INTRODUCTION

Pecan [Carya illinoinensis (Wangenh.) K. Koch] is an important woody nut crop and had raised great attentions these years for its high phenolic contents (Zhang, Peng, & Li, 2015). It is reported that tree nuts are among the best sources of natural antioxidants and the content of antioxidants in pecan nut ranked as the highest of tree nuts (Wu et al., 2004). Phenolics in pecan kernel were reported to...
have excellent antioxidant capacities (Biomhoff, Carlsen, Anderson, & Jacobs, 2006; de la Rosa et al., 2014; Prado et al., 2014; Flores-Cordova et al., 2017; Hilbig, Alves, et al., 2018; Jia et al., 2018; Robbins, Gong, Wells, Greenspan, & Pegg, 2015; de la Rosa, Alvarez-Parrilla, & Shahidi, 2011; Villarreal-Lozoya, Lombardini, & Cisneros-Zevallos, 2007), daily consuming of pecan nut is good to chronic diseases like inflammatory (Robbins, Greenspan, & Pegg, 2016), hyperlipidemia (Dominguez-Avila et al., 2015), and can protect human brain, blood, and liver from the damage of oxidant (Dominguez-Avila et al., 2015; Müller et al., 2013; Reckziegel et al., 2011). Phenolic metabolites in other organs of pecan trees also play critical roles in the defense of pathogens, injuries, and environment stresses. Such as metabolites in pecan leaves might play important roles in the resistant of scab (Lei et al., 2018).

The most important part of pecan is by no means the edible part of the fruit—the kernel. Pecan fruit consists of pericarp and the seed (nut). The seed includes the inner flesh kernel and the brown pellicle wrapped around the flesh which was called testa. The epicarp and mesocarp were undivided in pecan and together they formed the shuck, while the shell is the endocarp. Besides kernel, other organs of pecan trees also have different values and can be used. Pecan shell is the by-product of food industry. Pecan shells aqueous extract can protect mice from oxidative damage induced by cigarette smoke exposure and reduced the locomotor activity and anxiety symptoms induced by smoking withdrawal (Reckziegel et al., 2011), it also has hepatoprotective activity against ethanol-induced liver damage (Müller et al., 2013). The oxidative properties of margarines supplemented with pecan nutshell extracts, rosemary extract, and butylated hydroxytoluene (BHT) were investigated, while pecan nutshell extracts had equally effects with the other two antioxidants and may be considered as a natural product replacement for the synthetic antioxidant BHT (Engler Ribeiro, de Britto Policarpi, Dal Bo, Barbetta, & Block, 2017). The anti proliferative and anti-tumor activity of pecan shells and their relationship with phenolics were also investigated (de la Rosa et al., 2014; Hilbig, Policarpi, et al., 2018). Chopped pecan shells were used for making tea in Brazil and were thought to have the diuretic and digestive effects (Prado et al., 2014; Engler Ribeiro et al., 2017). Flavonols in pecan bark and leaf were reported to have antidiabetic and hepatoprotective actives (Abdallah, Salama, Abd-Elrahman, & El-Maraghy, 2011; Gad, Ayoub, & Al Azizi, 2007). Many pecan organs besides kernel have the potential of being used in food or health food industry. The bioactives of phenolics in different pecan organs had attracted interests and had been investigated, but the composition and the distribution of these phenolic constituents in most pecan organs were still unclear.

Similar to walnut, pecan also belongs to waste-heavy materials for that about 70% of the fruit weights are shells and shucks (Han et al., 2018). Harvesting pecan nuts produced a lot of shucks, while cracking pecan nuts produced huge amount of shells. Pecan trees tend to produce excessive male inflorescence, which easily led to the over consume of tree nutrition. Remove over-bearing male inflorescence can protect the tree and will produce by-products. Pruning and grafting will also produce by-products such as leaves, branches, and sometimes barks. Active phenolics were contained in these by-products, but their compositions were not well understood. A comprehensive metabolic investigation of phenolics in various organs of pecan is needed for better utilization of each part. On the other side, the distribution of secondary metabolites in plants is organ-specific. They usually share the same upstream metabolic pathway. And then, due to the differences in enzymes activity or type of each organ, these metabolites tend to have different synthetic and catabolic rates or have different chemical modification reactions such as methylation or glycosylation, which led to diverse structures to play diverse functions. So, a comprehensive metabolic investigation of phenolics in different pecan organs is also needed for better understanding of the organ-specific distribution of these active metabolites in pecan.

By far, the phenolics in pecan were mostly studied in kernels (Gong & Pegg, 2017; Jia et al., 2018; Robbins et al., 2015; Robbins, Ma, Wells, Greenspan, & Pegg, 2014; de la Rosa et al., 2011), while studies in shell (Hilbig, Alves, et al., 2018), leaf (Gad et al., 2007; Ishak, Ahmed, Abd-Alia, & Saleh, 1980; Lei et al., 2018), and bark (Abdallah et al., 2011) were much less. There is no report of the rest of the organs. The ultra-high-performance liquid chromatography coupled with hybrid linear ion trap and Orbitrap mass spectrometer (UHPLC-LTQ-Orbitrap MS^n) is the newest mass technic which has many advantages such as high sensitive and accuracies, while using ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometer (UHPLC-QQQ-MS^n) can get a better result of quantity. In this paper, we used these methods of both rapid identification and accuracy quantification to get a comprehensive survey of the distribution of phenolic metabolites in various pecan organs.

## 2 | MATERIALS AND METHODS

### 2.1 Materials and chemicals

Eight different organs of pecan, including kernel (without testa), testa, shell, shuck, leaf, branch, bark, and flower (male inflorescence), were collected in October 2018 (except the flower were in May 2019) at the scientific orchards of the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences. Samples were collected from healthy adult trees of cultivar "Pawnee." Six biological replicates were prepared for each organ, and each biological replicate contained samples from three trees. After transported back to the laboratory, the testa was peeled manually from the surface of kernels and was stored at 4°C, so as samples of other parts. Before use, all samples were powdered and homogenized in liquid nitrogen with mortars and pestles.

Menthol, acetic acid, n-hexane, acetonitrile (HPLC grade), and formic acid were purchased from ANPEL Laboratory Technologies. Standard reference compounds, including (+)-catechin, ellagic acid, and quercetin, were purchased from Nanjing Spring & Autumn Biological CO., Ltd.
2.2 | Sample extraction

Samples were extracted according to the methods of Regueiro et al. (2014) and Robbins et al. (2014) with slight modifications. The whole experiment was carried out in a dark room illuminated by a red light. Pecan samples (500 mg) and 1 ml mixed solution of methanol/water/acetic acid (70/29.5/0.5, v/v/v) were placed together in a 10-ml centrifuge tube, ultrasonic extracted for 5 min on ice bath, and centrifuged at 8,000 g for 10 min at 4°C (Hettich, Andreas Hettich GmbH & Co. KG). The supernatants were transferred into clean centrifuge tubes. Then, the mixed solutions were added again into the tubes with the kernel residues and ultrasonic extracted again with the same method. The supernatants were combined, and 1 ml n-hexane was added, vortexed for 1 min, centrifuged at 8,000 g for 5 min at 4°C for the purpose of defat. This defat process was also repeated again. The methanol layers were collected, combined and stored at -20°C until further analysis.

2.3 | UHPLC-LTQ-Orbitrap MS^n qualification

The phenolic metabolites were separated through an ACQUITY UPLC HSS T3 column (2.1 mm x 100 mm, 1.8 µm, Waters) on the Dionex Ultimate 3000 UHPLC system (Thermo Scientific) at 35°C. The mobile phases were 0.1% (v/v) formic acid (A) and acetonitrile (B), conducted as optimized procedure: 0–1 min, 95% A; 1–5 min, 95%–83% A; 5–12 min, 83%–70% A; 12–15 min, 70%–58% A. The flow rate was 0.4 ml/min, and the injection volume was 2 µl. The qualification was performed on the connected LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific) equipped with an electrospray ionization source (ESI) source. The parameters were set as follows: capillary voltage, 3 kV; source temperature, 120°C; desolvation temperature, 350°C; cone voltage, 50 V; cone gas flow, 50 L/hr; desolvation gas flow, 600 L/hr; Rutin was used as the lock mass. The mass range was m/z 100–2,000.

