Systematically Exploring the Chemical Ingredients and Absorbed Constituents of *Polygonum capitatum* in Hyperuricemia Rat Plasma Using UHPLC-Q-Orbitrap HRMS

Huanyu Guan 1,†, Pengfei Li 2,†, Qian Wang 1, Fanli Zeng 1, Daoping Wang 1,3, Mei Zhou 1,3, Meng Zhou 1, Xun He 1, Shanggao Liao 1,4 and Weidong Pan 1,3,*

Abstract: *Polygonum capitatum* as an ethnic medicine has been used to treat urinary tract infections, pyelonephritis and urinary calculi. In our previous study, *P. capitatum* was found to have anti-hyperuricemia effects. Nevertheless, the active constituents of *P. capitatum* for treating hyperuricemia were still unclear. In this study, an ultra-high-performance liquid chromatography coupled to quadrupole/orbitrap high-resolution mass spectrometry (UHPLC-Q-Orbitrap HRMS) was used to comprehensively detect the chemical ingredients of *P. capitatum* and its absorbed constituents in the plasma of hyperuricemia rats for the first time. Xcalibur 3.0 and Compound Discoverer 2.0 software coupled to mzCloud and ChemSpider databases were utilized for qualitative analysis. A total of 114 chemical components including phenolics, flavonoids, tannins, phenylpropanoids, amino acids, amides and others were identified or tentatively characterized based on the exact mass, retention time and structural information. Compared to the previous *P. capitatum* study, an additional 66 different components were detected. Moreover, 68 related xenobiotics including 16 prototype components and 52 metabolites were found in the plasma of hyperuricemia rats. The metabolic pathways included ring fission, hydrolysis, decarboxylation, dehydroxylation, methylation, glucuronidation and sulfation. This work may provide important information for further investigation on the active constituents of *P. capitatum* and their action mechanisms for anti-hyperuricemia effects.

Keywords: *Polygonum capitatum*; UHPLC-Q-Orbitrap HRMS; chemical profiling; metabolites

1. Introduction

Hyperuricemia (HUA) is one of the most common metabolic conditions characterized by abnormally increased serum urate levels. Long-term HUA is a main etiologic factor for the deposition of monosodium urate crystals (MSU) in joints and soft tissues resulting in gout [1]. Moreover, HUA is associated with incidences of hypertension, diabetes, obesity and chronic kidney disease [2]. Allopurinol, febuxostat and benz bromarone were selected as anti-hyperuricemic agents, although these agents exhibited some adverse effects including hypersensitivity, cardiovascular mortality risk and hepatic toxicity [3–5].

Traditional Chinese medicine (TCM) and ethnic medicine have been applied to treat hyperuricemia and gout for over thousands of years with their own unique advantages. TCM and ethnic medicine were considered important resources for discovering multitarget drugs for the treatment of hyperuricemia and gout. *Polygonum capitatum* Buch.-Ham. ex
D. Don, named Touhualiao in Chinese, was utilized as Miao ethnic medicine in China to treat urinary tract infections, pyelonephritis and urinary calculi [6]. In our previous study, *P. capitatum* was found to reduce serum urate levels to treat hyperuricemia and gouty arthritis without renal toxicities. The underlying action mechanism of *P. capitatum* involved inhibiting the expression and function of xanthine oxidase and decreasing the expressions of glucose transporter 9 (GLUT9) and urate transporter 1 (URAT1) [7]. Despite the remarkable efficacy of *P. capitatum* for anti-hyperuricemia and anti-gouty arthritis, the active constituents of *P. capitatum* related to the pharmacological effect are still not clear.

A range of the active constituents of TCM and ethnic medicine is an essential prerequisite for executing pharmacological effects. Profiling the chemical ingredients, the absorbed constituents and metabolites is beneficial for elucidating the pharmacological materials of TCM and ethnic medicine. Traditional separation technologies have been used to isolate and obtain pure components from *P. capitatum* including triterpenes [8], phenolics [8–10], flavonoids [8–10], lignans [8,9], alkaloids [10] and tannins [11], although the technology was time-consuming and it was often difficult to acquire the substances at low concentrations. Only partial phenolics of *P. capitatum* were identified by ultra-high-performance liquid chromatography–photodiode array detection coupled with triple quadrupole mass spectrometry (UHPLC-PDA-QqQ-MS) [12] and UHPLC with time-of-flight mass spectrometry (UHPLC-TOF-MS) [13]. These were not sufficient for the research on the active constituents and action mechanisms of *P. capitatum* for anti-hyperuricemia and anti-gouty arthritis. Therefore, it is necessary to comprehensively identify and characterize the chemical constituents of *P. capitatum* and its absorbed components in hyperuricemia rats.

UHPLC coupled with quadrupole/orbitrap high-resolution mass spectrometry (UHPLC-Q–Orbitrap HRMS) technology provided a sensitive and high-resolution platform for the analysis of chemical constituents at µg/kg concentration levels in complex matrix samples. The data-dependent acquisition mode of Q-Orbitrap HRMS provides MS/MS spectra with accurate mass data [14,15] which are beneficial for identifying and characterizing unknown compounds in TCM and ethnic medicine. Q-Orbitrap HRMS was also a powerful analytical technology for elucidating the metabolism of TCM and ethnic medicine in vivo due to its high sensitivity, high resolution and fast scanning capability.

In this study, a UHPLC-Q-Orbitrap HRMS method was employed to systematically clarify the chemical constituents of *P. capitatum* for the first time. Additionally, the prototypes and metabolites of *P. capitatum* in hyperuricemia rat plasma were also analyzed by the UHPLC-Q-Orbitrap HRMS technology based on neutral loss and metabolism types of representative components. Ultimately, 114 chemical constituents were tentatively identified or characterized from *P. capitatum*. Compared to the previous *P. capitatum* study using LC-MS, additional 66 different components were detected in this study. Among these, two new compounds were found, and 7 compounds were discovered in *P. capitatum* for the first time. A total of 68 related xenobiotics including 16 prototypes and 52 metabolites were detected in the hyperuricemia rats. Among them, 14 prototypes and 50 metabolites were reported for the first time. This study helped illustrate the active components and action mechanisms of *P. capitatum* for anti-hyperuricemia and anti-gouty arthritis.

