Seasonal changes of metabolites in phloem sap from

*Broussonetia papyrifera*

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**Keywords**: metabolites; phloem sap; sugars; organic acid; laticifers

**Running title**: metabolites in phloem sap of *Broussonetia papyrifera*
Seasonal changes of metabolites in phloem sap from *Broussonetia papyrifera*

**Abstract** Gas chromatography-Mass spectrometry (GC-MS) were employed to analyze the whole metabolites in phloem sap of *Broussonetia papyrifera* and the seasonal changes of content of these metabolites were also investigated. Thirty-eight metabolites were detected in *BP* phloem exudates. The highest content (44.59 mg g$^{-1}$) of total metabolites was presented in March. High contents of organic acids and sugars were detected in *BP* phloem exudates from all growing months. Smaller amounts of fatty acids and alcohols were also detected in *BP* phloem exudates. Interestingly, some metabolites, such as PI3 kinase inhibitor, Chlorogenic acid, Chelerythrine and palmitic acid, which have properties of bioactivity to anticancer and anti-inflammation, were also detected. Quinic acid was the most abundant organic acid, representing up to 86.3% (average value) of all organic acids. D-fructose, D-glucose, and sucrose were the major soluble sugars in phloem saps and the maximum of sugars content was 19.76 mg g$^{-1}$ (average value) in November. Seasonal changes of contents of metabolites were different among individuals. The metabolites analysis double confirmed that the *BP* phloem sap can be serviced as an important resource for synthesis of pharmaceutical and human health products.

**Keywords:** metabolites; phloem sap; sugars; organic acid; laticifers; GC-MS

**Introduction**

Well-known as its fast growing, good adaptability, strong germinating ability and outstanding regeneration capability, *Broussonetia papyrifera* L. Vent. (*BP*) is widely planted in East Asia, the United States, and the Pacific Islands[1,2]. Similar to other tree species from Moraceae, *BP* has more abundant sap from its phloem tissue. Previous studies reported that some bioactive ingredients
were extracted from the BP phloem sap and they were proved to be beneficial to antioxidant[3], anti-inflammatory and anticancer[4-6] and shown inhibitory activity against aromatase[7], tyrosinase[8], α-Glucosidase[9], and anticholinesterase[10]. Consequently, the BP phloem sap is a promising natural resource for synthesis of new medicines and health products. Otherwise, BP phloem is a good choice for pulping and manufacturing of high quality paper[11,12]. To understand the chemical composition in phloem sap is beneficial to improvement of paper industrial.

Phloem sap is produced from the secondary metabolites of tree and relative to tree physiology. Fresh phloem sap commonly consists of polychemical compositions, which is produced from metabolic active of photosynthate. These chemical ingredients play key roles on tree physiology, such as resistance of pest infestation [13,14], maintenance of osmotic pressure between conductivity cells[15,16], and adjustment of the balance of inorganic ions[16]. Metabolic profiling is an effective technology for investigating the chemical composition in plant tissue[17-21]. However, limited studies focusing on the metabolites and their seasonal changes of BP phloem sap were found. As well-known, tree physiology is regulated by endogenous and exogenous factors. As the most important exogenous regulatory factors, the temperature, illumination and precipitation changes in different growing months. In addition, the phloem sap is a secretion from laticifer cells in tree phloem tissue, which is divided yearly from cambium[22]. Many studies have proved that the anatomy structure in phloem formation shows a seasonal dynamic change during growing years[23-29]. Therefore, it was hypothesized that there were seasonal changes on the chemical compositions and their concentration of the BP phloem sap. Based on the mentioned previously, the aim of this work was to investigate the chemical compositions in BP phloem sap by GC-MS and
intended to reveal the seasonal changes of these compounds in Nanjing city of China during the 2016 growing season.

**Experimental**

**Phloem sap collection**

Five healthy *Broussonetia papyrifera* trees were selected from campus of Nanjing Forestry University (NJFU) (32°04’57.4” N, 118°49’00.3” E), Nanjing, China. The average diameter at breast height was 17.6 cm and the average tree age was 11.5 years. Phloem saps were collected between 10 a.m. to 12 a.m. at Mar. 28, Apr. 22, May 20, Jun.15, Jul.22, Sep.20, Oct.21, and Nov.15. Sampling of phloem saps was simple and feasible because phloem saps are mainly stored in axial laticifer cells. The cell types in *BP* phloem is shown in Fig 1. *BP* phloem generally contained sieve tubes, laticifers, phloem rays, phloem fibers, and crystals. Even though in the region of nonconducting phloem featured with collapsed sieve tubes, latidifer cells still scatter intact[22,30,31]. Briefly, after peeling off the outset bark (approximately 2 cm²), the phloem was gashed by disinfected scalpel at a 45° angle to tree growth direction. The saps were natural flowed down into prepared tubes and frozen by liquid N₂ immediately and stored in -20°C for future use. In order to avoid the interaction on saps from each month, sampling position on tree truck was spiral and interval 10 cm between each other at all directions.

**Pre-treatment of the sap**

Phloem saps were extracted by the mixture solution of methanol, chloroform and deionized water according to the former reports[19,21]. The freeze saps were finely grinded under liquid N₂ condition and 50 ± 3mg sample was mixed with 1mL methanol (-20°C pre-chilled) in 1.5mL centrifuge tube. Then 50μl ribitol (CAS No.488-81-3, 2mg mL⁻¹ in ddH₂O), which was selected as
internal standard for compounds quantitative analysis, was added and the mixture was incubated for 20min at 70°C and 950rpm min⁻¹. Following by 15min centrifuge at 10000rpm min⁻¹, 500μl supernatant was transferred to a new tube and mixed with equal volume of chloroform (-20°C pre-chilled) and deionized water (4°C pre-chilled), and then incubated for 20min at 37°C at and 950rpm min⁻¹. After 15min centrifuging at 4000rpm min⁻¹, about 500μl supernatant was transferred to a new tube and stored at -20°C for further test. Ribitol and extraction reagents were purchased from Sigma-Aldrich (USA) and Nanjing chemical reagent Co., Ltd, China, respectively.

![Fig.1 Cross section and cells in BP phloem collected in October, 2016. C: cambium; X: xylem; L: laticifers; CP: conducting phloem; NP: nonconducting phloem; Cr: crystals; PR: phloem rays; PF: phloem fibers; St: sieve tubes. Bar is 200μm.](image)

**Derivatization and GC-MS**

The frozen pure phloem sap was dried under vacuum at -60°C and derivatized with N-methyl-N-trimethsilyl-trifluoroacetamide (MSTFA) (CAS No.24589-78-4). Briefly, the dried samples were mixed with 50μl Methoxyamine hydrochloride (CAS No.593-56-6, dissolved in pyridine, CAS No.110-86-1, 20mg ml⁻¹) and incubated 2 hours at 37°C, 950rpm min⁻¹. Then 100μl MSTFA (containing 20μl ml⁻¹ alkanes) was added and kept 30 min at 37°C, 950rpm min⁻¹ and
overnight at room temperature. All the derivatization reagents were purchased from Sigma-Aldrich (USA). 0.4μL derivatized samples were injected into GC-MS (TRACE DSQ, USA) fitted with a DB-5MS column (30m ×0.32 mm×0.25μm). The flow rate for the He (99.999%) carrier gas was 1.0mL min⁻¹. The detail parameters for GC test were as follows: the initial temperature was held at 50°C for 1min, and then increased to 300°C at a rate of 10°C min⁻¹, held for 5min. Both the injector and the detector temperatures were 250°C. EI ion and ionization voltage was pre-set at 70 eV and scan range was 20-500 amu.

**MS peak identification and data analysis**

The Gas chromatogram was observed using the MS Workstation version 6.9.3 and the online result was searched with the NIST (National Institute of Standard and Technology, USA) mass spectrum data, and referred to related literature for metabolites identification and classification[17,20,21]. The relative content of metabolites was calculated using the equation of $C_x=\frac{(A_x/A_i)\times0.05\times2\text{mg mL}^{-1}}{m_0 \text{ (mg g}^{-1})}$, where $C_x$ is the relative content of identified metabolite; $A_x$ is the peak area of identified metabolite; $A_i$ is the peak area of internal standard; $m_0$ is the dry weight of phloem tissue[18,21]. Excel 2016 was used to draw the charts.

**Results and discussion**

**Chemical composition in phloem sap of BP**

Metabolites of the exudates from the BP phloem were analyzed by GC-MS after being derivatized by MSTFA. Thirty-eight metabolites were identified and their concentration was calculated by the mass of each metabolite with drying weight of phloem tissue. The mean values of the total concentration of metabolites and the percentage composition of BP phloem sap are shown in Fig. 2. The Highest (44.59mg g⁻¹) concentration of total metabolites occurred in March and the
Total concentration decreased to the lowest value (6.78 mg g\(^{-1}\)) in May, then increased to 23.01 mg g\(^{-1}\) in June. In July, the total concentration of metabolites was 9.59 mg g\(^{-1}\), which was the secondary lowest during the whole growing season. The total concentration increased to 20.96 mg g\(^{-1}\) in September and decreased slightly in October. The Phloem sap collected in November had the secondary highest concentration of total metabolites.

As shown in Fig.2, all identified metabolites can be classified into organic acids, sugars, fatty acids and alcohols. In all growing months, organic acids and sugars represented on the average of 86.6% of the overall metabolite concentration of the \textit{BP} phloem sap. The Previous work stated that a wide range of sugars and organic acids can be detect by the MSTFA derivatized method [32]. Fatty acids and alcohols were abundant compositions of the \textit{BP} phloem sap exudates, both of them representing 1%-7% of the total metabolites during all growing months. Other metabolites such as...
PI3-Kinase Inhibitor, silanamine, were also identified, although they represented less than 1% of the total. The changes of percentages of different metabolites depended on the growing month. The highest percentage of organic acid presented in May (66% of the total metabolites) and lowest percentage existed in November (22% of the total metabolites). On the contrary, the lowest percentage of sugars presented in May (17% of the total metabolites) and the highest percentage existed in November (72% of the total metabolites). Both the highest percentage of fatty acids and alcohols occurred in September (7% of the total metabolites).

Seasonal changes of all metabolites

To estimate the seasonal variation of all metabolites in BP phloem sap, five trees were selected for sampling monthly. Qualitative and quantitative analysis of all identified metabolites performed on GC-MS. The chemical composition and their content changes during whole growing season 2016 are shown in Table 1 and Table 2. As mentioned previously, metabolites were discussed as two groups, i.e., organic acids and sugars, as well as others.

Organic acids

As shown in Table 1, sixteen organic acids were detected in the BP phloem sap and their compositions and contents changed depending on growing month. In all months, the quininic acid was the most abundant organic acid, representing up to 86.3% (average value) of all organic acids. It was showed the highest content (19.643 mg g⁻¹) of quininic acid at March (Table 1). But the highest percentage of quininic acid presented in November (Fig. 3). The seasonal changes of content of quininic acid were similar to that in total concentration of all metabolites. Quininic acid, which was used as an astringent and starting material for the synthesis of new pharmaceuticals, was also purified from *Eucalyptus globulus* [33], cinchona bark, and other plant products. Galactaric
acid, a precursor for synthesis of adipic acid[34], was the secondary abundant organic acid in the
BP phloem sap and its average percentage was 3.6% of all organic acid contents. Both the highest
content (0.737mg g\(^{-1}\)) and percentage (7.1%) of galactaric acid presented in June. But it was not
detected in September. Citric acid was another abundant organic acid in the BP phloem sap and its
highest content and percentage were 0.575 mg g\(^{-1}\) and 5.6%, respectively, in June. However, citric
acid was not detected in July, October and November (Table 1). Citric acid, an important industrial
organic acid, is widely used as an acidifier, flavoring and chelating agent, and is manufactured more
than million tons every year[35]. On the other hand, the seasonal difference of the citric acid
content is because it is an intermediate in the tricarboxylic acid cycle (TCA)[36], which is most
related to the tree physiology in different growing months. Lactic acid was detected in all growing
months and its highest content was 0.559mg g\(^{-1}\) in March, but the percentage of lactic acid in all
organic acids was the highest in October. Lactic acid is mainly employed in food additives,
pharmaceutical and cosmetics. Some other organic acids, such as oxalic acid, glycolic acid,
propanedioic acid and so forth, were detected, but all of their percentages were less than 2% of total
organic acids and some of them only existed in specific growing months (Table 1).

![Fig.3 Percentage composition of organic acids in the BP phloem saps and their season changes.](image-url)
Table 1. Organic acids detected in BP phloem sap and the season changes of their contents (n=5). Unit: mg g\(^{-1}\)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid</td>
<td>0.559±0.062</td>
<td>0.128±0.016</td>
<td>0.068±0.008</td>
<td>0.052±0.005</td>
<td>0.031±0.007</td>
<td>0.230±0.039</td>
<td>0.447±0.053</td>
<td>0.078±0.012</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>0.157±0.032</td>
<td>---</td>
<td>0.049±0.006</td>
<td>---</td>
<td>0.025±0.002</td>
<td>0.120±0.039</td>
<td>0.079±0.018</td>
<td>0.049±0.013</td>
</tr>
<tr>
<td>Glycolic acid</td>
<td>0.144±0.029</td>
<td>0.054±0.011</td>
<td>0.041±0.004</td>
<td>0.057±0.011</td>
<td>---</td>
<td>0.016±0.001</td>
<td>0.034±0.010</td>
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</tr>
<tr>
<td>Propanedioic acid</td>
<td>0.079±0.018</td>
<td>0.078±0.025</td>
<td>0.050±0.003</td>
<td>0.191±0.042</td>
<td>---</td>
<td>0.024±0.003</td>
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</tr>
<tr>
<td>1-Hydroxyisovaleric acid</td>
<td>0.392±0.063</td>
<td>0.159±0.047</td>
<td>0.095±0.009</td>
<td>0.130±0.033</td>
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</tr>
<tr>
<td>Butanedioic acid</td>
<td>0.192±0.025</td>
<td>---</td>
<td>0.126±0.023</td>
<td>0.052±0.007</td>
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</tr>
<tr>
<td>Benzoic acid</td>
<td>---</td>
<td>---</td>
<td>0.018±0.000</td>
<td>---</td>
<td>0.030±0.002</td>
<td>0.037±0.009</td>
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</tr>
<tr>
<td>2-Butenedioic acid</td>
<td>---</td>
<td>---</td>
<td>0.215±0.048</td>
<td>0.085±0.016</td>
<td>---</td>
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</tr>
<tr>
<td>Malic acid</td>
<td>0.486±0.071</td>
<td>0.070±0.002</td>
<td>0.072±0.012</td>
<td>0.105±0.027</td>
<td>0.019±0.000</td>
<td>---</td>
<td>0.041±0.016</td>
<td>---</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.235±0.039</td>
<td>0.353±0.039</td>
<td>0.121±0.036</td>
<td>0.575±0.055</td>
<td>---</td>
<td>0.110±0.035</td>
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<td>---</td>
</tr>
<tr>
<td>Ribonic acid</td>
<td>---</td>
<td>0.190±0.022</td>
<td>0.048±0.009</td>
<td>0.239±0.038</td>
<td>0.050±0.004</td>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td>Shikimic acid</td>
<td>0.156±0.018</td>
<td>---</td>
<td>0.217±0.059</td>
<td>---</td>
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</tr>
<tr>
<td>Quinic acid</td>
<td>19.634±2.336</td>
<td>6.915±0.319</td>
<td>3.106±0.116</td>
<td>7.575±0.407</td>
<td>3.148±0.085</td>
<td>7.576±0.526</td>
<td>10.842±1.338</td>
<td>5.662±0.527</td>
</tr>
<tr>
<td>L-Threonic acid</td>
<td>0.094±0.015</td>
<td>0.069±0.025</td>
<td>0.038±0.004</td>
<td>0.219±0.036</td>
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<td>---</td>
</tr>
<tr>
<td>5-O-Feruloylquinic acid</td>
<td>0.317±0.068</td>
<td>---</td>
<td>0.117±0.020</td>
<td>0.447±0.052</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.035±0.005</td>
</tr>
<tr>
<td>Galactaric acid</td>
<td>0.534±0.092</td>
<td>0.398±0.051</td>
<td>0.095±0.018</td>
<td>0.737±0.049</td>
<td>0.153±0.028</td>
<td>---</td>
<td>0.206±0.045</td>
<td>0.152±0.033</td>
</tr>
<tr>
<td>Total (Mean value)</td>
<td>22.980</td>
<td>8.416</td>
<td>4.478</td>
<td>10.467</td>
<td>3.426</td>
<td>8.107</td>
<td>11.687</td>
<td>5.977</td>
</tr>
</tbody>
</table>