2.4 | UHPLC-QQQ-MS^n quantification

The quantification of phenolic metabolites was performed on the Waters Acquity UPLC system (Waters, Corp.) using the same column and chromatographic conditions as used in previous qualification. Phenolic metabolites were quantified on the AB SCIEX Triple Quad 6500 plus (AB SCIEX Corp.) equipped with an ESI source. The MS was conducted under multi reaction monitoring (MRM) mode, and the parameters were set as follows: capillary voltage, 3.5 kV; source temperature, 150°C; desolvation temperature, 400°C; cone voltage, 50 V; cone gas flow, 50 L/hr; desolvation gas flow, 1,000 L/hr; collision gas flow, 0.15 ml/min. The declustering potential and collision energy were set to match the MRM of each marker. The dwell time was automatically set by the MultiQuant software. The raw data were processed with MultiQuant 3.0.2 software.

2.5 | Total phenolic content

The total phenolic content was measured according to the methods of our previous report (Jia et al., 2018). Briefly, methanol extract (10 µl) was mixed with 2 ml of 7.5% (w/v) sodium carbonate and 2.5 ml of 10% (v/v) Folin–Ciocalteu reagent, and put in water bath at 50°C for 15 min in the dark. The reaction solutions were cooled to room temperature, and then, the absorbance was measured at 760 nm using UV spectrophotometer (Shimadzu UV-2100, Shimadzu Corporation). Ellagic acid was used as standard reference, and the results were expressed as milligrams of ellagic acid equivalents (EAE) per gram of defatted kernel weight (mg EAE g⁻¹). The bland solution was made by pure methanol and treated along with samples under the same protocol. All samples were measured in triplicates on three biological replicates.

2.6 | Antioxidant capacities

2.6.1 | 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The antioxidant capacities were firstly measured by the DPPH free radical scavenging assay according to the methods of previous report (Jia et al., 2018). Briefly, 4 ml of DPPH radical solution (39.43 mg DPPH in 1 L methanol) was mixed with 10 µl of methanol extract and kept in dark for 30 min. Then, absorbance was measured at 515 nm with UV spectrophotometer. The bland solution was made by pure methanol and treated along with samples under the same protocol. All samples were measured in triplicates on three biological replicates. Absorbance of blank was subtracted from each sample. Trolox was used as standard reference, and the results were expressed as μmol trolox equivalents (TE) per gram of defatted kernel weight (μmol TE g⁻¹).

2.6.2 | 2, 2’-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammomium salt (ABTS) free radical scavenging assay

Then, the antioxidant capacities were measured by the ABTS free radical scavenging assay according to previous reports of Salvador, Podestá, Block, and Ferreira (2016) and Prado et al. (2013). Briefly, 38.36 mg of ABTS was dissolved in 10 ml deionized water to get the ABTS⁺ solution (7.0 mM). Then, the ABTS⁺ solution was mixed with 2.45 mM potassium persulphate solution on the ratio of 1:1 (v/v). The mixture was kept under dark for at least 16 hr and diluted with ethanol to absorbance of 0.70 ± 0.05 at 734 nm with UV spectrophotometer before use. Then, 40 µl methanol extracts of samples were mixed with 2 ml ABTS⁺ solution, and the absorbance was measured at 734 nm after rested still for 6 min. The bland solutions were made by pure methanol and treated along with samples under the same protocol. All samples were measured in triplicates on three biological replicates.
biological replicates. Absorbance of blank was subtracted from each sample. Trolox was used as standard reference, and the results were expressed as μmol trolox equivalents per gram of defatted kernel weight (μmol TE g⁻¹).

2.7 | Method validation and statistical analysis

Commercial standard compounds were used to validate the linearity, precision, repeatability, and stability of the optimized method. At least six appropriate concentrations of standard solution in duplicate were prepared to obtain the linearity. The LOD (limit of detection) and LOQ (limit of qualification) for each analyte were acquired at a S/N of 3 and 10, respectively. Six injections of mixed standard solutions, including the highest, middle, and lowest concentration of linearity, were used to assess the precision and reproducibility. Six independent replicates were studied for each sample solution. Sample stability was assessed by analyzing it at 0, 2, 4, 8, 12, and 24 hr. The accuracy of the method was performed by adding the corresponding marker compounds with high (120% of the known amounts), middle (same as the known amounts), and low (80% of the known amounts) levels to 0.25 g of samples analyzed previously. Triplicate experiments were performed at each level. The average recoveries were estimated by the formula: recovery (%) = ([amount found – original amount]/amount added) × 100 (Zhang et al., 2019).

The MS data were processed by the Xcalibur (version 4.1) software, and PCA was performed by the SPSS (version 18.0) software.

3 | RESULTS AND DISCUSSION

3.1 | Method validation

The liquid chromatographic method had been optimized, and the final parameters were chosen under the consideration of both separation resolution and analytical efficiency. Commercial standard compounds were used to verify the optimized analytical method (Table S1). Results showed that the method had good linearity over wide ranges with regression coefficients (r²) over .99. The LOD and the LOQ were 0.0009 μg/ml and 0.0029 μg/ml for catechin, 0.0008 μg/ml and 0.0026 μg/ml for ellagic acid, 0.0008 μg/ml and 0.0028 μg/ml for quercetin, respectively. The precision, repeatability, and stability of the method were also studies using standard compounds (Table S2). The low relative standard deviations (RSDs) demonstrated that this method is precise, repeatable, and stable and can be used in the following experiment.

3.2 | Identification of phenolic metabolites in different pecan organs

A total of 97 phenolic metabolites have been identified from 8 pecan organs, including 57 hydrolysable tannins, 19 condensed tannins, 1 complex tannin, 18 flavonols, and 2 other compounds (Figure 1a, Table 1). There were significant differences in the numbers of metabolites identified in different pecan organs (Table 1). The number of phenolic compounds identified from leaves was largest among all organs, followed by testa, while the least phenolic compounds were identified from shells. This result showed the difference in the complexity of phenolic compounds among different pecan organs, similar result can be seen in the principal component analysis (PCA) too (Figure 1b). The samples of testa and leaf were far from the others, which showed the specificity of phenolic compounds in these organs. The samples of kernel, shell, branch, and bark were close to each other, which showed the similarity of composition between them. The phenolic compositions of kernel were quite different from those of testa. Of the 97 phenolic metabolites, 30 were found in all organs, 34 in only one organ, and 21 in only two organs (Table 1).

3.2.1 | Identification of hydrolysable tannins and their distribution

The major phenolic compounds identified were hydrolysable tannins, accounting for about two-thirds of the total number (Figure 2). Peak 74 with molecular ion at m/z 300.9998 ([M–H]⁻) was identified.
| Peak No. | RT (min) | Compound identification | Formula | [M−H] (m/z) | MS/MS fragments (m/z) | Δm (ppm) | K | T | SL | SK | BK | BH | L | F |
|---------|---------|-------------------------|---------|-------------|----------------------|----------|---|---|----|----|----|----|---|---|
| 1       | 0.72    | HHDP-glucose            | C_{20}H_{18}O_{14} | 481.0641     | 301–275              | -4.78    | √ | √ | √  | √  | √  | √  | √ | √ |
| 2       | 0.95    | HHDP-glucose            | C_{20}H_{18}O_{14} | 481.0632     | 301–275              | -2.91    | √ | √ | √  | √  | √  | √  | √ | √ |
| 3       | 1.10    | HHDP-glucose            | C_{20}H_{18}O_{14} | 481.0628     | 301–275              | -2.08    | √ | √ | √  | √  | √  | √  | √ | √ |
| 4       | 1.31    | Gallic acid hexoside    | C_{13}H_{16}O_{10} | 331.0647     | 169                  | 5.44     | √ | √ | √  | √  | √  | √  | √ | √ |
| 5       | 1.42    | Gallic acid hexoside    | C_{13}H_{16}O_{10} | 331.0647     | 169                  | 5.44     | √ | √ | √  | √  | √  | √  | √ | √ |
| 6       | 1.43    | Pterocaritin B‡         | C_{39}H_{32}O_{26} | 915.1122     | 871–781–733–569–301  | -1.97    | √ | √ | √  | √  | √  | √  | √ | √ |
| 7       | 1.6     | Valoneoyl-glucose       | C_{23}H_{32}O_{19} | 649.0694     | 605–481–301           | -2.62    | √ | √ | √  | √  | √  | √  | √ | √ |
| 8       | 1.72    | Valoneoyl-glucose       | C_{23}H_{32}O_{19} | 649.0694     | 605–481–301           | -2.62    | √ | √ | √  | √  | √  | √  | √ | √ |
| 9       | 2.47    | Pedunculagin/casuariin  | C_{34}H_{24}O_{22} | 783.0695     | 481–301               | -1.79    | √ | √ | √  | √  | √  | √  | √ | √ |
| 10      | 2.57    | Procyanidin trimer (C1)| C_{46}H_{36}O_{18} | 865.1987     | 739–713–695–577–575–287 | -0.81    | √ | √ | √  | √  | √  | √  | √ | √ |
| 11      | 3.00    | Azaleatin rhamnoside    | C_{22}H_{18}O_{11} | 461.1101     | 315                  | -3.69    | √ | √ | √  | √  | √  | √  | √ | √ |
| 12      | 3.02    | Pedunculagin/casuariin  | C_{34}H_{24}O_{22} | 783.0690     | 481–301               | -1.15    | √ | √ | √  | √  | √  | √  | √ | √ |
| 13      | 3.21    | Pedunculagin/casuariin  | C_{34}H_{24}O_{22} | 783.0689     | 481–301               | -1.02    | √ | √ | √  | √  | √  | √  | √ | √ |
| 14      | 3.69    | 1 (E)C and 1 (E)GC B linkage | C_{30}H_{26}O_{13} | 593.1309     | 467–425–407–289     | -2.36    | √ | √ | √  | √  | √  | √  | √ | √ |
| 15      | 3.87    | Pedunculagin/casuariin  | C_{34}H_{24}O_{22} | 783.0630     | 481–301               | 6.51     | √ | √ | √  | √  | √  | √  | √ | √ |
| 16      | 3.92    | Neochlorogenic acid‡    | C_{16}H_{18}O_{9}  | 353.0885     | 191–179–135           | -3.40    | √ | √ | √  | √  | √  | √  | √ | √ |
| 17      | 4.05    | Glansrin C‡             | C_{41}H_{26}O_{26} | 933.0653     | 631–451–301           | -2.04    | √ | √ | √  | √  | √  | √  | √ | √ |
| 18      | 4.25    | Pedunculagin/casuariin  | C_{34}H_{24}O_{22} | 783.0690     | 481–301               | -1.15    | √ | √ | √  | √  | √  | √  | √ | √ |
| 19      | 4.53    | Praecoxin A/platycariin | C_{41}H_{26}O_{27} | 951.0789     | 907–783–481–301       | -5.15    | √ | √ | √  | √  | √  | √  | √ | √ |
| 20      | 4.73    | Procyanidin dimer B linkage | C_{30}H_{28}O_{12} | 577.1364     | 451–425–407–289       | -3.12    | √ | √ | √  | √  | √  | √  | √ | √ |
| 21      | 4.81    | Procyanidin dimer B linkage | C_{30}H_{28}O_{12} | 577.1364     | 451–425–407–289       | -3.12    | √ | √ | √  | √  | √  | √  | √ | √ |
| 22      | 4.85    | Praecoxin A/platycariin | C_{41}H_{26}O_{27} | 951.0759     | 907–783–481–301       | -2.00    | √ | √ | √  | √  | √  | √  | √ | √ |

(Continues)
| Peak No. | RT (min) | Compound identification | Formula | Compound name | MS/MS fragments (m/z) | Δm (ppm) | K | T | SL | BK | BH | L | F |
|---------|---------|-------------------------|---------|--------------|----------------------|----------|---|---|----|----|----|---|---|
| 23      | 4.92    | Pedunculagin/casuariin isomer | C_{34}H_{24}O_{22} | 783.0630 | 481–301 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 24      | 5.01    | Procyanidin dimer B linkage | C_{30}H_{26}O_{12} \_ B | 577.1372 | 633–435–301 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 25      | 5.12    | Tellimagrandin I | C_{34}H_{24}O_{22} | 785.0845 | 633–483–301 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 26      | 5.25    | Pterocarbin A \_ B | C_{46}H_{36}O_{30} | 1,067.1257 | 1,023–933–765–377–301 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 27      | 5.28    | Procyanidin trimmer (C1) | C_{45}H_{38}O_{18} | 865.2026 | 739–713–695–577–575–287 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 28      | 5.36    | Stenophyllanin A/B isomer‡ | C_{56}H_{40}O_{31} | 1,207.1541 | 917–873–855 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 29      | 5.57    | Pterocarbin A \_ B | C_{46}H_{36}O_{30} | 1,067.1268 | 1,023–933–765–377–301 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 30      | 5.59    | Procyanidin trimmer (C1) | C_{44}H_{36}O_{20} | 665.2020 | 739–695–577–575–287 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 31      | 5.53    | Tris-O-degalloyl rugosin | C_{34}H_{26}O_{18} | 633.0762 | 481–301 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 32      | 5.53    | Tris-O-degalloyl rugosin | C_{34}H_{26}O_{18} | 633.0762 | 481–301 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 33      | 5.57    | Chinanthiamin A2 | C_{60}H_{50}O_{24} | 1,153.2637 | 865–713–577–575–413 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 34      | 5.72    | Casuarin/casuariin | C_{41}H_{28}O_{26} | 935.0845 | 907–873–855 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 35      | 5.76    | Strictinin/isostictinin | C_{41}H_{28}O_{26} | 935.0845 | 907–873–855 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 36      | 5.93    | Chinanthiamin A2 | C_{60}H_{50}O_{24} | 1,153.2637 | 865–713–577–575–413 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 37      | 5.97    | Paeonol A/palatinarin | C_{41}H_{28}O_{26} | 935.0845 | 907–873–855 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 38      | 6.02    | Tellimagrandin I | C_{34}H_{26}O_{22} | 785.0865 | 633–483–301 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 39      | 6.06    | Brevifolin carboxylic acid | C_{34}H_{26}O_{22} | 281.0355 | 247 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 40      | 6.18    | Glaucrin A\_ B | C_{41}H_{28}O_{26} | 935.0845 | 907–873–855 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 41      | 6.26    | Stenophyllanin A/B isomer‡ | C_{56}H_{40}O_{31} | 1,207.1541 | 917–873–855 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| 42      | 6.33    | Valoneic acid dilactone | C_{21}H_{10}O_{13} | 469.0109 | 425–301 | 6.51 | √ | √ | √ | √ | √ | √ | √ |
| Peak No. | RT (min) | Compound identification | Formula | [M−H] (m/z) | MS/MS fragments (m/z) | Δm (ppm) | K | T | SL | SK | BK | BH | L | F |
|---------|---------|-------------------------|---------|-------------|----------------------|----------|---|---|---|---|----|----|---|---|
| 43      | 6.38    | Procyanidin dimer B linkage | C_{30}H_{26}O_{12} | 577.1357 | 451–425–407–289 | -1.91    | √ | √ |    |    |    |    |   |   |
| 44      | 6.45    | Valoneic acid dilactone  | C_{21}H_{26}O_{13} | 469.0067 | 425–301 | -5.12 |    |    |    |    |    |    |   |   |
| 45      | 6.54    | Reginin A/D isomer⩢ | C_{21}H_{26}O_{48} | 858.0762[M−2H]^{2−} (MW 1717) | 933–783–633–481–301 | -6.81 |    |    |    |    |    |    |   |   |
| 46      | 6.65    | Procyanidin trimer (C1) | C_{45}H_{38}O_{18} | 865.1995 | 739–713–695–577–575–287 | -1.73 |    |    |    |    |    |    |   |   |
| 47      | 6.66    | Cinnamottannin A2       | C_{66}H_{50}O_{24} | 1,153.2667 | 865–713–577–575–413 | -4.60 |    |    |    |    |    |    |   |   |
| 48      | 6.69    | Glansrin C⩢            | C_{41}H_{26}O_{26} | 933.0674 | 631–451–301 | -4.29 |    |    |    |    |    |    |   |   |
| 49      | 6.74    | Reginin A/D isomer⩢ | C_{75}H_{50}O_{48} | 858.0759[M−2H]^{2−} (MW 1717) | 933–783–633–481–301 | -5.94 |    |    |    |    |    |    |   |   |
| 50      | 6.98    | Casurinincasuaricin isomer⩢ | C_{41}H_{26}O_{26} | 935.0723 | 783–633–481–301 | 7.27 |    |    |    |    |    |    |   |   |
| 51      | 7.03    | Rugosin C/glansrin A isomer⩢ | C_{41}H_{26}O_{26} | 1,103.0906 | 1.059–935–757–633–301 | -5.17 |    |    |    |    |    |    |   |   |
| 52      | 7.04    | Procyanidin dimer B linkage | C_{30}H_{26}O_{12} | 577.1360 | 451–425–407–289 | -2.43 |    |    |    |    |    |    |   |   |
| 53      | 7.17    | Cinnamottannin A3      | C_{75}H_{62}O_{30} | 1,441.3262 | 1,289–1,153–1,151–865–577–575 | -0.97 |    |    |    |    |    |    |   |   |
| 54      | 7.21    | Glansrin C⩢            | C_{41}H_{26}O_{26} | 933.0576 | 631–451–301 | 6.22 |    |    |    |    |    |    |   |   |
| 55      | 7.23    | Pedunculagin/casuarin isomer | C_{30}H_{26}O_{22} | 783.0722 | 481–301 | -5.24 |    |    |    |    |    |    |   |   |
| 56      | 7.47    | Azaleatin galactose     | C_{22}H_{22}O_{12} | 477.1046 | 315 | -2.72 |    |    |    |    |    |    |   |   |
| 57      | 7.52    | Quercetin galloyl hexoside | C_{29}H_{16}O_{36} | 615.0945 | 463–301 | 6.67 |    |    |    |    |    |    |   |   |
| 58      | 7.56    | Rugosin C/glansrin A isomer⩢ | C_{41}H_{26}O_{26} | 1,103.0907 | 1.059–935–757–633–301 | -5.26 |    |    |    |    |    |    |   |   |
| 59      | 7.60    | Ellagic acid pentose    | C_{7}H_{14}O_{12} | 433.0428 | 301 | -4.85 |    |    |    |    |    |    |   |   |
| 60      | 7.60    | Azaleatin glucoside     | C_{22}H_{22}O_{12} | 477.1049 | 315 | -3.35 |    |    |    |    |    |    |   |   |
| 61      | 7.72    | Quercetin galloyl hexoside | C_{29}H_{16}O_{36} | 615.1016 | 463–301 | -4.88 |    |    |    |    |    |    |   |   |
| 62      | 7.78    | Cinnamottannin A3      | C_{75}H_{62}O_{30} | 1,441.3235 | 1,289–1,153–1,151–865–577–575 | 0.90 |    |    |    |    |    |    |   |   |
| 63      | 7.79    | Azaleatin arabinoside  | C_{21}H_{20}O_{11} | 447.0946 | 315 | -4.25 |    |    |    |    |    |    |   |   |
| Peak No. | RT (min) | Compound identification                      | Formula     | [M−H] (m/z) | MS/MS fragments (m/z) | Δm (ppm) | K | T | SL | SK | BK | BH | L | F |
|---------|----------|----------------------------------------------|-------------|-------------|-----------------------|-----------|---|---|----|----|----|----|---|---|
| 64      | 7.81     | Casuarinin/casuarictin isomer‡               | C_{41}H_{28}O_{26} | 935.0841    | 783–633–481–301       | −5.35     | ✓ |   |    |    |    |    |   |   |
| 65      | 7.91     | Azaleatin rhamnoside                         | C_{22}H_{22}O_{11} | 461.1106    | 315                   | −4.77     | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |   |   |
| 66      | 7.92     | Quercetin galloyl hexoside                   | C_{28}H_{24}O_{36} | 615.1019    | 463–301               | −5.36     | ✓ |   |    |    |    |    |   |   |
| 67      | 7.93     | Casuarinin/casuarictin isomer‡               | C_{41}H_{28}O_{26} | 935.0767    | 783–633–481–301       | 2.57      |   |   |    |    |    |    |   |   |
| 68      | 7.96     | Quercetin galloyl hexoside                   | C_{28}H_{24}O_{36} | 615.1019    | 463–301               | −5.36     | ✓ |   |    |    |    |    |   |   |
| 69      | 7.97     | Methyl ellagic acid galactose                 | C_{21}H_{18}O_{13} | 477.0682    | 315–300               | −2.72     |   |   |    |    |    |    |   |   |
| 70      | 7.97     | Heterophyllin D‡                             | C_{82}H_{54}O_{52} | 934.0767[M−2H] | 1,568–1,085–783–633–451–301 | −7.12     | ✓ | ✓ |    |    |    |    |   |   |
| 71      | 8.00     | Cinnamattinin A3                             | C_{77}H_{62}O_{30} | 1,441.322   | 1,289–1,153–1,151–865–577–575 | 1.94      |   |   |    |    |    |    |   |   |
| 72      | 8.11     | Procyanidin dimer A linkage                  | C_{28}H_{24}O_{12} | 575.1202    | 449–423–407–289       | −2.09     | ✓ |   |    |    |    |    |   |   |
| 73      | 8.19     | Quercetin hexoside                           | C_{21}H_{18}O_{12} | 463.0900    | 301                   | −4.97     | ✓ | ✓ | ✓ |    |    |    |   |   |
| 74      | 8.22     | Ellagic acid                                 | C_{20}H_{16}O_{9} | 300.9998    | 257–229               | −4.65     | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |   |   |
| 75      | 8.31     | Quercetin hexoside                           | C_{21}H_{18}O_{12} | 463.0897    | 301                   | −4.32     | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |   |   |
| 76      | 8.39     | Methyl ellagic acid glucose                  | C_{21}H_{18}O_{13} | 477.0690    | 315–300               | −4.40     | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |   |   |
| 77      | 8.46     | Quercetin hexoside                           | C_{21}H_{18}O_{12} | 463.0891    | 301                   | −3.02     | ✓ | ✓ | ✓ |    |    |    |   |   |
| 78      | 8.64     | Eucalbanin A/comusmin B isomer‡              | C_{48}H_{30}O_{30} | 1,085.0780  | 933–783–633–451–301   | −3.32     | ✓ |    |    |    |    |    |   |   |
| 79      | 8.79     | (Epi) catechin galate                        | C_{22}H_{16}O_{10} | 441.0837    | 331–289–271–169       | −3.40     |   |   |    |    |    |    |   |   |
| 80      | 8.93     | Caryatin sulfate                             | C_{19}H_{14}O_{10}S | 409.0248    | 329                   | −4.65     | ✓ | ✓ |    |    |    |    |   |   |
| 81      | 9.07     | Quercetin pentose                            | C_{20}H_{16}O_{11} | 433.0792    | 301                   | −4.85     | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |   |   |
| 82      | 9.15     | Eucalbanin A/comusmin B isomer‡              | C_{48}H_{30}O_{30} | 1,085.0781  | 933–783–633–451–301   | −3.41     | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |   |   |
| 83      | 9.28     | Methyl ellagic acid pentose                  | C_{20}H_{16}O_{12} | 447.0581    | 315–300–198–161       | −3.80     | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |   |   |
| 84      | 9.35     | Quercetin pentose                            | C_{20}H_{16}O_{11} | 433.0784    | 301                   | −3.00     | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |   |   |
| 85      | 9.46     | Ellagic acid rhamnoside                      | C_{20}H_{16}O_{12} | 447.0579    | 301                   | −3.36     | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |   |   |
| Peak No. | RT (min) | Compound identification | Formula | [M−H] (m/z) | MS/MS fragments (m/z) | Δm (ppm) | K | T | SL | SK | BK | BH | L | F |
|---------|----------|-------------------------|---------|-------------|-----------------------|----------|---|---|----|----|----|----|---|---|
| 86      | 9.58     | Quercetin rhamnoside    | C_{21}H_{20}O_{11} | 447.0949    | 301                   | -4.92    | √ | √ | √  | √  | √  | √  | √ | √ |
| 87      | 9.70     | Caryatin                | C_{27}H_{14}O_{7}  | 329.0671    | 314–301               | -3.04    | √ | √ | √  | √  | √  | √  | √ | √ |
| 88      | 9.74     | Ellagic acid galloyl pentose | C_{26}H_{16}O_{16} | 585.0536    | 433–301               | -3.25    | √ | √ |    |    |    |    |   |   |
| 89      | 9.95     | Procyanidin dimer A linkage | C_{30}H_{24}O_{12} | 575.1204    | 449–423–407–289       | -2.43    | √ |    |    |    |    |    |   |   |
| 90      | 10.15    | Methyl ellagic acid     | C_{15}H_{9}O_{8}   | 315.0148    | 301                   | -2.22    | √ | √ | √  | √  | √  | √  | √ | √ |
| 91      | 10.34    | Ellagic acid galloyl pentose | C_{26}H_{18}O_{16} | 585.0545    | 433–301               | -4.79    |    |    |    |    |    |    |   |   |
| 92      | 10.87    | Dimethyl ellagic acid rhamnoside | C_{22}H_{20}O_{12} | 475.0888    | 329                   | -2.32    | √ | √ | √  | √  | √  | √  | √ | √ |
| 93      | 11.59    | Methyl ellagic acid galloyl pentose | C_{27}H_{20}O_{16} | 599.0689    | 447–433–315–301       | -2.67    |    |    |    |    |    |    |   |   |
| 94      | 11.78    | Caryatin methyl ether   | C_{16}H_{16}O_{7}  | 343.0830    | 329                   | -3.50    |    |    |    |    |    |    |   |   |
| 95      | 11.93    | Methyl ellagic acid galloyl pentose | C_{27}H_{20}O_{16} | 599.0687    | 447–433–315–301       | -2.34    | √ | √ | √  | √  | √  | √  | √ | √ |
| 96      | 12.17    | Methyl ellagic acid galloyl pentose | C_{27}H_{20}O_{16} | 599.0687    | 447–433–315–301       | -2.34    | √ | √ | √  | √  | √  | √  | √ | √ |
| 97      | 12.55    | Methyl ellagic acid galloyl pentose | C_{27}H_{20}O_{16} | 599.0685    | 447–433–315–301       | -2.00    | √ | √ | √  | √  | √  | √  | √ | √ |

| Total number | 41  | 58  | 35  | 41  | 40  | 53  | 60  | 42  |

†K, kernel; T, testa; SL, shell; SK, shuck; BK, bark; BH, branch; L, leaf; F, flower (male inflorescence); E, epi; C, catechins; GC, gallocatechin.
‡These compounds were identified for the first time in pecan.
FIGURE 2  UHPLC-LTQ-Orbitrap MS total ion current chromatograms in negative ion mode of eight pecan organs
as ellagic acid by comparing its fragments with previous reports and its $t_R$ value with standard compound. Ellagic acid pentose (peak 59) and ellagic acid rhamnose (peak 85) can be assured by the fragment ion at $m/z$ 301, which represent the ellagic acid aglycone, and a loss of 132 Da (pentose) and 146 Da (deoxyhexose) in the MS² spectrum. Methyl ellagic acid derivatives (peaks 69, 76, 83, and 90) can be recognized by the ions at $m/z$ 315 and loss of a methyl group, while two sequential losses of methyl groups gave evidence of peak 92 to be dimethyl ellagic acid derivative. According to the fragment pattern, peak 92 was assigned as dimethyl ellagic acid rhamnose, while this is the first report of this compound in pecan. Peak 83 had the same molecular weight (MW) of 447 with peak 85, but their fragment patterns were different. Peak 83 showed fragment ions at $m/z$ 315 ([M−H−132]$^-$, loss of pentose) and 300 ([M−H−132−15]$^-$, loss of pentose and methyl group), indicated that it was methyl ellagic acid pentose. All these compounds except peak 69 were found in all organs.

Hexahydroxydiphenoyl (HHDP) group is a basic structure of ellagitannins. HHDP-glucose (peaks 1, 2, and 3, [M−H]$^-$, $m/z$ 481) were identified in all organs and they eluted really fast (Figure 2). Seven metabolites (peaks 9, 12, 13, 15, 18, 23, and 55) were identified as pedunculagin/casuariin isomer (bis-HHDP-glucose) according to the corresponding precursor ion at $m/z$ 783 ([M−H]$^-$), with its fragmentation ions at 481 ([M−H−302]$^-$, loss of HHDP) and 301 ([M−H−482]$^-$, loss of HHDP-glucose). These metabolites also distributed in all organs. Peak 6 ([M−H]$^-$, $m/z$ 915) was tentatively assigned as pterocarmin B, which possessed more pentose than pedunculagin/casuariin isomer. This ellagitannin had been found in Pterocarya stenoptera and walnut before (Fukuda, Ito, & Yoshida, 2003; Nonaka, Ishimaru, Azuma, Ishimatsu, & Nishioka, 1989); it was found in samples of leaves and branches in this experiment, this is the first report in pecan.

Gallotannins had also been found in the earlier eluents. The deprotonated molecular ions at $m/z$ 331.0647 and the fragments at 169 indicated that peaks 4 and 5 contained gallic acid and hexose moieties. Peak 4 had only been detected in leaf sample, while peak 5 only in male inflorescence sample.

More hydrolyzed tannins identified in pecan were more complicated and mixed with both HHDP and gallotannin groups. Peaks 42 and 44 ([M−H]$^-$, $m/z$ 469) were identified as valoneic acid dilactone, which contained one ellagic acid moiety ([M−H−301] in MS² spectrum) and one gallic acid moiety (loss of 168 in MS² spectrum) conjugated with C-C bond. They were found only in pecan leaves in this experiment.

Metabolites detected at $m/z$ 585 (peaks 88 and 91) and $m/z$ 599 (peaks 93, 95, 96, and 97) were assigned as ellagic acid galloyl pentose and methyl ellagic acid galloyl pentose, respectively.

On the basis of HHDP-glucose ($m/z$ 481), metabolites with one to three galloyl substituents were found at $m/z$ 633 (peaks 31 and 35), 785 (peaks 25 and 38), and 935 (peaks 34, 50, 64, and 67) and were assigned as strictinin/isostrictinin isomer, tellimagrandin I, and casuarinin/casuarictin isomer. This is the first report of casuarinin/casuarictin isomer in pecan. Casuarinin and previous mentioned casuarin were first isolated from Casuarina stricta (Okuda, Yoshida, Ashida, & Yazaki, 1982), and they were widely distributed in many species including Juglandaceae families (Okuda, Yoshida, Hatano, Yazaki, & Ashida, 1980) and were building blocks of C-glycosidic tannins (Okuda, Yoshida, Hatano, & Ito, 2009).

On the basis of pedunculagin/casuariin isomer (bis-HHDP-glucose, $m/z$ 783), metabolites with one and two galloyl substituents were found at $m/z$ 933 (peaks 17, 40, 48, and 54) and 1,085 (peaks 78 and 82) and were assigned as glansrin C and eucalbanin A/cornusin B isomer. Glansrin C and eucalbanin A/cornusin B isomer had been detected from walnut before (Regueiro et al., 2014), and this is the first report of these two tannins in pecan. Peaks 19, 22, and 37 produced [M−H]$^-$ ions at $m/z$ 951 and had similar series of ions at $m/z$ 783, 633, 481, and 301 in MS² spectrum, indicated they contained an extra group of gallic acid and they were assigned as praeceoxin A/platycarin isomer.

One more galloyl group linked to the HHDP unit will form valoneoyl, tergalloyl, or macaranoyl groups (Okuda et al., 2009). Peaks 7 and 8 had the deprotonated molecular ion ([M−H]$^-$) at $m/z$ 649.0694, with its fragments at $m/z$ 605 ([M−H−44]$^-$, loss of carboxyl), 481 ([M−H−168]$^-$, loss of gallic acid) and 301, were identified as valoneoyl-glucose, though tergalloyl-glucose or macaranoyl-glucose were also possible. Through analysis of the fragmentation patterns, peaks 51 and 58 ([M−H]$^-$, 1,103) can be assigned as rugosin C/glanSRin A isomer which contained one more valoneoyl group on the basis structure of strictinin/isostrictinin isomer. This is the first report of rugosin C/glanSRin A isomer in pecan.

Peaks 26 and 29 generated [M−H]$^-$ ions at $m/z$ 1,067 and gave fragment ions at $m/z$ 933 ([M−H−134]$^-$, loss of pentose) showed the existence of an extra pentose on the basis of glansrin C. Combined this information with literature reports, they were determined to be pterocarmin A. This compound had been found in walnut before (Regueiro et al., 2014), while this is the first report in pecan.