2. Results and Discussion
2.1. UHPLC-Q–Orbitrap HRMS Analysis of *P. capitatum* Extract

UHPLC-Q-Orbitrap HRMS method was employed to profile the chemical constituents in *P. capitatum* extract. Under the optimized UHPLC-Q-Orbitrap HRMS conditions, the total ion current (TIC) chromatograms of *P. capitatum* extract in negative and positive ion modes are shown in Figure 1. The elemental compositions for the compound and the fragment ion were predicted within a mass tolerance of ±5 ppm. The chemical structures of the components in *P. capitatum* were elucidated by comparing their retention time, exact mass and structural information with those of authentic standards or available literature data. As a result, a total of 114 components from *P. capitatum* were unambiguously identified or tentatively characterized (Table S1 in Supplementary Materials), including 30 phenolic...
acids, 38 flavonoids, 16 phenylpropanoids, 10 tannins, 10 phenolics, 3 amino acids, 3 amides and 4 others. The chemical structures of the detected constituents were shown in Figure S1 in Supplementary Materials. Among these compounds, compounds 59, 77, 84, 96, 97, 110 and 112 were found in P. capitatum for the first time. Although positive and negative ion modes were employed, more peak signals and higher sensitivities were obtained in the negative ion mode. The fragment ions in the negative and positive ion modes are listed in Table S1.

Figure 1. The TIC chromatograms of P. capitatum extract in negative (NEG) and positive (POS) ion modes.

2.1.1. Phenolic Acids

The mass signals for phenolic acids in negative ion mode were observed. The quasi-molecular ions of phenolic acids preferred to produce the corresponding product ions by neutral loss of H$_2$O, CO and CO$_2$. Compounds 4 and 14 were unambiguously identified as gallic acid and protocatechuic acid, respectively, by comparing their retention time and mass data with those of reference standards. The [M − H]$^-$ of gallic acid at m/z 169.0133 lost a unit of CO$_2$ to form the product ion at m/z 125.0233 and subsequently discarded a H$_2$O unit and a CO group to yield the ions at m/z 107.0126 and 97.0283. The [M − H]$^-$ ion of compound 55 was 14.0158 Da (CH$_3$) more massive than that of gallic acid. The ions at m/z 168.0055 and 165.0187 were formed from the [M − H]$^-$ ion through losing -CH$_3$ and H$_2$O. Moreover, the prominent ion at m/z 139.0390 was assigned as [M − H − CO$_2$]$^-$. Compound 55 was tentatively identified as the reported 4-O-methylgallic acid [12]. Similarly, compounds 50 and 52 gave the [M − H]$^-$ ions at m/z 197.0448 and 197.0449 with the same predicted molecular formulae (C$_9$H$_4$O$_5$$^-$), which was C$_2$H$_4$ moiety more massive than that of gallic acid. Notably, the product ions at m/z 169.0133 of compound 50 corresponding to [M − H − C$_2$H$_4$]$^-$ indicated the existence of ethyl moiety. The fragment ion at m/z 125.0233 ([M − H − C$_2$H$_4$ − CO$_2$]$^- $) was also observed in the tandem mass spectrometry (MS$^2$) of compound 50. Therefore, compound 50 was tentatively identified as the reported ethyl gallate [16]. Different from the fragmentation pattern of compound 50, compound 52 generated the product ions at m/z 182.0212 ([M − H − CH$_3$]$^- $) and 166.9976 ([M − H − 2 × CH$_3$]$^- $) suggesting the presence of two methyl groups in compound 52.
Compound 52 was assigned as syringic acid [17]. Compounds 17 and 35 eluted at 3.20 min and 7.07 min showed additional hexoside groups (162 Da) compared to compounds 52 and 50. The fragmentation pathways of the aglycone ions were similar to those of compounds 52 and 50. Compounds 17 and 35 were tentatively identified as syringic acid-O-hexoside and ethyl gallate-O-hexoside. Compound 13 gave a [M – H]⁻ ion at m/z 329.0858 and exhibited the fragment ions at m/z 167.0341 ([M – H – hexose sugar]⁻), 152.0105 ([M – H – hexose sugar – CH₃]⁻), 123.0440 ([M – H – hexose sugar – CO₂]⁻) and 108.0205 ([M – H – hexose sugar-CO₂ – CH₃]⁻) assigned as the moiety of vanillic acid. Based on the above observation, compound 13 was tentatively identified as vanillic acid-O-hexoside. Compound 34 exhibited the quasi-molecular ion at m/z 481.0985 ([M – H]⁻) which was 152.0127 Da (C₇H₄O₄) more massive than that of compound 13. The [M – H]⁻ ion of compound 34 lost a vanillic acid residue and a dehydrated hexose moiety in succession to form the ions at m/z 313.0564 and 169.0133. Notably, the characteristic fragment ions at m/z 169.0133, 125.0232 and 97.0282 assigned as gallic acid were observed in the MS² spectrum of compound 34, which primarily implied a galloyl residue connected to the vanillic acid-O-hexoside. Due to the existence of vanillic acid [17] and 2-methoxy-4-hydroxyphenol-1-O-β-D-(6'-O-galloyl) glucopyranoside [10] in P. capitatum, compound 34 was tentatively identified as vanillic acid-4-O-(6'-O-galloyl)-glucopyranoside.

2.1.2. Flavonoids

Flavonoids refer to a class of natural compounds possessing a chemical skeleton of C6-C3-C6. A total of 38 flavonoids were detected in P. capitatum, including flavonols, flavan-3-ols and flavanones. Compounds 26, 67, 69, 75, 76, 93, 105 and 107 were confirmed as (+)-catechin, myricetin, quercetin-3-O-(2′′-O-galloyl)-β-D-glucopyranoside, rutin, quercetin-3-O-β-D-glucopyranoside, quercitrin, quercetin and 3′′-O-galloylquercitrin compared by authentic reference standards.