All values were calculated by dividing the concentration of each metabolites by the dry weight of phloem tissue. Values are given as mean ±SD (n=5). Not detected is showed as “---”.

**Sugars and other metabolites**

Natural sugars are the fundamental metabolites from photosynthesis in tree growth and provide energy and precursor for tree physiology and biosynthesis of other organic compounds. Sucrose, D-fructose, D-glucose, D-psicose, and D-lactulose were detected in the BP phloem sap. The highest content of total sugars was 19.761mg g\(^{-1}\) (average value) in November and the lowest was only 1.123mg g\(^{-1}\) (average value) in May (Table 2). The content and percentage of individual sugar was depending on different growing months. D-fructose was the most abundant sugar, representing almost over 40% of the soluble sugars in March, July, October, and November (Fig. 4). On the other hand, sucrose was the most abundant sugar in other four growing months. D-fructose content showed the maximum (7.614mg g\(^{-1}\)) and minimum (0.134mg g\(^{-1}\)) (average value) in November and
May, respectively. Similarly, the highest content of D-glucose presented in November and the lowest value in May. Phloem sap collected from September contained the highest content of sucrose (7.344 mg g\(^{-1}\), average value) and nearly occurring to 80% of all sugars in this month (Table 2 and Fig.4). According to previous studies, sucrose can be converted into glucose and fructose by sucrose synthase[37]. In addition, more abundant of sugars in phloem is the reason for phloem sap-sucking insects[13,14].

Two fatty acids, palmitic acid and stearic acid, were detected in the \textit{BP} phloem sap in all growing months (Table 1). Moreover, it was shown the same seasonal change patterns. The highest content of both of them presented in March. Pascual et al. (2017) reported that the palmitic acid strongly boosted the metastatic potential of CD36+ in mouse models of human oral cancer cells[38]. Stearic acid is chemical industrial resource and wildly used in the production of cosmetics and lubricants. In addition, glycerol monostearate, 1-monopalmitin, and octadecenoic acid were detected in the \textit{BP} phloem sap from partly growing months. Glycerol monostearate, a food additive and control release agent in pharmaceuticals, was also detected in the \textit{BP} phloem sap, especially in March (0.229 mg g\(^{-1}\)) and June (0.151 mg g\(^{-1}\)) (average value).

![Fig.4 Percentage composition of sugars in the \textit{BP} phloem saps and their season changes.](image-url)
Table 2. Sugars and other metabolites detected in $BP$ phloem sap and their season changes (n=5). Unit: mg g$^{-1}$.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D-Fructose</strong></td>
<td>6.52±0.368</td>
<td>0.464±0.076</td>
<td>0.134±0.062</td>
<td>1.289±0.094</td>
<td>2.256±0.117</td>
<td>0.491±0.094</td>
<td>2.198±0.118</td>
<td>7.614±1.039</td>
</tr>
<tr>
<td><strong>D-Glucose</strong></td>
<td>4.002±0.207</td>
<td>0.653±0.085</td>
<td>0.139±0.055</td>
<td>1.354±0.083</td>
<td>1.915±0.105</td>
<td>0.599±0.069</td>
<td>1.392±0.301</td>
<td>7.147±1.146</td>
</tr>
<tr>
<td><strong>Sucrose</strong></td>
<td>0.997±0.093</td>
<td>1.166±0.117</td>
<td>0.648±0.073</td>
<td>3.533±0.136</td>
<td>0.932±0.075</td>
<td>7.344±1.225</td>
<td>1.191±0.057</td>
<td>4.406±0.317</td>
</tr>
<tr>
<td><strong>D-Psicose</strong></td>
<td>---</td>
<td>0.231±0.045</td>
<td>---</td>
<td>2.161±0.226</td>
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</tr>
<tr>
<td><strong>D-Lactulose</strong></td>
<td>---</td>
<td>0.448±0.063</td>
<td>0.210±0.054</td>
<td>1.512±0.110</td>
<td>---</td>
<td>1.011±0.095</td>
<td>0.499±0.082</td>
<td>0.593±0.062</td>
</tr>
<tr>
<td><strong>Total Sugars</strong> (mean value)</td>
<td>11.754</td>
<td>2.962</td>
<td>1.123</td>
<td>9.851</td>
<td>5.103</td>
<td>9.445</td>
<td>5.282</td>
<td>19.761</td>
</tr>
<tr>
<td><strong>Palmitic acid</strong></td>
<td>1.32±0.115</td>
<td>0.262±0.059</td>
<td>0.164±0.038</td>
<td>0.195±0.042</td>
<td>0.159±0.038</td>
<td>0.338±0.073</td>
<td>0.087±0.022</td>
<td>0.194±0.056</td>
</tr>
<tr>
<td><strong>Stearic acid</strong></td>
<td>0.829±0.064</td>
<td>0.154±0.044</td>
<td>0.079±0.021</td>
<td>0.116±0.019</td>
<td>0.061±0.026</td>
<td>0.083±0.020</td>
<td>0.031±0.006</td>
<td>0.069±0.014</td>
</tr>
<tr>
<td><strong>1-Monopalmitin</strong></td>
<td>0.29±0.037</td>
<td>0.124±0.038</td>
<td>0.033±0.006</td>
<td>0.112±0.031</td>
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</tr>
<tr>
<td><strong>Octadecenoic acid</strong></td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.283±0.047</td>
<td>0.960±0.091</td>
<td>---</td>
<td>---</td>
<td>0.455±0.072</td>
</tr>
<tr>
<td><strong>Glycerol monostearate</strong></td>
<td>0.22±0.055</td>
<td>0.095±0.012</td>
<td>0.032±0.000</td>
<td>0.151±0.028</td>
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</tr>
<tr>
<td><strong>D-Pinitol</strong></td>
<td>0.12±0.021</td>
<td>---</td>
<td>---</td>
<td>0.132±0.021</td>
<td>---</td>
<td>0.049±0.022</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Myo-Inositol</strong></td>
<td>0.31±0.048</td>
<td>0.128±0.047</td>
<td>0.045±0.008</td>
<td>0.519±0.099</td>
<td>0.148±0.039</td>
<td>0.261±0.034</td>
<td>0.034±0.016</td>
<td>0.169±0.048</td>
</tr>
<tr>
<td><strong>PI3-Kinase Inhibitor</strong></td>
<td>0.12±0.036</td>
<td>0.059±0.005</td>
<td>0.040±0.002</td>
<td>0.069±0.017</td>
<td>---</td>
<td>0.115±0.056</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Chlorogenic acid</strong></td>
<td>3.427±0.229</td>
<td>0.111±0.031</td>
<td>---</td>
<td>0.390±0.059</td>
<td>---</td>
<td>---</td>
<td>0.058±0.013</td>
<td>---</td>
</tr>
<tr>
<td><strong>Chelerythrine</strong></td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.142±0.044</td>
<td>---</td>
<td>---</td>
<td>0.032±0.002</td>
<td>---</td>
</tr>
<tr>
<td><strong>Pentasiloxane</strong></td>
<td>0.08±0.021</td>
<td>---</td>
<td>0.030±0.001</td>
<td>0.057±0.014</td>
<td>0.014±0.000</td>
<td>0.062±0.027</td>
<td>0.024±0.009</td>
<td>0.019±0.000</td>
</tr>
<tr>
<td><strong>Silanol, trimethyl-, phosphate (3:1)</strong></td>
<td>2.73±0.205</td>
<td>1.248±0.115</td>
<td>0.622±0.072</td>
<td>0.742±0.092</td>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td><strong>Silanol, trimethyl-</strong></td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Glycerol</strong></td>
<td>0.12±0.062</td>
<td>---</td>
<td>0.026±0.004</td>
<td>0.034±0.008</td>
<td>0.039±0.003</td>
<td>0.094±0.033</td>
<td>0.081±0.025</td>
<td>0.046±0.007</td>
</tr>
<tr>
<td><strong>3-Acetamidocoumarin</strong></td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.043±0.016</td>
<td>0.064±0.011</td>
<td>---</td>
</tr>
<tr>
<td><strong>1-Hexanol</strong></td>
<td>0.09±0.037</td>
<td>---</td>
<td>0.019±0.001</td>
<td>---</td>
<td>0.033±0.001</td>
<td>---</td>
<td>0.067±0.021</td>
<td>0.048±0.005</td>
</tr>
<tr>
<td><strong>Linalool</strong></td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.183±0.029</td>
<td>1.175±0.085</td>
<td>0.050±0.006</td>
<td>0.411±0.063</td>
<td>---</td>
</tr>
<tr>
<td><strong>Glycelral</strong></td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.275±0.062</td>
<td>0.456±0.069</td>
</tr>
</tbody>
</table>

All values were calculated by dividing the concentration of each metabolites by the dry weight of phloem tissue. Values are given as mean ±SD (n=5). Not detected is showed as “---”.

Alcohols also are an important substrate and intermediate products in cycle of metabolism. Myo-inositol, an important intermediate in plants, was proved to have positive physiological effects on humans[39,40]. It was shown the highest content (0.519mg g$^{-1}$, average value) of myo-inositol in June and there was distinct various in different months. Low content of D-pinitol was detected in March, June, and September. Many studies reported that the D-pinitol was a bioactive ingredient in biological and medical activities[41,42].
Interestingly, PI3 kinase inhibitor, a type of medical drug for anticancer and anti-inflammation[43-46], was detected in the BP phloem sap. Compared to other months, the higher contents of PI3K inhibitor were 0.121mg g\(^{-1}\) and 0.115mg g\(^{-1}\) (average values) in March and September, respectively. Chlorogenic acid, another potential medical drug in anti-inflammatory and reduction in blood pressure[47,48], was also detected in the BP phloem sap and its maximum was 3.427mg g\(^{-1}\) in March (Table 2). Linalool content showed the highest value of 0.411mg g\(^{-1}\) in November, which was proved to be an antifungal activity substance[49]. Chelerythrine, a benzophenanthridine alkaloids with potent anti-inflammatory effects in vivo[50], was 0.142mg g\(^{-1}\) in July.

**Conclusions**

Chemical composition of the exudates from BP phloem were analyzed by GC-MS after being derivatized using MSTFA. Thirty-eight metabolites were detected, and the total concentration of these metabolites were different depending on growing month. The highest total metabolites concentration was 44.59mg g\(^{-1}\) in March and the lowest one was 6.78mg g\(^{-1}\) in May. Organic acids and sugars were the most abundant metabolites in the BP phloem sap from whole growing months. Small amounts of fatty acids and alcohols were also detected in BP phloem exudates. Seasonal changes of the content of metabolites were different among individuals. The highest content of organic acids was 22.98mg g\(^{-1}\) (average value) in March and quinic acid was the most abundant organic acid, representing up to 86.3% (average value) of all organic acids. In November, it was shown the maximum of sugars content of 19.76 mg g\(^{-1}\) (average value) and D-fructose, D-glucose, and sucrose were the major soluble sugars. Interestingly, some metabolites, such as PI3 kinase inhibitor, Chlorogenic acid, Chelerythrine, and palmitic acid, which have bioactivity to anticancer
and anti-inflammation, were also detected and showed different seasonal variations. The metabolite analysis double confirmed that the BP phloem sap can be serviced as an important resource for synthesis of pharmaceutical and human health products.

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Author’s contribution

Conceived and designed the experiments: JT Shi, JY Luo.

Perform the experiments: HC Liu, JT Shi.

Analyzed the data: JT Shi, HC Liu, JY Luo, LP Cai.

Wrote the manuscript and approved the final version: JT Shi, HC Liu, JY Luo, LP Cai.

Conflict of interest

The authors declare that they have no conflict of interest.

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