Several ellagitannins were identified through their doubly charged ions ([M−H]$^2^-$) which were confirmed by the isotopic peaks (Figure 3), such as peak 32 showed [M−2H]$^2^-$ ion at $m/z$ 707.0638 indicated its MW of 1,415. Meanwhile, the fragment ions at $m/z$ 1,114, 934, 783, 633, 481, and 301 in MS² spectrum showed that it was composed of two ellagitannins, casuarinin/casuariin isomer, and HHDP-glucose (Figure 4). This ellagitannin was identified as tris-O-degalloyl rugosin F isomer and was found in pecan testa and kernel in this experiment. Peaks 45 and 49 generated doubly charged ions at $m/z$ 858.0762 [M−2H]$^2^-$ and similar fragment ions at $m/z$ 933, 783, 633, 481, and 301. This meant that these ellagitannins had a MW of 1,717 and were composed of two structures, tellimagrandin I and glansrin C, so they were identified as reginin A/D isomer. This ellagitannin was found only in pecan testa in this experiment. The MW of peak 70 is the highest among the peaks we identified. Its doubly charged ion was found at $m/z$ 934.0767 [M−2H]$^2^-$, indicating that its MW was 1,869. Through the analysis of fragment ions, it was found that this ellagitannin was composed of tellimagrandin I and eucalbanin A/cornusin B isomer, so it was identified as heterophyllinin D. All these high MW
**FIGURE 3**  The doubly charged ions of heterophyllin D (a), reginin A/D isomer (b), and tris-O-degalloyl rugosin F isomer (c)
**Figure 4** MS/MS fragmentation spectrum of a tris-O-degalloyl rugosin F isomer

**Figure 5** Deduced metabolic pathway of part of the hydrolysable tannins in pecan organs. Different colors of metabolites represented different structure categories: brown, ellagitannins; blue, hydrolysable tannins mixed with both HHDP and galloyl groups; pink, complex tannin. K, kernel; T, testa; SL, shell; SK, shuck; BK, bark; BH, branch; L, leaf; F, flower (male inflorescence)
ellagitannins were reported the first time in pecan. Through this study, the tannin profile and each route of tannin metabolic pathway of pecan had been expanded (Figure 5), which laid a foundation for future researches and uses.

3.2.2 Identification of condensed tannins and their distribution

A series of condensed tannins were identified in these organs. Peak 27 ($t_r = 5.28$ min) was assured as (+)-catechin, the basic building blocks of CTs, by the molecular ion at $m/z$ 289.0726 and comparing with commercial standard. This compound was found in all organs. The (epi)catechin gallate (peak 79) was only found in testa at 8.79 min in minimum quantity and cannot be found in other organs. There were two kinds of linkage between the building blocks of procyanidins, in which B type is more common because of less space resistant. Five metabolites (peaks 20, 21, 24, 43, and 52) were identified as procyanidin dimer B linkage (PD2B) according to the deprotonated molecular ion at $m/z$ 577 ([M−H]−) and the fragment ion at $m/z$ 289 ([M−H−288, loss of a catechin moiety]). Peaks 20 and 43 were found in all organs. Two metabolites (peaks 72 and 89) were identified as procyanidin dimer A linkage according to the molecular ion at $m/z$ 575 ([M−H]−), two hydrogen atoms less than PD2B. They can only be found in branch, testa, and bark. Peak 14 was also procyanidin dimer but contained one (epi)catechin and one (epi)galocatechin. Procyanidin trimer (peaks 10, 30, and 46), tetramer (peaks 33, 36, and 47), and pentamer (peaks 53, 62, and 71) were also identified, in which pentamer were only found in shuck.

3.2.3 Identification of flavonols and their distribution

All flavonoid metabolites identified in this experiment were flavonols, and most of them were eluted in the later part of elutes. There were three types of flavonol aglycones, quercetin, azaleatin, and caryatin, which contained their identical fragment ions at $m/z$ 301, 315, and 329 in the MS² spectrum, respectively. One tricky problem about flavonol identification in pecan is they have similar MW with ellagic acid, methyl ellagic acid, and dimethyl ellagic acid derivatives. Luckily, the high-accuracy MW can solve these problems.

For example, the high-accuracy MW of peaks 56 and 60 ([M−H]− 477.10) can help us discriminated them from peaks 69 and 76 ([M−H]− 477.06), their structures were determined as azaleatin hexoside instead of methyl ellagic acid hexoside according the MW and the fragment ions at $m/z$ 315. It is reported that the galactosides eluted earlier than its corresponding glucosides (Abad-García, Berrueta, Garmón-Lobato, Gallo, & Vicente, 2009), so peak 56 was tentatively assigned as azaleatin galactose, and peak 60 was tentatively assigned as azaleatin glucoside. Ishak et al. had isolated a series of azaleatin glycosides from pecan branch including glucoside, rhamnoside, arabinoside, diglucoside, and rutinoside (Ishak et al., 1980), and their structures were elucidated with NMR method, so this is the first report of azaleatin galactose in pecan, and this is also the first report of azaleatin glucoside in pecan bark.

The high-accuracy MW also helped us to identify peak 81 (experimental MW 433.0792) and 84 (experimental MW 433.0784) to be quercetin pentose (C₁₆H₁₀O₈, theoretical MW 433.0771) instead of ellagic acid pentose (peak 59, C₁₇H₁₄O₁₀, theoretical MW 433.0407). Similarly, peaks 73, 75, and 77 were assigned as quercetin hexosides (C₂₁H₁₄O₁₂, theoretical MW 463.0877) according to their high-accuracy MW 463.09, instead of ellagic acid hexoside (C₂₀H₁₄O₁₃, theoretical MW 463.0513). Peaks 57, 61, 66, and 68 ([M−H]− 615) were assigned as quercetin galloyl hexose instead of ellagic acid galloyl hexose. A series of quercetin glycosides had been isolated from pecan leaves and elucidated with NMR method, including glucoside, rhamnoside, arabinoside, galactoside, and galloyl galactoside (Abdallah et al., 2011; Gad et al., 2007; Ishak et al., 1980).

The molecular ion at $m/z$ 329 and the fragment ions at $m/z$ 314 and 301 of peak 87 were consistent with the dimethyl ellagic acid (C₁₆H₁₀O₆, theoretical MW 329.0297) which had been reported in pecan kernel, but the experimental MW of 329.0671 denied this deduction, instead it complied with caryatin (C₁₅H₁₄O₇, theoretical MW 329.0661).

The high-accuracy MW of both peaks 63 and 86 were 447.09, suggested their molecular formula to be C₂₃H₂₀O₁₁. Combined with information of fragment ion at $m/z$ 315, peak 63 can be tentatively assigned as azaleatin arabinoside, which had been previously reported in pecan branches (Ishak et al., 1980). Peak 86 had the same molecular formula but different structure, the fragment ion at $m/z$ 301 suggested it contained a quercetin aglycone and a rhamnoside; therefore, it was assigned as quercetin rhamnoside, which had been reported in pecan leaves (Gad et al., 2007). Azaleatin arabinoside had been found in branch and bark, while quercetin rhamnoside had been found in all organs. So, there were four peaks (peaks 63, 83, 85, and 86) had similar MW of 447, but their structures were all different with each other, which were azaleatin arabinoside, methyl ellagic acid pentose, ellagic acid rhamnoside, and quercetin rhamnoside. With powerful tools of both the high-accuracy MW and the fragment patterns in MS² spectrum, we can discriminate complicate phenolic metabolites with similar structures in pecan organs clearly and efficiently.

Flavonoids reported in pecan previously were all isolated from pecan leaves, branches or barks, their structures were elucidated by NMR methods, so this is the first report of identifying them in other organs and this is also the first report of identifying them with LCMS method. Through comprehensive survey of pecan organs, some distribution patterns of flavonols in pecan were found (Figure 6). The flavonol aglycones quercetin, azaleatin, and caryatin found in pecan have similar structures, methylation of the 6-hydroxy group of quercetin forms azaleatin, and further methylation of the 3-hydroxy group of azaleatin forms caryatin. A lot of flavonols can only be found in bark, leaf, or branch, especially azaleatin derivatives and caryatin derivatives. These results might indicate that more
methylations might occur in organs which had more chances to face biotic stresses like insects or diseases.

3.2.4 | Identification of other phenolic compounds and their distribution

The structure of peaks 28 and 41 ([M−H]−, 1,207) was much special, they were identified as complex tannins, which means a flavonol-based motif linked with ellagitannins through C-C bond (Okuda et al., 2009). By analysis of the fragmentation pattern, they were identified as stenophyllan A/B isomer, which contained two HHDP, one galloyl and one epicatechin groups. This is the first report of this complex tannin in pecan. Brevifolin carboxylic acid (peak 39) and neochlorogenic acid (peak 16) were also assigned according to their high-accuracy MWs, fragmentation patterns and compared with literature reports (Regueiro et al., 2014). Neochlorogenic acid was found only in pecan leaf in this experiment, and this is the first report of this compound in pecan.

3.3 | Content

In order to get more accurate result, we use the UHPLC-QQQ-MS equipment with MRM mode to carry out the quantitative analysis (Table 2). The precursor and product ions of selected compounds for MRM quantification were showed in Table S3. Catechin and ellagic acid were quantified with standard compounds. Other phenolics were semi quantified with catechin and ellagic acid according to their structural characteristics, which means that ellagic acid derivatives using ellagic acid as reference, while condensed tannins and flavonols using catechin.

Several phenolics’ contents were really high in pecan, such as ellagic acid pentose in testa (2,882.85 µg/g), ellagic acid rhamnoside in branch (2,590.89 µg/g) and testa (1,727.29 µg/g), HHDP-glucose in testa (1,161.76 µg/g), and caryatin in branch (1,156.38 µg/g).

The predominant phenolics in different pecan organs were different, such as the top five phenolics in pecan testa were ellagic acid pentose (2,882.85 µg/g), ellagic acid rhamnoside (1,727.29 µg/g), HHDP-glucose (peak 3, 1,161.76 µg/g), HHDP-glucose (peak 1, 957.46 µg/g) and pedunculagin/casuariin isomer (1,161.76 µg/g), while the top five in bark were caryatin (665.17 µg/g), methyl ellagic acid (42.56 µg/g), methyl ellagic acid glucose (14.15 µg/g), ellagic acid pentose (9.65 µg/g), and ellagic acid rhamnoside (4.80 µg/g), but ellagic acid pentose was one of the top five phenolics in all organs.

The total content of hydrolysable tannins was significantly high in pecan testa (9,786.08 µg/g), branch (3,871.19 µg/g), leaf (1,949.78 µg/g), and kernel (1,261.41 µg/g), but low in bark and shuck. The total content of condensed tannins was much lower than that of hydrolysable tannins, their content was varying from 190.71 µg/g to 1.79 µg/g, highest content appeared in testa, while lowest in flower. Combined with previous qualification results, we can see that tannins tend to accumulate in pecan testa with both diverse structures and high contents.

The distribution of flavonols was much different with tannins, they were the prevailing phenolics in branch (1,223.18 µg/g), bark (667.80 µg/g), and leaf (308.64 µg/g), but rare in testa (10.04 µg/g) and kernel (3.29 µg/g). This result consisted with previously reports while most flavonols were isolated and identified firstly from
| Peak No. | Phenolic metabolites | Kernel | Testa | Shell | Shuck | Bark | Branch | Leaf | Flower |
|---------|----------------------|--------|-------|-------|-------|------|--------|------|--------|
| 74      | Ellagic acid         | 4.96 ± 0.04 | 29.75 ± 2.38 | 19.73 ± 3.96 | 2.04 ± 0.65 | 0.27 ± 0.06 | 6.57 ± 1.05 | 1.29 ± 0.03 | 3.56 ± 0.92 |
| 90      | Methyl ellagic acid  | 27.00 ± 9.80 | 369.64 ± 53.53 | 76.28 ± 10.43 | 7.76 ± 0.66 | 42.56 ± 5.53 | 41.33 ± 36.06 | 21.33 ± 0.92 | 8.76 ± 2.66 |
| 59      | Ellagic acid pentose | 515.77 ± 167.20 | 2,882.85 ± 831.87 | 390.92 ± 36.55 | 59.61 ± 7.72 | 9.65 ± 1.98 | 356.53 ± 75.30 | 447.62 ± 6.76 | 39.99 ± 7.23 |
| 85      | Ellagic acid rhamnoside | 273.87 ± 129.00 | 1,727.29 ± 304.49 | 111.71 ± 42.21 | 6.31 ± 1.06 | 4.80 ± 0.36 | 2,590.89 ± 229.48 | 268.85 ± 9.23 | 40.64 ± 5.27 |
| 83      | Methyl ellagic acid pentose | 71.81 ± 20.42 | 349.10 ± 87.0  | 57.79 ± 7.69 | 9.06 ± 0.92 | 2.33 ± 0.08 | 256.25 ± 53.71 | 26.06 ± 5.24 | 42.9 ± 2.71 |
| 92      | Dimethyl ellagic acid rhamnoside | 52.02 ± 15.58 | 280.46 ± 26.15 | 224.80 ± 52.68 | 3.37 ± 0.34 | 0.58 ± 0.09 | 8.78 ± 3.18 | 12.85 ± 0.47 | 1.07 ± 0.07 |
| 76      | Methyl ellagic acid galloyl pentose | 2.45 ± 0.24 | 4.43 ± 1.23 | 44.93 ± 12.56 | 62.38 ± 29.83 | 14.15 ± 4.30 | 123.78 ± 15.60 | 363.90 ± 21.71 | 189.30 ± 24.31 |
| 3       | HHDP-glucose         | 7.68 ± 18.14 | 1,161.76 ± 322.21 | 53.49 ± 21.77 | 0.85 ± 0.23 | 0.15 ± 0.06 | 258.06 ± 78.58 | 297.41 ± 4.66 | 62.97 ± 8.25 |
| 2       | HHDP-glucose         | 5.87 ± 0.70 | 3.20 ± 0.75 | 0.18 ± 0.09 | 0.02 ± 0.04 | 0.01 ± 0.0003 | 1.19 ± 0.48 | 0.77 ± 0.04 | 0.19 ± 0.04 |
| 1       | HHDP-glucose         | 71.51 ± 24.84 | 957.64 ± 138.33 | 48.41 ± 22.52 | 2.25 ± 0.24 | 0.21 ± 0.07 | 126.45 ± 14.93 | 131.30 ± 9.38 | 24.50 ± 3.70 |
| 12      | Pedunculagin/ casuarin isomer | 59.50 ± 35.37 | 828.07 ± 28.96 | 30.40 ± 16.31 | 2.45 ± 0.50 | 0.10 ± 0.02 | 100.60 ± 15.33 | 83.50 ± 0.73 | 95.80 ± 14.55 |
| 18      | Pedunculagin/ casuarin isomer | 0.12 ± 0.03 | 1.08 ± 0.32 | 0.03 ± 0.001 | 0.01 ± 0.01 | ND | 0.18 ± 0.06 | 2.37 ± 0.09 | 0.08 ± 0.003 |
| 97      | Methyl ellagic acid galloyl pentose | 4.40 ± 0.94 | 79.17 ± 8.67 | 3.90 ± 1.16 | 0.29 ± 0.05 | 0.05 ± 0.01 | 1.69 ± 0.80 | 1.60 ± 0.18 | 0.82 ± 0.36 |
| 95      | Methyl ellagic acid galloyl pentose | 4.46 ± 2.33 | 645.46 ± 88.63 | 54.62 ± 16.06 | 9.19 ± 0.83 | 0.56 ± 0.07 | 13.30 ± 3.74 | 69.65 ± 3.68 | 16.03 ± 2.55 |
| 96      | Methyl ellagic acid galloyl pentose | 4.38 ± 0.33 | 53.73 ± 9.26 | 6.07 ± 1.45 | 0.75 ± 0.12 | 0.06 ± 0.01 | 1.44 ± 0.65 | 6.27 ± 0.43 | 1.53 ± 0.39 |
| 8       | Valoneoyl-glucose    | 0.29 ± 0.14 | 2.93 ± 0.53 | 0.13 ± 0.09 | 0.01 ± 0.01 | ND | 0.35 ± 0.06 | 1.28 ± 0.17 | 0.10 ± 0.01 |

(Continues)
| Peak No. | Phenolic metabolites                  | Kernel   | Testa      | Shell       | Shuck      | Bark       | Branch     | Leaf       | Flower     |
|---------|--------------------------------------|----------|------------|-------------|------------|------------|------------|------------|------------|
| 25      | Tellimagrandin I                     | 3.62 ± 1.55 | 48.99 ± 3.12 | 1.35 ± 0.35 | 0.05 ± 0.01 | 0.01 ± 0.01 | 0.95 ± 0.53 | 1.30 ± 0.09 | 0.67 ± 0.07 |
| 34      | Casuarinin/casuarictin isomer        | 0.67 ± 0.30 | 30.39 ± 3.59 | 4.80 ± 0.47 | 0.44 ± 0.06 | 0.03 ± 0.01 | 0.88 ± 0.29 | 4.20 ± 0.22 | 3.41 ± 1.00 |
| 22      | Praecoxin A/platycariin isomer       | 4.90 ± 0.90 | 80.06 ± 11.47 | 14.38 ± 1.98 | 2.47 ± 0.41 | 0.28 ± 0.03 | 1.03 ± 0.26 | 47.60 ± 3.03 | 5.66 ± 1.51 |
| 37      | Praecoxin A/platycariin isomer       | 5.39 ± 0.91 | 78.72 ± 3.06 | 15.71 ± 1.09 | 2.76 ± 0.33 | 0.31 ± 0.02 | 3.68 ± 0.14 | 50.68 ± 6.27 | 5.21 ± 1.44 |
| 19      | Praecoxin A/platycariin isomer       | 2.64 ± 1.37 | 41.66 ± 7.81 | 12.75 ± 0.57 | 2.37 ± 0.39 | 0.21 ± 0.03 | 1.13 ± 0.28 | 127.85 ± 3.77 | 8.84 ± 2.57 |
| 41      | Stenophyllanin A/B isomer            | 29.7 ± 7.17 | 129.88 ± 27.79 | 9.87 ± 1.24 | 5.10 ± 1.60 | 0.49 ± 0.23 | 24.03 ± 3.17 | 9.10 ± 1.18 | 21.55 ± 7.59 |
|         | Total content of hydrolysable tannins|          | 1.261.41 | 9.786.08 | 1.086.24 | 169.74 | 76.81 | 3.871.19 | 1.949.78 | 561.26 |
|         | Condensed tannins                    |          |            |             |             |             |             |             |             |             |
| 27      | (+)-Catechin                         | 6.02 ± 0.65 | 35.61 ± 3.25 | 2.67 ± 1.28 | 29.99 ± 4.08 | 1.90 ± 0.34 | 4.10 ± 1.91 | 5.40 ± 0.66 | 0.80 ± 0.04 |
| 43      | Procyanidin dimer B linkage          | 3.51 ± 0.47 | 50.50 ± 3.06 | 1.85 ± 0.65 | 56.63 ± 1.62 | 3.59 ± 0.45 | 2.43 ± 1.22 | 3.85 ± 0.51 | 0.84 ± 0.01 |
| 20      | Procyanidin dimer B linkage          | 0.94 ± 0.12 | 13.58 ± 1.17 | 0.63 ± 0.22 | 5.86 ± 0.41 | 0.65 ± 0.15 | 0.27 ± 0.06 | 1.13 ± 0.06 | 0.03 ± 0.0007 |
| 24      | Procyanidin dimer B linkage          | 0.01 ± 0.0006 | 0.12 ± 0.02 | 0.01 ± 0.01 | 0.02 ± 0.003 | 0.01 ± 0.002 | ND | ND | 0.01 ± 0.003 |
| 14      | 1(E)C and 1(E)GC B linkage           | 2.86 ± 0.25 | 90.90 ± 6.48 | 31.19 ± 10.00 | 5.78 ± 0.78 | 0.19 ± 0.06 | 0.24 ± 0.11 | 5.95 ± 0.54 | 0.11 ± 0.04 |
|         | Total content of condensed tannins   |          | 13.34 | 190.71 | 36.35 | 98.28 | 6.34 | 7.04 | 16.33 | 1.79 |
|         | Flavonols                            |          |            |             |             |             |             |             |             |             |
| 65      | Azaleatin rhamnoside                 | 0.54 ± 0.10 | 5.46 ± 0.35 | 0.18 ± 0.05 | ND | ND | 0.07 ± 0.003 | 0.52 ± 0.01 | 0.07 ± 0.002 |

(Continued)
**Table 2** (Continued)

| Peak No. | Phenolic metabolites | Kernel | Testa | Shell | Shuck | Bark | Branch | Leaf | Flower |
|----------|----------------------|--------|-------|-------|-------|------|--------|------|--------|
| 87       | Caryatin             | 2.30 ± 1.59 | 1.04 ± 1.00 | 16.19 ± 14.73 | 6.23 ± 0.83 | 665.17 ± 127.07 | 1,156.38 ± 378.92 | 0.33 ± 0.15 | 0.20 ± 0.05 |
| 75       | Quercetin hexoside   | 0.07 ± 0.07 | 0.44 ± 0.11 | 0.11 ± 0.06 | 5.38 ± 1.59 | 0.17 ± 0.01 | 0.69 ± 0.11 | 57.33 ± 9.57 | 5.27 ± 0.90 |
| 86       | Quercetin rhamnoside | 0.38 ± 0.13 | 3.10 ± 0.40 | 0.48 ± 0.25 | 4.86 ± 1.18 | 2.46 ± 0.39 | 66.04 ± 4.90 | 250.46 ± 31.17 | 23.61 ± 3.37 |

| Total content of flavonoids | 3.29 | 10.04 | 16.96 | 16.47 | 667.80 | 1,223.18 | 308.64 | 291.5 |
| Total content of phenolics quantified with LCMS | 1,278.04 | 9,986.83 | 1,139.55 | 284.49 | 750.95 | 5,101.41 | 2,274.75 | 592.20 |

**Total phenolic content (mg EAE g\(^{-1}\))**

- DPPH (\(\mu\)mol TE g\(^{-1}\))
  - 71.49 ± 20.43
  - 9,435.93 ± 624.49
  - 3,269.21 ± 46.03
  - 109.63 ± 25.03
  - 416.54 ± 1.18
  - 243.38 ± 2.46
  - 547.28 ± 3.30
  - 127.39 ± 1.92
  - 190.15 ± 2.52

- ABTS (\(\mu\)mol TE g\(^{-1}\))
  - 182.85 ± 27.13
  - 8,493.96 ± 1,860.77
  - 3,439.00 ± 90.36
  - 2.71.81 ± 16.48
  - 1,020.05 ± 175.43
  - 721.54 ± 64.70
  - 1,407.17 ± 324.07
  - 1,119.02 ± 125.70

**CONCLUSIONS**

The composition and distribution of phenolic metabolites in pecan kernels were analyzed for the first time. A rapid qualitative method of QQQ-Orbitrap MS was established. Ninety-seven phenolics were identified from eight pecan organs. Twelve phenolics were found for the first time in pecan, including dimethyl ellagic acid, isoquercitrin, kaempferol, dihydroquercetin, and citric acid. With the help of high-accuracy MS phenolic metabolites with similar MW and structures in pecan organs can be discriminated more clearly and accurately. Combined with previous qualitative information, we have identified 33 phenolic metabolites in different pecan organs. These results were mainly focused on pecan kernels, so this study can be extended to other organs. The composition and distribution of phenolic metabolites in pecan kernels and testa from different regions, varieties, and extraction methods provide a theoretical basis for using these organs as functional foods.
ACKNOWLEDGMENTS
The authors would like to thank the financial support of the National Natural Science Foundation of China (No. 31200502) and the Independent R & D projects of the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences (JSPKLB202055); the Qiong Lan Project and Postgraduate Research & Practice Innovation Program of Jiangsu Province; the Six talents peak project in Jiangsu Province; and the Jiangsu Provincial Public Health Service Subsidy of Traditional Chinese Medicine in 2019 "National Chinese Medicine Resources Census Project" (Finance Society [2019]39).

CONFLICT OF INTEREST
The authors declare that they have no conflict of interests.

ETHICAL APPROVAL
The human and animal testing was unnecessary in the current study.

ORCID
Pei Liu https://orcid.org/0000-0003-2664-0551
Xiaodong Jia https://orcid.org/0000-0002-7059-4188

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Xu M, Liu P, Jia X, et al. Metabolic profiling revealed the organ-specific distribution differences of tannins and flavonoids in pecan. Food Sci Nutr. 2020;8:4987–5006. https://doi.org/10.1002/fsn3.1797