Generally, flavonoid O-glycosides usually lose saccharide moieties to produce the corresponding aglycone ions. Most of the flavonoid O-glycosides in P. capitatum were characterized by galloyl groups attached to monosaccharide residues. In the MS² spectra, the loss of a galloyl moiety (152 Da) was the characteristic fragmentation pattern of flavonoid O-glycosides in P. capitatum. For flavonoid aglycones, retro-diels-alder (RDA) fragmentation reaction and neutral losses of H₂O (18 Da), CO (28 Da) were involved. As the isomers of quercetin-3-O-(2′′-O-galloyl)-β-D-glucopyranoside (compound 69), compounds 79 and 97 showed the [M – H]⁻ ions at m/z 615.0987 (err. 1.02 ppm) and 615.0988 (err. 1.23 ppm) with the same predicted molecular formulae (C₂₆H₂₁O₁₆⁻). Compound 79 gave a similar fragment pattern to that of compound 69, where the [M – H]⁻ ion continuously lost a galloyl unit and a hexose residue to form the ion at m/z 463.0886 and 301.0352. The fragment ions at m/z 151.0027 and 107.0126 were generated from the aglycone ion through the RDA fragmentation reaction. Compound 79 was tentatively identified as the reported quercetin-3-O-(3′′-O-galloyl)-β-D-glucopyranoside [18]. The MS² spectrum for compound 97 showed the product ions at m/z 463.0881 and 316.0223 resulting from the loss of a galloyl unit and a rhamnosyl residue in succession. The fragment ions observed at m/z 164.0104 and 151.0027 were assigned as the [1,3-A]⁻ and [1,2-B]⁻ products of the RDA fragmentation pathway. The observed ions at m/z 463.0881, 316.0223, 164.0104 and 151.0027 were characteristic of myricetin-O-rhamnoside. Myricetin-3-O-rhamnoside-gallate has been identified as a main chemical constituent of Polygonum neofiliforme as homologous plant of P. capitatum [19]. Moreover, the compounds quercetin-3-O-(2′′-O-galloyl)-rhamnopyranoside and quercetin-3-O-(3′′-O-galloyl)-rhamnopyranoside were present in P. capitatum [18]. Compound 97 was tentatively identified as myricetin-3-O-(2′′-O-galloyl)-rhamnopyranoside or myricetin-3-O-(3′′-O-galloyl)-rhamnopyranoside. The proposed mass fragmentation pathway of compound 97 is shown in Figure 2.
Compound 87 detected at 14.50 min showed a quasi-molecular ion at m/z 629.0785 (C_{28}H_{21}O_{17}^-) and lost a galloyl moiety to generate the product ions at m/z 477.0678. The above [M - H]^− ion and product ions of compound 87 were 13.9797 Da higher than the corresponding ions of compound 79. The observed ions at m/z 327.0357 and 175.0240 of compound 87 suggested a glucuronic acid moiety was connected to the galloyl group. Furthermore, the ions at m/z 301.0353, 151.0027 and 107.0126 were assigned as quercetin. Therefore, compound 87 was tentatively identified as a new compound, quercetin 3-O-galloylglucuronide. Similarly, compounds 102 and 104 were tentatively identified as the reported quercetin-3-O-(6‴-O-trans-feruloyl)-β-D-galactopyranoside [20] and quercetin-3-O-(4‴-O-acetyl)-α-L-rhamnopyranoside [21], respectively. Compound 49 eluted at 8.63 min exhibited an [M - H]^− ion at m/z 735.1563 and gave the product ions at m/z 583.1091 and 447.0930 through losing a protocatechuoyl moiety and a galloyl group in succession, suggesting the existence of protocatechuoyl and galloyl residues. In addition, the characteristic ions at m/z 447.0930, 301.0348 and 243.0295 in the MS^2 spectra implied the presence of quercetin-O-rhamnoside. Although the positions of the protocatechuoyl moiety and the galloyl group were unclear, the finding of quercetin-3-O-(2‴-O-protocatechuoyl)-rhamnoside...
and 3′′-O-galloylquercitrin in this plant implied the protocatechuoyl and galloyl moieties were connected to the monosaccharide residue of compound 49. Therefore, compound 49 was tentatively identified as a new compound, quercetin-3-O-(protocatechuoyl-galloyl)-rhamnose.

2.1.3. Phenylpropanoids

Phenylpropanoids in *P. capitatum* were divided into phenylpropanoid sucrose esters, lignans and chromones derivatives. Compound 111 (*t_R* = 18.36 min) produced an [M − H]⁻ ion at *m/z* 735.2141 (C_{34}H_{32}O_{16}, err. 1.31). In the MS² spectrum, the fragment ions at *m/z* 559.1661 (C_{24}H_{31}O_{15}) and 175.0392 (C_{10}H_{2}O_{3}) were produced via the loss of the dehydrated feruloyl group. The ion at *m/z* 559.1661 lost an acetylated hexosyl residue to generate the ion at *m/z* 337.0931, and subsequently discarded another hexosyl residue to form a feruloyl moiety (*m/z* 193.0498, C_{10}H_{2}O_{4}⁻). Compound 111 was tentatively identified as the reported bistoroside B [22]. Compared to compound 111, compound 84 (*t_R* = 14.16 min, *m/z* 559.1665, [M − H]⁻, C_{24}H_{31}O_{15}) exhibited the absence of a feruloyl residue and showed a similar mass fragmentation pathway in compound 111. Compound 84 was tentatively identified as 6′-acetyl-6(or 3)-feruloylsucrose. This compound was firstly found in the *Polygonum* genus. The mass data and the fragmentation pathway of 6′-acetyl-6(or 3)-feruloylsucrose was proposed for the first time in this study (Figure 3A).

Similarly, compound 112 (*t_R* = 18.36 min, *m/z* 777.2244, [M − H]⁻, C_{36}H_{41}O_{19}⁻) showed an additional acetyl moiety (42.0103 Da) compared to compound 111. Based on the reported mass data [23], compound 112 was tentatively identified as smilaside A. Compound 110 possessed an [M − H]⁻ ion at *m/z* 705.2040 (C_{35}H_{32}O_{17}⁻) and gave the product ions at *m/z* 559.1662 (C_{24}H_{31}O_{15}⁻), 163.0389 (C_{9}H_{2}O_{3}) and 145.0284 (C_{9}H_{3}O_{2}⁻), suggesting the existence of a coumaroyl moiety. The characteristic ions of 6′-acetyl-6(or 3)-feruloylsucrose at *m/z* 559.1662, 337.0943, 193.0499 and 175.0392 were also observed in the MS² spectrum of compound 110. Compound 110 was tentatively identified as 6′-acetyl-3(or 6)-feruloyl-6(or 3)-coumaroylsucrose (Figure 3B).

Two isomeric compounds, 77 and 90, were eluted at 13.20 and 14.83 min possessing identical molecular formulae (C_{26}H_{33}O_{11}⁻). For compound 77, the fragment ions at *m/z* 359.1498, 344.1259 and 329.1027 in the MS² spectrum were generated via losing a hexosyl moiety and subsequent discarding two methyl groups. The RDA fragment ion at *m/z* 241.0500 was corresponding to the cleavage of 8-8' bond (Figure 3C). Due to the presence of isolariciresinol-9′-O-β-D-xlyopyranoside in *P. capitatum*, compound 77 was tentatively identified as isolariciresinol-9′-O-glucopyranoside. Compound 90 neutrally lost a pentose residue (132 Da) to form the aglycone ion at *m/z* 389.1605 and lost three methyl groups in succession to generate the ions at *m/z* 374.1370, 359.1136 and 344.0892, respectively, implying the existence of three methoxy groups in the aglycone moiety. Therefore, compound 90 was tentatively identified as 3(or 5′)-methoxyisolariciresinol-9′-O-xlyopyranoside.

2.1.4. Tannins

Tannins identified in *P. capitatum* in this study were classified into proanthocyanidins and ellagitannins. Proanthocyanidins were condensed tannins composed of oligomers and polymers of flavan-3-ol moieties linked mainly through 4-8' bonds. RDA reaction, heterocyclic ring fission (HRF) and quinone methide (QM) cleavage were the main mass fragmentation patterns of proanthocyanidins [24]. Ellagitannins belonging to hydrolysable tannins consisted of hexahydroxydiphenoyl (HHDP) groups and related acyl groups. The characteristic fragment ion at *m/z* 300.9991 (C_{14}H_{25}O_{8}⁻) in the MS² spectrum of ellagitannin was corresponding to an ellagic acid moiety.

A pair of isomer compounds 25 and 32 both showed the deprotonated ions at *m/z* 577.1348 ([M − H]⁻, C_{36}H_{25}O_{12}⁻) and the fragment ions at *m/z* 425.0877, 451.1039 and 289.0717 generated through RDA fragment reaction, HRF fragmentation and QM cleavage, respectively (Figure 4A), which suggested that compounds 25 and 32 were the reported procyanidin B1/B2 [25]. Compounds 43, 51 and 53 all produced the deprotonated ions at
m/z 729.1458 (C_{37}H_{29}O_{16}^-) and lost a galloyl group to generate the fragment ions at m/z 577.1352 (C_{30}H_{25}O_{12}^-). The subsequent fragmentation pathways of the ion at m/z 577.1352 were similar to those of compounds 25 and 32. Compounds 43, 51 and 53 exhibited an additional galloyl residue compared to compounds 25 and 32. Since catechin-3-O-gallate had been found in *P. capitatum*, compounds 43, 51 and 53 were tentatively identified as 3(or 3′)-O-galloyl(epi)catechin-(4,8′)-(epi)catechin.

Figure 3. Cont.
The characteristic fragment ion at m/z 257.0089 ([M−H]−) showed the fragment ions at m/z 219.0293 and 203.0343 resulting from the successive or simultaneous loss of H2O and CO groups in their MS2 spectra. Compound 110 at tR 5.58 min gave a deprotonated ion at m/z 329.1027. Since brevifolin has previously been found in P. capitatum, the product ion at m/z 275.0197 ([M−H]−) was tentatively attributed to phyllanthusiin C. The MS2 spectrum of phyllanthusiin C revealed the intense product ion at m/z 257.0197 ([M−H]−) and the fragment ions at m/z 231.0291 ([M−CH3−H2O]−), 247.0243 ([M−H−CH3]−) and 309.0245 ([M−H−CO2]−). The subsequent fragmentation pathway of the ion at m/z 257.0089 ([M−H]−) exhibited an [M−H−2H2O−CO]− ion at m/z 191.0342 and 147.0440. Since brevifolin has previously been found in P. capitatum, compound 110 was tentatively identified as brevifolin. Compound 84 at tR 6.71 min gave a deprotonated ion at m/z 300.9991 (C14H5O8) and the fragment ions at m/z 289.0717 generated through RDA fragment reaction, HRF fragmentation and QM cleavage were the main mass fragmentation pathways (Figure 4C) of phyllanthusiin C. The fragmentation pathway (Figure 4C) of phyllanthusiin C was proposed for the first time.

**Figure 3.** The fragmentation patterns of compounds 84 (A), 110 (B) and 77 (C).

**Figure 4.** The fragmentation pathways of compounds 25 or 32 (A), 64 (B) and 33 (C). The dotted purple and green lines represent the cleavage positions of compound 33 in MS2 spectrum.
Compound 64 (t_R = 11.36 min) had a [M – H]^− ion at m/z 937.0953 (C_{41}H_{29}O_{28}^-) and gave the fragment ions at 893.1055 and 785.0838 generated from the loss of a CO_2 unit and a galloyl residue, respectively. The product ion at m/z 785.0838 continuously discarded a galloyl residue to form the ion at m/z 615.0617. In addition, the ions at m/z 300.9991 and 275.0198 were assigned to the moieties of ellagic acid and urolithin M5. Compound 64 was tentatively identified as davidin. The proposed mass fragment pathway of compound 64 was shown in Figure 4B. Compound 33 (t_R = 6.71 min) exhibited an [M – H]^− ion at m/z 925.0955 (C_{40}H_{28}O_{26}^-). The MS² spectra for compound 33 revealed the intense product ion at m/z 605.0789 resulting from the neutral loss of a dehydrated HHDP residue (C_{14}H_{24}O_9). The characteristic fragment ions at 615.0638 and 309.0245 were generated through losing a C_{13}H_{20}O_9 moiety, which implies the existence of the residue region composed of D- and E-rings. Compound 33 was tentatively attributed to phyllanthusin C. The MS² fragmentation pathway (Figure 4C) of phyllanthusin C was proposed for the first time.

2.1.5. Other Phenolics

Ten phenolics with a small molecular mass (<350 Da) and less than 15 carbons were tentatively identified in P. capitatum. The common fragment characteristic of phenolics is a successively or simultaneous loss of H_2O and CO groups in their MS² spectra. Compound 29 at t_R 5.58 min gave a deprotonated ion at m/z 247.0246 (C_{12}H_{26}O_6^-). The MS² spectrum of compound 29 showed the fragment ions at m/z 219.0293 and 203.0343 resulting from the neutral loss of CO and CO_2 unit from quasi-molecular ion, respectively. Furthermore, the ion at m/z 219.0293 went on losing a CO and a CO_2 unit in succession to form the ions at 191.0342 and 147.0440. Since brevifolin has previously been found in P. capitatum in the reported literature [20], compound 29 was tentatively identified as brevifolin. Compound 46 gave a quasi-molecular ion at m/z 275.0197 ([M – H]^−, C_{13}H_{29}O_7^-) and the fragment ions at m/z 257.0089 ([M – H – H_2O]^−), 247.0243 ([M – H – CO]^−), 231.0291 ([M – H – CO_2]^−), 229.0138 ([M – H – H_2O – CO]^−) and 203.0343 ([M – H – CO – CO_2]^−). A lactone moiety or a carboxyl group was implied to be present in compound 46. In addition, the ion at m/z 191.0341 was generated through an RDA fragmentation reaction from the ion at m/z 231.0291. Compound 46 was tentatively identified as urolithin M5. Urolithin M5 was an intestinal bacterial metabolite of ellagitannin davidin from P. capitatum [26] and was also found in natural higher plants from diverse families [27,28]. The compound might be biosynthesized through the polyketide pathway [29,30] by endophytic fungi residing in raw P. capitatum. Compound 8 (t_R = 2.09 min) gave an [M – H]^− ion at m/z 243.0507 (C_{10}H_{11}O_7^-) and exhibited the characteristic ions of gallic acid moiety at m/z 169.0134, 125.0233 and 107.0125. The glycerol residue at m/z 91.0388 was found in the spectrum of compound 8. Based on the reported literature [31], compound 8 was tentatively identified as galloyl-glycerol.

2.1.6. Amino Acids and Amides

Three Amino acids and 3 amides were identified or characterized in P. capitatum. Compound 6 (t_R = 2.03 min, [M + H]^+), m/z 166.0864) was tentatively identified as the reported phenylalanine [12]. Compound 12 (t_R = 2.37 min) gave an [M + H]^+ ion at m/z 328.1386 (C_{13}H_{23}O_7N^+) and lost a fructose moiety to form the characteristic ion of phenylalanine (m/z 166.0859 and 120.0808). Comparing the mass information of compound 12 to those in the reported literature [32], compound 12 was tentatively identified as fructose-phenylalanine. Compound 96 eluted at 15.82 min showed a quasi-molecular ion at m/z 330.1335 (C_{18}H_{20}NO_2^+) and gave the base-peak ion at m/z 177.0547 assigned to ferulic acid through neutral loss of an octopamine residue. The ions at m/z 145.0284 and 117.0383 were formed through successively losing CH_2O and CO from the ion at m/z 177.0547. Moreover, the ion at m/z 194.0815 was generated through carbon-nitrogen bond cleavage (Figure 5A). Compound 96 was tentatively assigned as N-feruloyloctopamine. For compound 101 (t_R = 17.58 min, [M + H]^+, C_{18}H_{20}NO_4^+), a tyramine moiety replaced the
octopamine moiety compared to 96. The MS² data of compound 101 was similar to that of compound 96. Compound 101 was tentatively assigned as N-feruloyltyramine (Figure 5A).

Figure 5. The fragmentation patterns of compounds 96, 101 (A) and 106 (B).

2.1.7. Others

Compound 106 eluted at 17.80 min produced an [M + H]⁺ ion at m/z 309.0863 (C₁₇H₁₃O₄N₂⁺), suggesting compound 106 might be an alkaloid. In the MS/MS analysis, the fragment ions at m/z 281.0915, 263.0811 and 235.0862 were generated via ring fission and lost CO, H₂O and CO units in succession. Furthermore, the [M + H]⁺ ion of
compound 106 lost a C₂H₂O₂ moiety of hydroxymethylfuran ring and underwent RDA fragment reaction to yield the fragment ion at m/z 180.0804. The product ion at m/z 206.0835 was formed though losing C₂H₃O₂- and CO₂ from the [M + H]+ ion. Compound 106 was tentatively assigned as flazin. The proposed mass fragment pathway of flazin was shown for the first time in the study (Figure 5B). Compound 41 (tR = 7.67 min, [M – H]−, m/z 387.1660) generated the aglycone ion at m/z 207.1021 (C₁₂H₁₅O₃−) through losing hexose sugar, and further lost one molecule of CO₂ to form the ion at m/z 163.1118. Compound 41 was tentatively identified as the reported 12-hydroxyjasmonic acid glucoside [10].

2.2. UHPLC-Q-Orbitrap HRMS Analysis of the Prototype Compounds in Hyperuricemia Rat Plasma

The TIC chromatograms and mass data of rat plasma from hyperuricemia and drug-treated groups were compared to analyze P. capitatum-related exogenous components. The peaks that appeared at the same positions in the TIC chromatograms of both the dosed rat plasma and the herb extract but not in the chromatogram of the model rat plasma were regarded as prototype constituents. As a result, 16 prototype components of P. capitatum were found in hyperuricemia rat plasma. The detailed mass information is shown in Table S1. Among these, ellagic acid, 5,7-dihydroxychromone, quercetin-3-O-glucuronide, quercitrin, 3,3′-di-O-methyllellagic acid, flazin, salidoside, 3,4,5-trimethoxyphenol-1-O-β-D-glucopyranoside, fructose-phenylalanine, nudiposide, quercetin-3-O-β-D-galactoside, quercetin-3-O-β-D-glucopyranoside, kaempferol-4-O′-rutinoside, N-feruloyltyramine and afzelin were found in rat plasma after oral administration of P. capitatum extracts for the first time.

2.2. UHPLC-Q-Orbitrap HRMS Analysis of the Prototype Compounds in Hyperuricemia Rat Plasma

The procedures for identification of metabolites included speculating probable metabolites according to the biotransformation rules of original compounds, extracting the [M – H]− or [M + H]+ ions of probable metabolites from dosed plasma in full-scan mass mode and analyzing the MS² information of the detected peak. The detected metabolic mechanism of P. capitatum in hyperuricemia rats involved ring fission, hydrolysis, decarboxylation, dehydroxylation, methylation, glucuronidation and sulfation. In this study, a total of 52 metabolites of P. capitatum in rat plasma were tentatively identified. Among them, 50 metabolites were revealed for the first time. The details of the characterized metabolites are listed in Table 1.

2.3.1. Characterization of Phenolic-Related Metabolites

A total of 22 constituents were identified as phenolic-related metabolites, including 14 gallic acid-related (M1–M3, M5–M6, M7–M12, M14, M20 and M24), 3 syringic acid/ethyl gallate-related (M18, M19 and M26), 2 dimethylellagic acid-related (M43 and M47), a vanillic acid-related (M17) and a protocatechuic acid-related (M4) metabolites.

M18 (tR = 5.67 min) and M19 (tR = 5.94 min) exhibited the same quasi-molecular ion at m/z 373.0775 ([M – H]−, C₁₅H₁₇O₁₁−) and the same fragment ion at m/z 197.0448 by the cleavage of a dehydrated glucuronic acid moiety, suggesting the two metabolites were the glucuronidation products. Furthermore, M18 showed the characteristic fragment ions at m/z 197.0448, 169.0144 and 125.0233 of ethyl gallate, while M19 showed the fragment ions at m/z 197.0447, 182.0208, 166.9976 assigned to syringic acid. Therefore, M18 and M19 were identified as ethyl gallate glucuronide and syringic acid glucuronide, respectively. M20 (tR = 6.20 min) and M26 (tR = 7.02 min) exhibited the quasi-molecular ions [M-H]− at m/z 277.0022 which were 79.9573 Da (SO₃) more than that of 3,4-O-dimethylgallic acid or syringic acid and yielded the characteristic product ions of the two isomers at m/z 197.0449, 182.0213 and 166.9977. M20 and M26 were preliminarily assigned as 3,4-O-dimethylgallic acid sulfate or syringic acid-4-O-sulfate. To elucidate the exact conjugation site of M20 and M26, their ClogP values were calculated through ChemBioDraw Ultra 20.0 software. Generally, compounds with larger ClogP values tend to form longer retention times in reverse-phase chromatography. The ClogP value of 3,4-O-dimethylgallic acid...
sulfate (−0.53863) is smaller than that of syringic acid-4-O-sulfate (−0.18863). Thus, M20 and M26 were speculated as 3,4-dimethylylgallic acid sulfate and syringic acid-4-O-sulfate. M2 and M19 presented [M − H]− ions at m/z 359.0620 (C14H15O11−) and lost a dehydrated glucuronic acid residue, a methyl group and a CO2 unit to form the ions at m/z 183.0292, 168.0068 and 124.0154, implying M2 and M19 were the methylated and glucuronidated products of gallic acid. Considering both 4-O-methylgallic acid and the 3-O-methylgallic acid were the main methylated metabolites of gallic acid [33] and 3-OH position was easier to be glucuronidated due to the smaller steric hindrance, M2 with higher peak intensity was speculated as 4-O-methylgallic acid-3-O-glucuronide. M19 was tentatively identified as 3-O-methylgallic acid-4-O-glucuronide. Similarly, M7 lost an SO3 group to yield the aglycone ion assigned to methylated gallic acid. M7 was tentatively identified as methylgallic acid sulfate.

Table 1. The metabolites of P. capitatum in hyperuricemia rat plasma.

| Peak No. | Compounds                                      | Molecular Formula | tR  | Ion Mode | Precursor Ion | Error (ppm) | Product Ions         |
|---------|-----------------------------------------------|-------------------|-----|----------|---------------|-------------|----------------------|
| M1      | Gallic acid glucuronide                       | C13H14O11         | 1.93| negative mode | 345.0464     | 3.25        | 169.0132             |
| M2      | 4-O-methylgallic acid-3-O-glucuronide         | C14H16O11         | 1.93| negative mode | 359.0620     | 2.96        | 183.0292, 168.0068, 124.0154 |
| M3      | Gallic acid sulfate                           | C7H6O8S           | 2.13| negative mode | 248.9707     | 3.15        | 169.0132             |
| M4      | Protocatechelic acid sulfate                  | C7H6O7S           | 2.23| negative mode | 232.9757     | 2.62        | 153.0183, 109.0295   |
| M5      | 2-O-Methylpyrogallol sulfate                  | C7H6O6S           | 2.35| negative mode | 218.9963     | 2.31        | 139.0389, 124.0154   |
| M6      | 1-O-Methylpyrogallol-3-O-sulfate              | C7H6O6S           | 2.72| negative mode | 218.9964     | 2.72        | 139.0390, 124.0154   |
| M7      | Methylgallic acid sulfate                     | C8H8O8S           | 2.75| negative mode | 262.9866     | 3.63        | 183.0291, 168.0055   |
| M8      | Pyrogallol-1-O-sulfate or Pyrogallol-2-O-sulfate | C6H6O6S          | 2.85| negative mode | 204.9806     | 2.46        | 125.0233             |
| M9      | 2-O-methylpyrogallol-1-O-glucuronide         | C13H16O9          | 3.02| negative mode | 315.0725     | 4.51        | 139.0401, 124.0166, 113.0245 |
| M10     | 4-O-methylgallic acid or 3-O-methylgallic acid | C8H6O5           | 4.04| negative mode | 183.0291     | 1.64        | 168.0055, 124.0155   |
| M11     | Phenol sulfate                                | C6H6O4S           | 4.08| negative mode | 172.9905     | 1.24        | 93.0333              |
| M12     | Pyrogallol-1-O-glucuronide or Pyrogallol-2-O-glucuronide | C12H14O9         | 4.28| negative mode | 301.0564     | 3.39        | 125.0232             |
| M13     | Dehydroxylation and ring cleavage and sulfation of catechin (3-hydroxyphenylacetic acid sulfate) | C8H6O6S         | 4.32| negative mode | 230.9964     | 2.79        | 187.0063, 151.0393, 107.0490, 79.9560 |
| M14     | 1-O-Methylpyrogallol-2-O-sulfate              | C7H6O6S           | 4.74| negative mode | 218.9968     | 4.82        | 139.0402, 124.0166   |
| M15     | Glucuronidation of 5,7-Dihydroxychromone      | C15H14O10         | 5.35| negative mode | 353.0510     | 2.00        | 177.0183             |
| M16     | m-Coumaric acid sulfate or p-coumaric acid sulfate | C9H8O6S         | 5.50| negative mode | 242.9966     | 3.27        | 163.0392, 119.0493   |
Table 1. Cont.

| Peak No. | Compounds                                      | Molecular Formula | tR   | Ion Mode          | Precursor Ion | Error (ppm) | Product Ions                                    |
|---------|------------------------------------------------|------------------|------|-------------------|---------------|-------------|-----------------------------------------------|
| M17     | Sulfation of vanillic acid                     | C₈H₆O₇S          | 5.63 | negative mode     | 246.9914      | 2.92        | 167.0340, 152.0107,                           |
| M18     | Ethyl gallate glucuronide                      | C₁₅H₁₈O₁₁        | 5.67 | negative mode     | 373.0775      | 2.61        | 197.0448, 169.0144, 125.0233                  |
| M19     | Syringic acid glucuronide                      | C₁₅H₁₈O₁₁        | 5.94 | negative mode     | 373.0775      | 2.61        | 197.0447, 182.0208, 166.9976                  |
| M20     | 3,4-O-dimethylgallic acid sulfate              | C₉H₁₀O₈S         | 6.20 | negative mode     | 277.0022      | 3.49        | 197.0449, 182.0213, 166.9977                  |
| M21     | 5-(3',4'-Hydroxyphenyl)-γ-valerolactone        | C₁₇H₂₀O₁₀        | 6.46 | negative mode     | 383.0979      | 1.64        | no fragment                                   |
| M22     | Methylcatechin glucuronide                     | C₂₂H₂₄O₁₂        | 6.64 | negative mode     | 479.1188      | 0.91        | 465.1025, 303.0863                            |
| M23     | Urolithin A glucuronide-sulfate                | C₁₉H₁₆O₁₃S       | 6.74 | negative mode     | 483.0233      | 1.10        | 403.0664, 227.0346                            |
| M24     | 3-O-methylgallic acid-4-O-glucuronide          | C₁₄H₁₆O₁₁        | 6.81 | negative mode     | 359.0617      | 2.29        | 183.0293, 168.0068, 124.0154                  |
| M25     | 5-(3',4'-Dihydroxyphenyl)-γ-valerolactone      | C₁₁H₁₂O₂S        | 6.88 | negative mode     | 287.0233      | 4.35        | 207.0504, 163.0764, 79.9574                   |
| M26     | Syringic acid-4-O-sulfate                      | C₉H₁₀O₈S         | 7.02 | negative mode     | 277.0021      | 3.16        | 197.0454, 182.0213, 166.9976                  |
| M27     | Quercitrin glucuronide                         | C₂₇H₂₈O₁₇        | 7.20 | negative mode     | 623.1252      | 1.55        | 447.0935                                      |
| M28     | Sulfation of 5,7-Dihydroxycromone              | C₉H₆O₇S          | 7.41 | negative mode     | 256.9760      | 3.85        | 177.0186, 133.0284                            |
| M29     | Trihydroxyflavanone glucuronide               | C₂₂H₂₀O₁₂        | 7.60 | negative mode     | 463.0884      | 2.76        | 287.0561                                      |
| M30     | 5-(3'-Hydroxyphenyl)-γ-valerolactone sulfate   | C₁₁H₁₂O₆S        | 8.09 | negative mode     | 271.0280      | 3.19        | 191.0706, 147.0820                            |
| M31     | Isolariciresinol-4 (or 4')-O-glucuronide       | C₂₆H₃₂O₁₂        | 8.39 | negative mode     | 535.1819      | 1.64        | 359.1497, 344.1266, 329.1036, 241.0507       |
| M32     | Isolariciresinol-4 (or 4')-O-glucuronide       | C₂₆H₃₂O₁₂        | 8.55 | negative mode     | 535.1822      | 2.21        | 359.1498, 344.1259                            |
| M33     | Glucuronidation of 3,5'-dimethoxy-isolariciresinol | C₂₈H₃₆O₁₄    | 8.72 | negative mode     | 595.2025      | -0.07       | 419.1709, 404.1469                            |
| M34     | Urolithin C glucuronide                        | C₁₉H₁₆O₁₁        | 9.47 | negative mode     | 419.0620      | 2.61        | 243.0300                                      |
| M35     | Isolariciresinol-9 (or 9')-O-glucuronide       | C₂₆H₃₂O₁₂        | 9.65 | negative mode     | 535.1821      | 2.00        | 359.1498                                      |
| M36     | Isolariciresinol-9 (or 9')-O-glucuronide       | C₂₆H₃₂O₁₂        | 9.86 | negative mode     | 535.1823      | 2.44        | 359.1504, 344.1267, 329.1029, 241.0507       |
| M37     | Sulfation of Isolariciresinol                  | C₂₀H₂₄O₆S        | 10.00| negative mode     | 439.1073      | 2.19        | 359.1497                                      |
| Peak No. | Compounds                        | Molecular Formula | tR  | Ion Mode        | Precursor Ion       | Error (ppm) | Product Ions                                                                 |
|---------|----------------------------------|-------------------|-----|-----------------|---------------------|------------|-------------------------------------------------------------------------------|
| M38     | Urolithin A glucuronide          | C_{19}H_{16}O_{10} | 10.13 | negative mode   | 403.0669            | 2.20       | 227.0345, 199.0388, 183.0446, 175.0239, 155.0491                              |
| M39     | Quercetin diglucuronide          | C_{27}H_{26}O_{19} | 11.42 | negative mode   | 653.0994            | 0.98       | 447.0671, 301.0345                                                           |
| M40     | Urolithin C sulfate              | C_{13}H_{12}O_{9}S | 12.80 | negative mode   | 322.9865            | 2.87       | 243.0297                                                                     |
| M41     | 3-O-Methylquercetin glucuronide sulfate | C_{22}H_{20}O_{16}S | 13.07 | negative mode   | 571.0396            | 1.57       | 491.0832, 315.0511, 300.0275, 148.0155                                     |
| M42     | Urolithin A sulfate              | C_{13}H_{12}O_{9}S | 13.60 | negative mode   | 306.9917            | 1.03       | 227.0346, 199.0398, 183.0446                                                 |
| M43     | 3,3′-Di-O-methylellagic acid glucuronide | C_{22}H_{18}O_{14} | 13.95 | negative mode   | 505.0623            | 2.10       | 329.0303, 314.0048, 298.9833, 270.9883                                     |
| M44     | Kaempferol glucuronide           | C_{21}H_{15}O_{12} | 15.11 | negative mode   | 461.0726            | 2.42       | 285.0402, 255.0293                                                           |
| M45     | Methylation of Ellagic acid      | C_{15}H_{10}O_{8}  | 15.82 | negative mode   | 315.0146            | 3.35       | 299.9910                                                                     |
| M46     | Naringenin glucuronide           | C_{21}H_{20}O_{11} | 15.82 | negative mode   | 447.0934            | 1.22       | 271.0612                                                                     |
| M47     | 3,3′-Di-O-methylellagic acid sulfate | C_{16}H_{10}O_{11}S | 16.48 | negative mode   | 408.9866            | 1.52       | 329.0304, 314.0062, 298.9833                                                  |
| M48     | Sulfation and loss of 2 × oxygen of catechin | C_{15}H_{14}O_{6}S | 16.86 | negative mode   | 321.0436            | 2.73       | 241.0866, 147.0440, 135.0440, 121.0283                                      |
| M49     | Kaempferol sulfate               | C_{15}H_{10}O_{9}S | 17.51 | negative mode   | 364.9972            | 2.77       | 285.0402, 255.0293                                                           |
| M50     | Methylation of Quercetin         | C_{22}H_{20}O_{13} | 18.03 | negative mode   | 491.0829            | 1.78       | 315.0516, 300.0273                                                           |
| M51     | Methylation of Procyanadin B1 or Procyanadin B2 | C_{31}H_{28}O_{12} | 20.28 | negative mode   | 591.1525            | 4.26       | no fragment                                                                  |
| M52     | Flazin methyl ether              | C_{18}H_{14}O_{4}N_{2} | 20.68 | positive mode   | 323.1024            | −0.85      | 263.0814, 206.0837, 180.0806                                                 |

M43 (tR = 13.95 min, m/z 505.0623, [M − H]−) and M47 (tR = 16.48 min, m/z 408.9866, [M − H]−) showed the same aglycone fragment ion at m/z 329.0303 through the loss of a dehydrated glucuronic acid and an SO3 unit, respectively. The ion at m/z 329.0303 discarded two methyl group in succession to yield the ions at m/z 314.0048 and 298.9833. The fragmentation behaviors of the aglycone ion at m/z 329.0303 was consistent with that of 3,3′-di-O-methylellagic acid. M43 and M47 was considered as 3,3′-di-O-methylellagic acid glucuronide and 3,3′-di-O-methylellagic acid sulfates.

2.3.2. Characterization of Flavonoid-Related Metabolites

Six flavonols-related, 2 flavaneone-related and 7 flavanols-related metabolites of P. capitatum were detected in dosed rat plasma. Sulfation, glucuronidation and methylation were the main metabolic pathways of the flavonoid. Moreover, ring fission and dehydroxylation were observed in the metabolic fate of flavanols (catechin or epicatechin).

M44 and M49 exhibited [M − H]− ions at m/z 461.0726 (C_{21}H_{17}O_{12}−) and 364.9972 (C_{15}H_{9}O_{6}S−), respectively. The same fragment ions at m/z 285.0402 of M44 and M49 were
corresponding to the loss of a dehydrated glucuronic acid and an SO₃ group from their [M – H]⁻ ions, respectively. The fragment at m/z 285.0402 furtherly lost a CH₂O moiety to form the ion at m/z 255.0293. The fragment pattern of the aglycone ion was consistent with that of kaempferol in P. capitatum extract. According to the reported literature [34], M₄₄ and M₄₉ were identified as kaempferol glucuronide and kaempferol sulfate, respectively. M₄₁ (tₚ = 13.07 min) produced a [M – H]⁻ ion at m/z 571.0396 (C₂₅H₁₉O₁₆S⁻) and dissociated into the fragment ions at m/z 491.0832 (C₂₂H₁₉O₁₃⁻) and 315.0511 (C₁₆H₁₁O₇⁻) ascribed to the continuous losses of an SO₃ unit and a glucuronic acid residue. The ion at m/z 315.0511 lost a methyl group to yield the product ion at m/z 300.0275 and furtherly was subjected to an RDA fragmentation reaction to form the ion at m/z 148.0155. The cracking path of the ion at m/z 315.0511 was similar to 3-O-methylquercetin [35]. M₄₁ was speculated as glucuronidation and sulfation of 3-O-methylquercetin.

Ring cleavage was a common metabolic pathway of (epi)catechin in vivo [36,37]. These metabolites generated through ring cleavage were further bio-transformed through sulfation or glucuronidation. M₁₃ (tₚ = 4.32 min) showed an [M – H]⁻ ion at m/z 230.9964 (C₈H₄O₆S⁻) and an aglycone ion at 151.0393 through loss of an SO₃ unit. The [M – H]⁻ ion produced the fragment ions at m/z 187.0063 (C₇H₂O₄S⁻) and 107.0490 (C₇H₂O⁻) by discarding a CO₂ group and an SO₃ group in succession, which was in high accordance with mass fragment pattern of 3-hydroxyphenylacetic acid sulfate [36]. M₁₃ was considered the metabolite of (epi)catechin through dehydroxylation, ring cleavage and sulfation (3-hydroxyphenylacetic acid sulfate). M₂₅ (tₚ = 6.88 min) showed an [M – H]⁻ ion at m/z 287.0233 (C₁₇H₁₁O₇S⁻) and an [aglycone – H]⁻ ion at m/z 207.0504 generated by the loss of an SO₃ moiety. The ion at m/z 163.0764 was yielded from the [aglycone – H]⁻ ion through losing a CO₂ unit. According to the reported metabolic pathway of (epi)catechin [38], M₂₅ was tentatively characterized as 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone sulfate. Compared to M₂₅, M₃₀ exhibited the absence of an oxygen atom (16 Da) and a similar fragment pattern to M₂₅. M₃₀ was tentatively assigned as 5-(3′-hydroxyphenyl)-γ-valerolactone sulfate or 5-(4′-hydroxyphenyl)-γ-valerolactone sulfate. