photodiode array detector was used to analyse the amount of DNJ in 10 µL of derivatised mulberry samples at a detection wavelength of 254 nm. Samples were eluted at a rate of 1 mL/min for 10 min in a 1:1 (v/v) mixture of acetonitrile, and 0.1% aqueous acetic acid and DNJ concentration was extrapolated from a standard curve (Kim *et al.,* 2003; Wei *et al.,* 2011).

*Extraction and determination of GABA concentration*

Previously reported methods were used with slight modifications to extract GABA and determine its concentration in mulberry leaf samples (Xia *et al.,* 2009). Briefly, 0.5 g of powdered mulberry leaf sample was dissolved in 2 mL of methanol for 15 min at room temperature. Samples were then centrifuged for 5 min at 5,000 rpm, and the supernatant was discarded. 2 mL of methanol was then added to the remaining solid sample, re-centrifuged for 5 min at 5,000 rpm, and the supernatant was discarded. The remaining mulberry leaf powder was baked in oven at 70°C for 30 min. The methanol was evaporated by adding 5 mL of water

and mixing by oscillation at 50°C for 2 h, then centrifuging for 15 min at 5,000 rpm. The supernatant volume was adjusted to 1 mL, and then frozen for storage. 1 mL of sample was added to 50 mL of 2 mol/L $AlCl_3$ solution, and the GABA concentration was determined at 645 nm.

*Extraction and determination of tannin concentration*

Tannin concentration was determined using previously reported methods (Wilson, 1984) with slight modifications. Briefly, 0.5 g of sample was dissolved in 10 mL of 70% ethanol, and extracted at 70°C using ultrasound at 80% power for 20 min, followed by centrifuging at 5,000 rpm for 5 min. Samples were extracted twice. Samples were adjusted to 50 mL with distilled water. Next, 1 mL of the sample was mixed with 25 mL of distilled water, 2.5 mL of Folin-Denis colour developer, and 10 mL of saturated $Na_2CO_3$ for 30 s to 8 min. The water volume was adjusted to 50 mL after 30 min. Absorbance was measured at 750 nm, and pure water was used as a blank.

*Extraction and determination of flavonoid concentration*

A previously reported aluminium nitrate method was used to determine flavonoid concentration with slight modifications (Zhishen *et al.,* 1999). Briefly, 0.5 g of mulberry leaf sample was dissolved in 100 mL of distilled water for 1 h, and extracted using a Soxhlet extractor. A known volume of sample extract or rutin, used as a reference substance, was placed in a 10 mL volumetric flask, and the volume was adjusted to 5 mL with distilled water. Next, 0.3 mL of 1:20 $NaNO_3$ was added and incubated for 5 min, followed by 3 mL of 1:20 $AlCl_3$. After incubating for an additional 6 min, 2 mL of 1 mol/L NaOH was added, and the final volume was adjusted to 10 mL with distilled water. A M8500 UV-Visible spectrophotometer was used to measure absorbance at 510 nm. Concentration was determined by extrapolation with a standard curve generated using rutin.

*Extraction and determination of polysaccharide concentration*

Polysaccharides were extracted using a previously published method (Ying *et al.,* 2011). Briefly, 0.250 g of mulberry leaf powder was dissolved in distilled water at a 15:1 mL/g ratio, and extracted using ultrasound (60 W for 20 min at 60°C). Polysaccharide concentration was measured using previously published methods by Ying *et al.* (2008). Briefly, 1 mL of extract was added to a 50 mL volumetric bottle, and the volume was adjusted to 20 mL. Next, 1 mL of 5% phenol solution was added, followed by 5 mL of concentrated sulphuric acid (added directly to the liquid surface without touching the wall of the test tube to make the test tube more stable). After letting the reaction stand for 10 min, the reaction was thoroughly mixed with a scroll oscillator, and then placed in a 30°C water bath for 20 min. The absorbance was then measured at 490 nm.

*Statistical analysis*

All results were analysed using SPSS statistical analysis software (Version 16.0, SPSS Inc.), and expressed as mean ± SD. To test for statistical significance, a one-way ANOVA with Tukey's test was performed for multiple comparisons. $p < 0.05$ was considered to be statistically significant.

## Results and discussions

*DNJ in 50 mulberry varieties*

DNJ concentrations of mulberry leaves from 50 mulberry varieties were determined and found to vary from 32.51 ± 1.84 to 154.11 ± 5.66 mg/100 g of dry weight (Table 1), and the average DNJ concentration was 85.58 mg/100 g dry weight. 'Shenglidaye' variety had the highest DNJ concentration, while 'Jiuwenlong' variety had the lowest (Table 1). The DNJ concentration ranged from 60 to 120 mg/100 g dry weight in 43 samples, was greater than 120 mg/100 g dry weight in four samples, and was less than 60 mg/100 g dry weight in three samples (Jiuwenlong, Hongxing 5, and Hongpiwasang). Thus, DNJ concentrations varied among mulberry varieties, with the DNJ content of the richest sample being 4.74-fold higher than the poorest sample.

A previous study investigating the DNJ content of young mulberry leaves from 35 Thai varieties reported a range of 30 to 170 mg/100 g dry weigh (Vichasilp *et al.,* 2012), which is very similar to our findings. Additional studies investigated DNJ contents in mature leaves reported concentrations as low as 0.1341 mg/g dry weight (Hu *et al.,* 2013), and as high as 2.159 mg/g dry weight (Zhang *et al.,* 2018a), greater than the highest DNJ values in our study. An important difference between our study and the previous studies is harvest time; the mulberry leaves used in this study were collected in May, whereas the previous studies collected leaves in August and October. Previous study estimated that 6 mg DNJ/60 kg human is needed to suppress high blood glucose levels, and commercial mulberry products contain less than 100 mg/100 g dry weight of DNJ, which is too low to provide therapeutic effects (Kimura *et al.,* 2007; Li *et al.,* 2018). Therefore, higher quality products enriched with DNJ would be desirable, and mulberry varieties with leaves rich in DNJ would be good sources of high-quality dry tea.

Table 1. The contents of the five bioactive ingredients in mulberry leaves in different varieties.

| No. | Variety | Morus spp. | Origin | DNJ (mg/100 g dry weight) | GABA (mg/100 g dry weight) | Tannin (g/100 g dry weight) | Flavonoid (mg/g dry weight) | Polysaccharide (g/100 g dry weight) |
|---|---|---|---|---|---|---|---|---|
| 1 | Husang | M. multicaulis Perr. | Zhejiang | 68.29 ± 1.42 $^{s-w}$ | 183.98 ± 10.41 $^{m-r}$ | 0.62 ± 0.04 $^{qr}$ | 16.20 ± 0.35 $^{qrs}$ | 11.53 ± 0.30 $^{k-q}$ |
| 2 | Huasang | M. cathayana Hemsl. | Anhui | 62.53 ± 1.92 $^{uv}$ | 427.12 ± 16.08 $^{a}$ | 1.49 ± 0.03 $^{a}$ | 31.57 ± 0.31 $^{a}$ | 7.55 ± 0.34 $^{v}$ |
| 3 | Mengluosang | M. multicaulis Perr. | Sichuan | 72.49 ± 2.68 $^{p-v}$ | 122.88 ± 5.58 $^{w}$ | 0.68 ± 0.03 $^{opqr}$ | 17.59 ± 0.19 $^{nopr}$ | 10.97 ± 0.31 $^{m-r}$ |
| 4 | Zhuangelou | M. alba Linn. | Sichuan | 93.39 ± 2.25 $^{h-j}$ | 229.04 ± 1.71 $^{ghij}$ | 0.78 ± 0.01 $^{klm}$ | 20.22 ± 0.39 $^{kl}$ | 11.71 ± 0.35 $^{i-p}$ |
| 5 | Sanbeitiwupi | M. alba Linn. | Sichuan | 76.98 ± 3.37 $^{o-u}$ | 184.84 ± 12.46 $^{l-r}$ | 0.60 ± 0.04 $^{r}$ | 15.06 ± 0.53 $^{st}$ | 10.60 ± 0.50 $^{m-s}$ |
| 6 | Jiuwenlong | M. multicaulis Perr. | Japan | 32.51 ± 1.84 $^{y}$ | 355.05 ± 15.03 $^{b}$ | 0.84 ± 0.01 $^{ijkl}$ | 15.68 ± 0.36 $^{rs}$ | 9.90 ± 0.37 $^{n-u}$ |
| 7 | Xiaoguansang | M. alba Linn. | Sichuan | 83.39 ± 1.37 $^{l-o}$ | 230.07 ± 8.46 $^{jhi}$ | 0.85 ± 0.06 $^{h-l}$ | 23.76 ± 0.66 $^{efg}$ | 7.96 ± 0.17 $^{uv}$ |
| 8 | Zhongsang5801 | M. atropurpurea Roxb. | Jiangsu | 78.09 ± 1.36 $^{o-t}$ | 148.06 ± 8.32 $^{r-w}$ | 0.89 ± 0.06 $^{e-j}$ | 16.20 ± 0.35 $^{qrs}$ | 11.12 ± 0.35 $^{l-q}$ |
| 9 | 48-1 | M. alba Linn. | Sichuan | 67.06 ± 1.84 $^{t-w}$ | 226.64 ± 14.60 $^{ghij}$ | 1.01 ± 0.02 $^{de}$ | 21.70 ± 0.18 $^{hij}$ | 8.67 ± 0.22 $^{r-v}$ |
| 10 | HuoSang | M. mizuho Hotta. | Zhejiang | 139.75 ± 3.39 $^{bc}$ | 308.11 ± 26.51 $^{cde}$ | 0.77 ± 0.02 $^{k-o}$ | 18.52 ± 0.26 $^{mno}$ | 9.54 ± 0.03 $^{p-v}$ |
| 11 | Husang 32 | M. multicaulis Perr. | Zhejiang | 72.03 ± 1.03 $^{q-v}$ | 162.22 ± 8.37 $^{q-v}$ | 0.65 ± 0.02 $^{pqr}$ | 17.28 ± 0.58 $^{opq}$ | 12.64 ± 0.23 $^{f-m}$ |
| 12 | Pingshi | M. atropurpurea Roxb. | Guangxi | 75.47 ± 1.87 $^{q-u}$ | 165.90 ± 10.03 $^{q-v}$ | 0.65 ± 0.04 $^{pqr}$ | 18.54 ± 0.77 $^{mno}$ | 10.23 ± 0.22 $^{n-u}$ |
| 13 | Yousang | M. multicaulis Perr. | Zhejiang | 95.10 ± 4.91 $^{g-j}$ | 178.96 ± 11.67 $^{n-s}$ | 0.71 ± 0.03 $^{m-q}$ | 24.93 ±0.23 $^{de}$ | 8.24 ± 0.10 $^{tuv}$ |
| 14 | Huanglusang | M. multicaulis Perr. | Hebei | 116.93 ± 1.41 $^{d-h}$ | 242.52 ± 6.88 $^{k-q}$ | 0.84 ± 0.01 $^{c}$ | 17.78 ± 0.29 $^{u}$ | 12.82 ± 0.12 $^{stuv}$ |
| 15 | Xinyizhilai | M. alba Linn. | Japan | 89.64 ± 2.58 $^{i}$ | 192.08 ± 9.04 $^{j-q}$ | 0.90 ± 0.02 $^{e-j}$ | 26.70 ± 0.22 $^{c}$ | 13.72 ± 0.75 $^{d-j}$ |
| 16 | Emeihuasang | M. multicaulis Perr. | Sichuan | 98.24 ± 2.35 $^{e}$ | 170.06 ± 8.64 $^{p-u}$ | 0.78 ± 0.03 $^{k-n}$ | 22.44 ± 0.17 $^{ghi}$ | 14.94 ± 0.30 $^{cdef}$ |
| 17 | Xinlunjiao | M. atropurpurea Roxb. | Guangdong | 101.98 ± 4.78 $^{d-h}$ | 274.74 ± 5.77 $^{ef}$ | 0.95 ± 0.02 $^{defg}$ | 20.17 ± 0.26 $^{kl}$ | 12.09 ± 0.52 $^{h-n}$ |
| 18 | Shanxitiansang | M. alba Linn. | Shanxi | 60.12 ± 0.72 $^{w}$ | 256.62 ± 18.66 $^{fgh}$ | 0.78 ± 0.03 $^{klmn}$ | 14.84 ± 0.11 $^{st}$ | 11.64 ± 0.95 $^{j-q}$ |
| 19 | Ganluo | M. alba Linn. | Sichuan | 75.47 ± 3.20 $^{o-u}$ | 129.83 ± 6.63 $^{vw}$ | 0.92 ± 0.04 $^{e-j}$ | 21.15 ± 0.15 $^{hijk}$ | 20.52 ± 0.24 $^{a}$ |
| 20 | Hongxing 5 | M. multicaulis Perr. | Anhui | 47.91 ± 0.73 $^{t-w}$ | 163.63 ± 11.18 $^{q-v}$ | 0.94 ± 0.02 $^{d-h}$ | 18.59 ± 0.25 $^{mno}$ | 17.12 ± 0.35 $^{ab}$ |
| 21 | Langzhonghey e | M. alba Linn. | Sichuan | 90.97 ± 2.40 $^{h-j}$ | 187.81 ± 9.72 $^{k-q}$ | 1.35 ± 0.03 $^{b}$ | 29.56 ± 0.57 $^{b}$ | 16.10 ± 1.45 $^{bc}$ |
| 22 | Yinghuachi | M. alba Linn. | Sichuan | 108.56 ± 0.95 $^{de}$ | 138.86 ± 10.72 $^{t-w}$ | 1.01 ± 0.03 $^{de}$ | 21.58 ± 1.04 $^{hijk}$ | 18.86 ± 1.12 $^{a}$ |
| 23 | Yidashoumusang | M. bombycis Koidz. | Japan | 71.45 ± 1.82 $^{q-v}$ | 222.77 ± 13.84 $^{hijk}$ | 1.02 ± 0.04 $^{d}$ | 19.48 ± 0.90 $^{lm}$ | 15.52 ± 0.87 $^{bcd}$ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 24 | Yu711 | M. multicaulis Perr. | Jiangsu | 83.75 ± 3.45 $^{k-n}$ | 209.83 ± 11.95 $^{i-o}$ | 0.76 ± 0.01 $^{lmno}$ | 13.76 ± 0.08 $^{tu}$ | 9.55 ± 0.47 $^{p-v}$ |
| 25 | Qinpifusang | M. multicaulis Perr. | Zhejiang | 106.27 ± 2.42 $^{d-g}$ | 312.51 ± 14.67 $^{cd}$ | 0.69 ± 0.03 $^{m-r}$ | 16.54 ± 0.37 $^{pqr}$ | 9.95 ± 0.72 $^{n-u}$ |
| 26 | 98-1 | M. alba Linn. | Sichuan | 67.77 ± 1.68 $^{t-w}$ | 233.04 ± 3.16 $^{ghi}$ | 0.94 ± 0.03 $^{d-h}$ | 18.62 ± 0.15 $^{mno}$ | 12.17 ± 0.42 $^{g-n}$ |
| 27 | Hongguo 1 | M. bombycis Koidz. | Shanxi | 77.20 ± 1.82 $^{o-u}$ | 176.64 ± 11.78 $^{o-s}$ | 0.72 ± 0.01 $^{m-q}$ | 17.78 ± 0.37 $^{nop}$ | 11.31 ± 0.62 $^{k-q}$ |
| 28 | Niujinsang | M. multicaulis Perr. | Hebei | 84.37 ± 1.27 $^{j-m}$ | 155.65 ± 5.53 $^{q-w}$ | 0.96 ± 0.03 $^{defg}$ | 19.45 ± 0.12 $^{lm}$ | 13.14 ± 0.41 $^{e-l}$ |
| 29 | Hongguo 2 | M. bombycis Koidz. | Shanxi | 79.00 ± 7.18 $^{n-s}$ | 176.13 ± 5.26 $^{o-t}$ | 0.89 ± 0.03 $^{ghij}$ | 12.86 ± 0.07 $^{u}$ | 10.06 ±0.24 $^{n-u}$ |
| 30 | Jiading | M. alba Linn. | Sichuan | 69.55 ± 3.68 $^{r-w}$ | 122.99 ± 10.02 $^{w}$ | 0.70 ± 0.02 $^{m-r}$ | 13.88 ± 0.54 $^{tu}$ | 12.70 ± 0.37 $^{f-m}$ |
| 31 | Zhaojiaosanhao | M. alba Linn. | Chongqing | 74.87 ± 2.57 $^{o-s}$ | 219.18 ± 9.91 $^{i-m}$ | 0.91 ± 0.03 $^{f-j}$ | 23.21 ± 0.47 $^{fg}$ | 10.75 ± 0.31 $^{m-s}$ |
| 32 | 213 | M. multicaulis Perr. | Chongqing | 112.20 ± 3.44 $^{d}$ | 263.37 ± 11.06 $^{fg}$ | 1.16 ± 0.03 $^{c}$ | 15.53 ± 0.30 $^{rs}$ | 10.76 ± 0.99 $^{m-s}$ |
| 33 | Beiyihao | M. alba Linn. | Chongqing | 80.02 ± 0.56 $^{l-p}$ | 276.22 ± 17.83 $^{def}$ | 0.91 ± 0.05 $^{f-j}$ | 18.98 ± 0.39 $^{lmn}$ | 8.61 ± 1.00 $^{stuv}$ |
| 34 | Yunnan 10 | M. alba Linn. | Yunnan | 134.02 ± 2.36 $^{c}$ | 162.56 ± 13.04 $^{q-v}$ | 0.99 ± 0.04 $^{def}$ | 24.38 ± 0.53 $^{def}$ | 15.07 ± 0.29 $^{cde}$ |
| 35 | Yun 2 | M. alba Linn. | Yunnan | 81.70 ± 4.81 $^{l-o}$ | 207.20 ± 6.73 $^{i-o}$ | 0.45 ± 0.02 $^{s}$ | 13.12 ± 0.08 $^{u}$ | 9.61 ± 0.25 $^{p}$ |
| 36 | Hongpiwasang | M. multicaulis Perr. | Hubei | 39.49 ± 0.31 $^{xy}$ | 180.44 ± 7.89 $^{n-r}$ | 0.69 ± 0.01 $^{m-r}$ | 13.74 ± 0.18 $^{tu}$ | 9.41 ± 0.11 $^{q}$ |
| 37 | Beichang 3 | M. alba Linn. | Chongqing | 70.01 ± 1.22 $^{r-w}$ | 177.02 ± 10.86 $^{o-s}$ | 0.68 ± 0.03 $^{m-r}$ | 21.13 ± 0.27 $^{ijk}$ | 10.50 ± 1.07 $^{o}$ |
| 38 | Jianchi | M. alba Linn. | Japan | 61.12 ± 0.72 $^{vw}$ | 232.25 ± 5.60 $^{ghi}$ | 0.66 ± 0.01 $^{pqr}$ | 15.70 ± 0.23 $^{rs}$ | 15.69 ± 0.22 $^{b}$ |
| 39 | Jinlong | M. alba Linn. | Japan | 66.30 ± 2.10 $^{uvw}$ | 176.8 ± 10.24 $^{o-s}$ | 0.73 ± 0.01 $^{mnop}$ | 17.80 ± 0.26 $^{nop}$ | 9.62 ± 0.17 $^{p}$ |
| 40 | Pansang | M. alba Linn. | Sichuan | 77.35 ± 0.93 $^{o-u}$ | 177.26 ± 5.70 $^{n-s}$ | 0.77 ± 0.04 $^{k-o}$ | 17.78 ± 0.46 $^{nop}$ | 13.89 ± 0.59 $^{c}$ |
| 41 | Jialingxin 9 | M. alba Linn. | Chongqing | 98.38 ± 4.40 $^{e-i}$ | 136.95 ± 8.83 $^{uvw}$ | 0.87 ± 0.01 $^{g-k}$ | 17.23 ± 0.50 $^{opq}$ | 10.17 ± 0.26 $^{n}$ |
| 42 | Kanwa | M. bombycis Koidz. | Sri Lanka | 106.48 ± 6.01 $^{def}$ | 316.51 ± 14.67 $^{c}$ | 1.16 ± 0.03 $^{c}$ | 18.35 ± 0.15 $^{mno}$ | 14.45 ± 0.65 $^{c}$ |
| 43 | Naxi 1 | M. alba Linn. | Sichuan | 89.83 ± 4.93 $^{ijk}$ | 242.47 ± 15.98 $^{fghi}$ | 1.35 ± 0.03 $^{b}$ | 23.16 ± 0.58 $^{fg}$ | 11.99 ± 0.23 $^{h}$ |
| 44 | Shenglidaye | M. multicaulis Perr. | Uzbekistan | 154.11 ± 5.66 $^{a}$ | 231.39 ± 11.34 $^{ghi}$ | .0.69 ± 0.01 $^{m-r}$ | 21.68 ± 0.19 $^{hij}$ | 15.50 ± 0.97 $^{b}$ |
| 45 | Fushe 1 | M. multicaulis Perr. | Jiangsu | 75.19 ± 1.11 $^{o-u}$ | 214.50 ± 4.62 $^{i-n}$ | 1.05 ± 0.03 $^{m-r}$ | 17.35 ± 0.32 $^{opq}$ | 13.12 ± 0.52 $^{e}$ |
| 46 | Gailiangshufan | M. alba Linn. | Japan | 96.24 ± 6.27 $^{fgji}$ | 221.88 ± 7.95 $^{h-l}$ | 0.94 ± 0.03 $^{d-i}$ | 22.58 ± 0.37 $^{gh}$ | 13.39 ± 1.36 $^{d}$ |
| 47 | Shigu 11-6 | M. alba Linn. | Sichuan | 79.81 ± 1.09 $^{m-q}$ | 410.55 ± 9.72 $^{a}$ | 1.15 ± 0.03 $^{c}$ | 20.36 ± 0.44 $^{jkl}$ | 9.78 ± 0.80 $^{n}$ |
| 48 | Leshanhua | M. alba Linn. | Sichuan | 149.39 ± 3.49 $^{ab}$ | 225.20 ± 0.68 $^{hij}$ | 0.70 ± 0.03 $^{m-r}$ | 17.30 ± 0.25 $^{opq}$ | 11.56 ± 0.67 $^{j}$ |
| 49 | Xintasang | M. multicaulis Perr. | Sichuan | 88.90 ± 3.12 $^{s-w}$ | 142.21 ± 11.70 $^{s-w}$ | 0.84 ± 0.02 $^{jkl}$ | 25.28 ± 1.17 $^{cd}$ | 9.49 ± 0.24 $^{p}$ |
| 50 | Ribengailiangshizi | M. alba Linn. | Japan | 112.55 ± 1.12 $^{p-u}$ | 171.66 ± 11.42 $^{p-u}$ | 0.92 ± 0.03 $^{e-j}$ | 21.68 ± 0.31 $^{hij}$ | 14.19 ± 0.48 $^{c}$ |

Data are means ± SD of triplicates ($n$ = 3). Different letters indicate statistically significant differences at $p < 0.05$ for each variety.

We found that 11 mulberry varieties in this study had more than 100 mg/100 g DNJ dry weight, notably the Shenglidaye (154.11 ± 5.66 mg/100 g dry weight), Leshanhua (149.39 ± 3.49 mg/100 g dry weight) and HuoSang (139.75 ± 3.39 mg/100 g dry weight) varieties. These varieties could be good candidates for further food or drug development.

*GABA in 50 mulberry varieties*

As shown in Table 1, GABA levels among the 50 examined mulberry leaves ranged from 122.88 ± 5.58 to 427.12 ± 16.08 mg/100 g dry weight. 'Huasang' variety had the highest GABA concentration, and 'Mengluosang' variety had the lowest. The average GABA concentration was 212.36 mg/100 g dry weight. Thirty-five samples had GABA contents ranging from 135 to 240 mg/100 g dry weight, 12 samples had GABA contents greater than 240 mg/100 g dry weight, and three samples (Mengluosang, Jiading, and Ganluo) had GABA levels less than 135 mg/100 g dry weight. The GABA content of the richest sample was 3.48-fold higher than that of the poorest sample, and GABA contents significantly differed among different varieties.

GABA has been used as a food constituent and a food supplement; it exhibits some important physiological functions such as the ability to reduce blood pressure (Suwanmanon and Hsieh, 2014), prevent chronic alcohol-related diseases (Oh *et al.,* 2003), inhibit cancer cell proliferation (Oh and Oh, 2004), and improve sleep (Yamatsu *et al.,* 2015). A previous study examined the GABA content of 23 varieties of fresh mulberry leaves, and found that it ranged from 0.325 to 1.224 mg/g (Xia *et al.,* 2009). In this study, we used a larger number of varieties (50); thus, representing more species and regions of origin, and generating more empirical data. This enabled us to obtain more precise information regarding the GABA contents among mulberry varieties. GABA is one of the important functional components of mulberry leaf. Inoue *et al.* (2003) found that the daily intake of 10 - 12 mg of GABA in 39 mildly hypertensive patients (16 females and 23 males) aged 28 - 81 years old for 12 weeks was effective; the highest GABA content obtained in this study is enough for some physiological benefits. Furthermore, we found that GABA was particularly rich in Huasang (427.12 ± 16.08 mg/100 g dry weight) and Shigu 11-6 (410.55 ± 9.72 mg/100 g dry weight) varieties, which makes them good candidates for development of GABA related food or drug products.

*Tannin in 50 mulberry varieties*

In the 50 examined mulberry samples, the tannin levels ranged from 0.45 ± 0.02 to 1.49 ± 0.03 g/100 g dry weight (Table 1). The average tannin concentration was 0.86 g/100 g dry weight. 'Huasang' variety had the highest tannin concentration, and 'Yun 2' variety had the lowest. Forty-one samples had tannin concentrations ranging from 0.60 to 1.05 g/100 g dry weight. The tannin content of the richest sample was 3.31-fold higher than that of the poorest sample. This is the first report of tannin concentrations of mulberry leaves which differ among varieties and regions of origin.

*Flavonoid in 50 mulberry varieties*

The flavonoid levels in the 50 varieties of mulberry examined ranged from 12.86 ± 0.07 to 31.57 ± 0.31 mg/g dry weight (Table 1). 'Huasang' variety had the highest flavonoid concentration, and 'Hongguo 2' variety had the lowest. The average flavonoid concentration was 19.27 mg/g dry weight. Five samples had flavonoid contents of more than 24.5 mg/g, and 33 samples had flavonoid contents ranging from 15.5 to 23 mg/g dry weight. The flavonoid content of the richest sample was 2.45-fold higher than that of the poorest sample.

Previous investigation of flavonoid contents in 12 varieties of Korean mulberry leaves reported a range from 7.485 to 12.979 mg/g dry weight, with the highest content in 'Cheong Su' variety (12.979 ± 0.1120 mg/g dry weight) (Ju *et al.,* 2018). Zhang *et al.* (2019) measured the flavonoid contents in 24 mulberry varieties, and found that they ranged from 31.037 ± 4.202 to 59.841 ± 15.598 mg/g dry weight, much higher than the levels found in this study. Our study may provide more precise information regarding the flavonoid contents of mulberry varieties based on the higher number examined.

*Polysaccharide in 50 mulberry varieties*

In the 50 mulberry samples examined, the polysaccharide levels ranged from 7.55 ± 0.34 to 20.52 ± 0.24 g/100 g dry weight (Table 1). The average polysaccharide concentration was 11.93 g/100 g dry weight. 'Ganluo' variety had the highest polysaccharide concentration, and 'Huasang' variety had the lowest. Thirty-two samples had polysaccharide contents ranging from 9.0 to 13.5 g/100 g dry weight. The polysaccharide content of the richest sample was 2.72-fold higher than that of the poorest sample. Our data demonstrate that polysaccharide concentrations greatly varied among different mulberry varieties. Among the 50 mulberry samples examined, three samples had polysaccharide contents greater than 16.5 g/100 g dry weight, including the Hongxing5, Ganluo, and Yinghuachi varieties.

Zhang *et al.* (2018a) measured polysaccharide contents in mulberry varieties, and found that they ranged from 50.97 to 116.62 mg/g dry weight, which is much lower than the concentrations found in this study. The differences are probably due to differences in place of origin and harvest time; the mulberry varieties in the previous study were collected from the mulberry fields of the Guizhou Sericulture Science and Technology Research Institute (Guizhou, China).

*Comparison of bioactive ingredient concentrations in different mulberry species*

The average DNJ, GABA, tannin, flavonoid, and polysaccharide contents are presented in Table 2. The samples with the highest DNJ content were species *M. alba* Linn., the samples with the highest GABA, tannin, and flavonoid content were species *M. cathayana* Hemsl., and the samples with the highest polysaccharide content were species *M. alba* Linn. Comparison between Table 2 and previous studies on the level of DNJ varied among species, from 0.2089 to 1.472 mg/g dry weight for *M. multicaulis* Perr., 0.5532 to 1.222 mg/g dry weight for *M. atropurpurea* Roxb., 0.7217 mg/g dry weight for *M. mizuho* Hotta, 0.4992 mg/g dry weight for *M. bombycis* Koidz., and 0.7131 mg/g dry weight for *M. cathayana* Hemsl (Hu *et al.,* 2013). The result showed that the DNJ and polysaccharide content was not significantly different when compared across species ($p > 0.05$). GABA, tannin, and flavonoid contents across species was significantly different ($p < 0.05$), and five bioactive ingredients were significantly difference within a single species.

*Comparison of bioactive ingredient concentrations in mulberry leaves originating from different regions*

As shown in Table 3, we can see the average of DNJ, GABA, tannin, flavonoid, and polysaccharide contents in mulberry leaves originating from Japan, Sri Lanka, Uzbekistan, and 11 different Chinese provinces or cities. The samples with the highest DNJ content were from Uzbekistan, the samples with the highest GABA, tannin, and flavonoid content were from Anhui, and the samples with the highest polysaccharide content were from Sichuan. When compared with Table 4, previous studies yielded different levels of DNJ among regions; 0.4750 mg/g dry weight from Sichuan, 0.3937 to 1.089 mg/g dry weight from Japan, 0.3657 to 1.376 mg/g dry weight from Zhejiang, 0.0741 to 0.3417 mg/g dry weight from Jiangsu, 0.4317 to 1.460 mg/g dry weight from Anhui, 0.7602 mg/g dry weight from Hebei, 0.9107 mg/g dry weight from Yunnan, 0.5532 to 1.061 mg/g dry weight from Guangxi, 0.8452 to 1.222 mg/g dry weight from Guangdong, and 0.1341 to 1.472 mg/g dry weight from Hubei (Hu *et al.*, 2013).

The present work showed that there were significant differences in five bioactive ingredient contents among varieties originating from the same province, city, or nation. However, GABA, tannin, flavonoid, and polysaccharide contents in mulberry samples originating from different regions were not significantly different ($p > 0.05$). DNJ concentrations were significantly different among varieties originating from different regions ($p < 0.05$).

*Classification of 50 mulberry varieties and evaluation of their economic value*

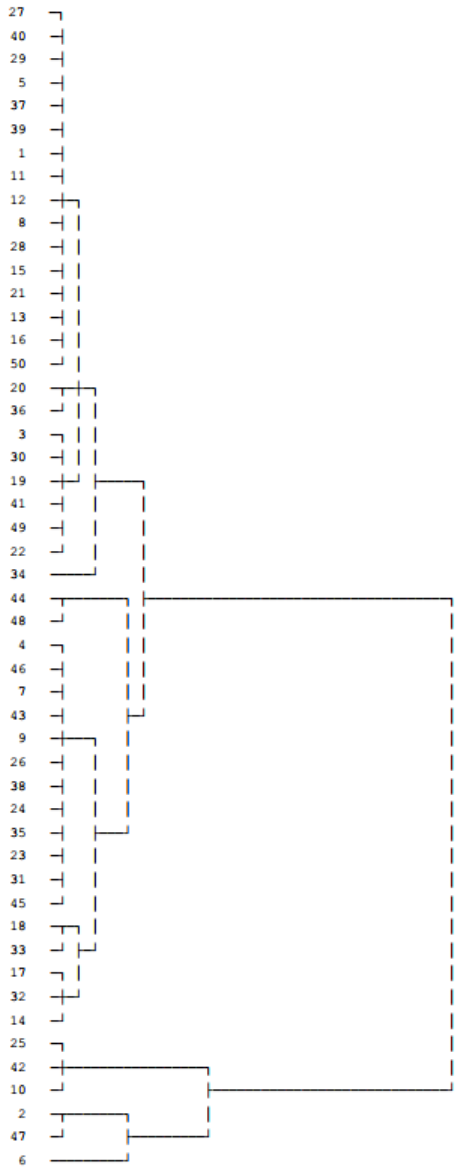


Figure 1. Cluster analysis dendrogram of mulberry leaf varieties based on bioactive ingredient content. Note: Varieties corresponding to the numbers are shown in Table 1.

Table 2. Comparison of bioactive ingredient concentrations and average concentrations in different mulberry species.

| ***Morus* spp.** | **Variety** | **Range of DNJ contents (mg/100 g dry weight)** | **Range of GABA contents (mg/100 g dry weight)** | **Range of tannin contents (g/100 g dry weight)** | **Range of flavonoid contents (mg/g dry weight)** | **Range of polysaccharide contents (g/100 g dry weight)** |
|---|---|---|---|---|---|---|
| *M. alba* Linn. | 25 | 60.12 ± 0.72 - 149.39 ± 3.49 | 122.99 ± 10.02 - 410.55 ± 9.72 | 0.45 ± 0.02 - 1.35 ± 0.03 | 13.12 ± 0.08 - 29.56 ± 0.57 | 7.96 ± 0.17 - 20.52 ± 0.24 |
| *M. multicaulis* Perr. | 16 | 32.51 ± 1.84 - 154.11 ± 5.66 | 122.88 ± 5.58 - 355.05 ± 15.03 | 0.62 ± 0.04 - 1.16 ± 0.03 | 13.74 ± 0.18 - 25.28 ± 1.17 | 8.24 ± 0.10 - 17.12 ± 0.35 |
| *M. bombycis* Koidz. | 4 | 71.45 ± 1.82 - 106.48 ± 6.01 | 176.13 ± 5.26 - 242.47 ± 15.98 | 0.72 ± 0.01 - 1.16 ± 0.03 | 12.86 ± 0.07 - 19.48 ± 0.90 | 10.06 ± 0.24 - 15.52 ± 0.87 |
| *M. atropurpurea* Roxb. | 3 | 78.09 ± 1.36 - 101.98 ± 4.78 | 148.06 ± 8.32 - 274.74 ± 5.77 | 0.65 ± 0.04 - 0.95 ± 0.02 | 16.20 ± 0.35 - 20.17 ± 0.26 | 10.23 ± 0.22 - 12.09 ± 0.52 |
| *M. cathayana* Hemsl. | 1 | 62.53 ± 1.92 | 427.12 ± 16.08 | 1.49 ± 0.03 | 31.57 ± 0.31 | 7.55 ± 0.34 |
| *M. mizuho* Hotta. | 1 | 139.75 ± 3.39 | 308.11 ± 26.51 | 0.77 ± 0.02 | 18.52 ± 0.26 | 9.54 ± 0.03 |
| *M. alba* Linn. | 25 | 86.18 | 206.76 | 0.87 | 20.10 | 12.38 |
| *M. multicaulis* Perr. | 16 | 84.24 | 205.58 | 0.81 | 18.36 | 11.82 |
| *M. bombycis* Koidz. | 4 | 83.53 | 223.01 | 0.95 | 17.12 | 12.84 |
| *M. atropurpurea* Roxb. | 3 | 85.18 | 196.23 | 0.83 | 18.30 | 11.15 |
| *M. cathayana* Hemsl. | 1 | 62.53 | 427.12 | 1.49 | 31.57 | 7.55 |
| *M. mizuho* Hotta. | 1 | 139.75 | 308.11 | 0.77 | 18.52 | 9.54 |

Table 3. Comparison of bioactive ingredient average concentrations in mulberry leaves originating from different regions.

| Origin | Variety | Average contents of DNJ (mg/100 g dry weight) | Average contents of GABA (mg/100 g dry weight) | Average contents of tannin (g/100 g dry weight) | Average contents of flavonoids (mg/g dry weight) | Average contents of polysaccharides (g/100 g dry weight) |
|---|---|---|---|---|---|---|
| Sichuan Prov., China | 16 | 86.82 | 198.36 | 0.90 | 20.59 | 12.62 |
| Japan | 7 | 75.69 | 224.64 | 0.86 | 19.95 | 13.15 |
| Chongqing city., China | 5 | 87.10 | 214.55 | 0.91 | 19.22 | 10.12 |
| Zhejiang Prov., China | 5 | 96.29 | 229.16 | 0.69 | 18.69 | 10.38 |
| Shanxi Prov., China | 3 | 72.11 | 203.12 | 0.80 | 15.16 | 11.00 |
| Jiangsu Prov., China | 3 | 79.01 | 190.80 | 0.90 | 15.77 | 11.26 |
| Anhui Prov., China | 2 | 55.22 | 295.38 | 1.22 | 25.08 | 12.34 |
| Hebei Prov., China | 2 | 100.65 | 199.09 | 0.90 | 18.62 | 12.98 |
| Yunnan Prov., China | 2 | 107.86 | 184.88 | 0.72 | 18.75 | 12.34 |
| Guangxi Prov., China | 1 | 75.47 | 165.90 | 0.65 | 18.54 | 10.23 |
| Guangdong Prov., China | 1 | 101.98 | 274.74 | 0.95 | 20.17 | 12.09 |
| Hubei Prov., China | 1 | 39.49 | 180.44 | 0.69 | 13.74 | 9.41 |
| Sri Lanka | 1 | 106.48 | 316.51 | 1.16 | 18.35 | 14.45 |
| Uzbekistan | 1 | 154.11 | 231.39 | 0.69 | 21.68 | 15.50 |

Table 4. Comparison of bioactive ingredient concentrations in mulberry leaves originating from different regions.

| Origin | Variety | Range of DNJ contents (mg/100 g dry weight) | Range of GABA contents (mg/100 g dry weight) | Range of tannin contents (g/100 g dry weight) | Range of flavonoid contents (mg/g dry weight) | Range of polysaccharide contents (g/100 g dry weight) |
|---|---|---|---|---|---|---|
| Sichuan Prov., China | 16 | 67.06 ± 1.84 - 149.39 ± 3.49 | 122.88 ± 5.58 - 410.55 ± 9.72 | 0.60 ± 0.04 - 1.35 ± 0.03 | 13.88 ± 0.54 - 29.56 ± 0.57 | 7.96 ± 0.17 - 20.52 ± 0.24 |
| Japan | 7 | 32.51 ± 1.84 - 112.55 ± 1.12 | 171.66 ± 11.42 - 355.05 ± 15.03 | 0.66 ± 0.01 - 1.02 ± 0.04 | 15.68 ± 0.36 - 26.70 ± 0.22 | 9.62 ± 0.17 - 15.69 ± 0.22 |
| Chongqing city., China | 5 | 70.01 ± 1.22 - 112.20 ± 3.44 | 136.95 ± 8.83 - 276.22 ± 17.83 | 0.68 ± 0.03 - 1.16 ± 0.03 | 15.53 ± 0.30 - 23.21 ± 0.47 | 8.61 ± 1.00 - 10.76 ± 0.99 |
| Zhejiang Prov., China | 5 | 68.29 ± 1.42 - 139.75 ± 3.39 | 162.22 ± 8.37 - 312.51 ± 14.67 | 0.62 ± 0.04 - 0.77 ± 0.02 | 16.20 ± 0.35 - 24.93 ± 0.23 | 8.24 ± 0.10 - 12.64 ± 0.23 |
| Shanxi Prov., China | 3 | 60.12 ± 0.72 - 79.00 ± 7.18 | 176.13 ± 5.26 - 256.62 ± 18.66 | 0.72 ± 0.01 - 0.89 ± 0.03 | 12.86 ± 0.07 - 17.78 ± 0.37 | 10.06 ± 0.24 - 11.64 ± 0.95 |
| Jiangsu Prov., China | 3 | 75.19 ± 1.11 - 83.75 ± 3.45 | 148.06 ± 8.32 - 214.50 ± 4.62 | 0.76 ± 0.01 - 1.05 ± 0.03 | 13.76 ± 0.08 - 17.35 ± 0.32 | 9.55 ± 0.47 - 13.12 ± 0.52 |
| Anhui Prov., China | 2 | 47.91 ± 0.73 - 62.53 ± 1.92 | 163.63 ± 11.18 - 427.12 ± 16.08 | 0.94 ± 0.02 - 1.49 ± 0.03 | 18.59 ± 0.25 - 31.57 ± 0.31 | 7.55 ± 0.34 - 17.12 ± 0.35 |
| Hebei Prov., China | 2 | 84.37 ± 1.27 - 116.93 ± 1.41 | 155.65 ± 5.53 - 242.52 ± 6.88 | 0.84 ± 0.01 - 0.96 ± 0.03 | 17.78 ± 0.29 - 19.45 ± 0.12 | 12.82 ± 0.12 - 13.14 ± 0.41 |
| Yunnan Prov., China | 2 | 81.70 ± 4.81 - 134.02 ± 2.36 | 162.56 ± 13.04 - 207.20 ± 6.73 | 0.45 ± 0.02 - 0.99 ± 0.04 | 13.12 ± 0.08 - 24.38 ± 0.53 | 9.61 ± 0.25 - 15.07 ± 0.29 |
| Guangxi Prov., China | 1 | 75.47 ± 1.87 | 165.90 ± 10.03 | 0.65 ± 0.04 | 18.54 ± 0.77 | 10.23 ± 0.22 |
| Guangdong Prov., China | 1 | 101.98 ± 4.78 | 274.74 ± 5.77 | 0.95 ± 0.02 | 20.17 ± 0.26 | 12.09 ± 0.52 |
| Hubei Prov., China | 1 | 39.49 ± 0.31 | 180.44 ± 7.89 | 0.69 ± 0.01 | 13.74 ± 0.18 | 9.41 ± 0.11 |
| Sri Lanka | 1 | 106.48 ± 6.01 | 316.51 ± 14.67 | 1.16 ± 0.03 | 18.35 ± 0.15 | 14.45 ± 0.65 |
| Uzbekistan | 1 | 154.11 ± 5.66 | 231.39 ± 11.34 | 0.69 ± 0.01 | 21.68 ± 0.19 | 15.50 ± 0.97 |

We performed hierarchical cluster analysis of mulberry leaf varieties based on the five bioactive ingredients (DNJ, GABA, tannin, flavonoid, and polysaccharide) measured in this study (Figure 1). The resulting dendrogram demonstrates that the mulberry varieties form three main clusters. One cluster had high GABA, tannin, and flavonoid concentrations. These varieties, including Huasang, Jiuwenlong, and Shigu 11-6, may have broad use in foods and therapeutics. Another cluster had high DNJ; mulberry leaves of these varieties were recommended to be processed as special teas, or raw materials for food products aimed at decreasing blood glucose. This cluster includes the Shenglidaye, Qinpifusang, and HuoSang varieties. The third cluster, composed of the remaining mulberry varieties, had lower levels of bioactive ingredients; thus, may be useful for silkworm rearing or as animal feed.

## Conclusions

The present work determined the DNJ, GABA, tannin, flavonoid, and polysaccharide levels in 50 different mulberry varieties representing six different species and originating from 14 different regions including Japan, Sri Lanka, Uzbekistan, and 11 Chinese provinces or cities. We found significant variation in the five bioactive ingredients in different varieties. The richest samples had 4.74 times more DNJ, 3.48 times more GABA, 3.31 times more tannin, 2.45 times more flavonoids, and 2.72 times more polysaccharides than the samples with the lowest concentrations. The five bioactive ingredients were significantly different among varieties originating from the same region. DNJ contents were significantly different in mulberry varieties originating from different regions, and GABA, tannin, and flavonoid contents were significantly different in mulberry varieties of different species. Huasang, Jiuwenlong, Shigu 11-6, Shenglidaye, Qinpifusang, and HuoSang varieties thus were identified as good sources for further utilisation.

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## References

Cai, D., Liu, M., Wei, X., Li, X., Wang, Q., Nomura, C. T. and Chen. S. 2017. Use of *Bacillus amyloliquefaciens* HZ-12 for high-level production of the blood glucose lowering compound, xxxx-deoxynojirimycin (DNJ), and nutraceutical enriched soybeans via fermentation. Applied Biochemistry and Biotechnology 181(3): 1108-1122.

Chang, L.-W., Juang, L.-J., Wang, B.-S., Wang, M.-Y., Tai, H.-M., Hung, W.-J., ... and Huang, M.-H. 2011. Antioxidant and antityrosinase activity of mulberry (*Morus alba* L.) twigs and root bark. Food and Chemical Toxicology 49(4): 785-790.

Do, H. J., Chung, J.-H., Hwang, J. W., Kim, O. Y., Lee, J.-Y. and Shin, M.-J. 2015. 1-deoxynojirimycin isolated from *Bacillus subtilis* improves hepatic lipid metabolism and mitochondrial function in high-fat-fed mice. Food and Chemical Toxicology 75: 1-7.

Hu, X.-Q., Jiang, L., Zhang, J.-G., Deng, W., Wang, H.-L. and Wei, Z.-J. 2013. Quantitative determination of 1-deoxynojirimycin in mulberry leaves from 132 varieties. Industrial Crops and Products 49: 782-784.

Inoue, K., Shirai, T., Ochiai, H., Kasao, M., Hayakawa, K., Kimura, M. and Sansawa, H. 2003. Blood-pressure-lowering effect of a novel fermented milk containing γ-aminobutyric acid (GABA) in mild hypertensives. European Journal of Clinical Nutrition 57(3): 490-495.

Jiang, Y.-G., Wang, C.-Y., Jin, C., Jia, J.-Q., Guo, X., Zhang, G.-Z. and Gui, Z.-Z. 2014. Improved 1-Deoxynojirimycin (DNJ) production in mulberry leaves fermented by microorganism. Brazilian Journal of Microbiology 45(2): 721-729.

Ju, W.-T., Kwon, O.-C., Kim, H.-B., Sung, G.-B., Kim, H.-W. and Kim, Y.-S. 2018. Qualitative and quantitative analysis of flavonoids from 12 species of Korean mulberry leaves. Journal of Food Science and Technology 55(5): 1789-1796.

Jung, S., Lee, M.-S., Choi, A.-J., Kim, C.-T. and Kim, Y. 2019. Anti-inflammatory effects of high hydrostatic pressure extract of mulberry (*Morus alba*) fruit on LPS-stimulated RAW264.7 cells. Molecules 24(7): article ID 1425.

Kim, J., Yun, E.-Y., Quan, F.-S., Park, S.-W. and Goo, T.-W. 2017. Central administration of 1-deoxynojirimycin attenuates hypothalamic endoplasmic reticulum stress and regulates food intake and body weight in mice with high-fat diet-induced obesity. Evidence-based Complementary and Alternative Medicine 2017: article ID 3607089.

Kim, J.-W., Kim, S.-U., Lee, H. S., Kim, I., Ahn, M. Y. and Ryu, K. S. 2003. Determination of 1-deoxynojirimycin in *Morus alba* L. leaves by

derivatization with 9-fluorenylmethyl chloroformate followed by reversed-phase high-performance liquid chromatography. Journal of Chromatography A 100(1-2): 93-99.

Kimura, T., Nakagawa, K., Kubota, H., Kojima, Y., Goto, Y., Yamagishi, K., ... and Miyazawa, T. 2007. Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. Journal of Agricultural and Food Chemistry 55(14): 5869-5874.

Li, E., Luo, X., Liao, S., Shen, W., Li, Q., Liu, F. and Zou, Y. 2018. Accumulation of gamma-aminobutyric acid during cold storage in mulberry leaves. International Journal of Food Science and Technology 53(12): 2664-2672.

Lin, S.-D., Mau, J.-L. and Hsu, C.-A. 2012. Bioactive components and antioxidant properties of gamma-aminobutyric acid (GABA) tea leaves. LWT - Food Science and Technology 46(1): 64-70.

Liu, L., Cao, J., Huang, J., Cai, Y. and Yao, J. 2010. Extraction of pectins with different degrees of esterification from mulberry branch bark. Bioresource Technology 101(9): 3268-3273.

Ma, Y., Lv, W., Gu, Y. and Yu, S. 2019. 1-deoxynojirimycin in mulberry (*Morus indica* L.) leaves ameliorates stable angina pectoris in patients with coronary heart disease by improving antioxidant and anti-inflammatory capacities. Frontiers in Pharmacology 10: article ID 569.

Oh, C.-H., and Oh, S.-H. 2004. Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. Journal of Medicinal Food 7(1): 19-23.

Oh, S.-H., Soh, J.-R. and Cha, Y.-S. 2003. Germinated brown rice extract shows a nutraceutical effect in the recovery of chronic alcohol-related symptoms. Journal of Medicinal Food 6(2): 115-121.

Shu, C., Zhao, H. D., Jiao, W. X., Fan, X. G. and Jiang, W. B. 2018. Research progress on the bioactivity of origin tannins. Science and Technology of Food Industry 17: 334-340.

Song, W., Wang, H.-J., Bucheli, P., Zhang, P.-F., Wei, D.-Z. and Lu, Y.-H. 2009. Phytochemical profiles of different mulberry (*Morus* sp.) species from China. Journal of Agricultural and Food Chemistry 57(19): 9133-9140.

Suwanmanon, K. and Hsieh, P.-C. 2014. Effect of γ-aminobutyric acid and nattokinase-enriched fermented beans on the blood pressure of spontaneously hypertensive and normotensive Wistar-Kyoto rats. Journal of Food and Drug Analysis 22(4): 485-491.

Vichasilp, C., Nakagawa, K., Sookwong, P., Higuchi, O., Luemunkong, S. and Miyazawa, T. 2012. Development of high 1-deoxynojirimycin (DNJ) content mulberry tea and use of response surface methodology to optimize tea-making conditions for highest DNJ extraction. LWT - Food Science and Technology 45(2): 226-232.

Vijayan, K., Saratchandra, B. and Teixeira da Silva, J. A. 2011. Germplasm conservation in mulberry (*Morus* spp.). Scientia Horticulturae 128(4): 371-379.

Wang, Y., Li, M., Yu, X., He, S., Wu, X. and Wang, Y. 2019. Mulberry leaf flavonoids protect against glucotoxicity-induced INS-1 cell apoptosis. Journal of Tradition Chinese Medicine 39(2): 153-159.

Wei, Z.-J., Zhou, L.-C., Chen, H. and Chen, G.-H. 2011. Optimization of the fermentation conditions for 1-deoxynojirimycin production by *Streptomyces lawendulae* applying the response surface methodology. International Journal of Food Engineering 7(3): article ID 16.

Wilson, M. F. 1984. Comparison of tannin levels in developing fruit buds of two orchard pear varieties using two techniques, Folin-Denis and protein precipitation assays. Journal of Chemical Ecology 10: 493-498.

Wu, S. 2019. Mulberry leaf polysaccharides suppress renal fibrosis. International Journal of Biological Macromolecules 124: 1090-1093.

Xia, Y. L., Xu, L. and Yang, C. F. 2009. Determination of gamma-aminobutyric acid in mulberry leaves. Chinese Agricultural Science Bulletin 25: 209-212.

Yamada, P., Ono, T., Shigemori, H., Han, J. and Isoda, H. 2012. Inhibitory effect of tannins from galls of *Carpinus tschonoskii* on the degranulation of RBL-2H3 cells. Cytotechnology 64(3): 349-356.

Yamatsu, A., Yamashita, Y., Maru, I., Yang, J., Tatsuzaki, J. and Kim, M. 2015. The improvement of sleep by oral intake of GABA and *Apocynum venetum* leaf extract. Journal of Nutritional Science and Vitaminology 61(2): 182-187.

Yang, X., Yang, L. and Zheng, H. 2010. Hypolipidemic and antioxidant effects of mulberry (*Morus alba* L.) fruit in hyperlipidaemia rats. Food and Chemical Toxicology 48(8-9): 2374-2379.

Ying, Z., Han, X. and Li, J. 2011. Ultrasound-assisted extraction of polysaccharides from mulberry leaves. Food Chemistry 127(3): 1237-1279.

Ying, Z., Li, J. and Han, X. 2008. Study on optimization of polysaccharide extraction from mulberry

leaves by response surface methodology. Journal of Chinese Institute of Food Science and Technology 4: 39-45.

Yoo, D. Y., Choi, J. H., Kim, W., Yoo, K.-Y., Lee, C. H., Yoon, Y. S., ... and Hwang, I. K. 2011. Effects of *Melissa officinalis* L. (lemon balm) extract on neurogenesis associated with serum corticosterone and GABA in the mouse dentate gyrus. Neurochemical Research 36: 250-257.

Yu, J., Cui, P.-J., Zeng, W.-L., Xie, X.-L., Liang, W.-J., Lin, G.-B. and Zeng, L. 2009. Protective effect of selenium-polysaccharides from the mycelia of *Coprinus comatus* on alloxan-induced oxidative stress in mice. Food Chemistry 117(1): 42-47.

Yu, Y., Li, H., Zhang, B., Wang, J., Shi, X., Huang, J., .... and Deng, Z. 2018. Nutritional and functional components of mulberry leaves from different varieties: evaluation of their potential as food materials. International Journal of Food Properties 21(1): 1495-1507.

Zhang, D.-Y., Wan, Y., Hao, J.-Y., Hu, R.-Z., Chen, C., Yao, X.-H., … and Li, L. 2018b. Evaluation of the alkaloid, polyphenols, and antioxidant contents of various mulberry cultivars from different planting areas in eastern China. Industrial Crops and Products 122: 298-307.

Zhang, F., Luo, Z.-F., Han, S.-Y. and Wang, X.-H. 2018a. Determination and comprehensive evaluation of three active substances in mulberry leaves of different varieties. North Sericulture 39: 8-11.

Zhang, H.-N., He, J.-H., Yuan, L. and Lin, Z.-B. 2003. *In vitro* and *in vivo* protective effect of *Ganoderma lucidum* polysaccharides on alloxan-induced pancreatic islets damage. Life Science 73(18): 2307-2319.

Zhang, L., Su, S., Zhu, Y., Guo, J., Guo, S., Qian, D., ... and Duan, J.-A. 2019. Mulberry leaf active components alleviate type 2 diabetes and its liver and kidney injury in db/db mice through insulin receptor and TGF-β/Smads signaling pathway. Biomedicine and Pharmacotherapy 112: article ID 108675.

Zhishen, J., Mengcheng, T. and Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64(4): 555-559.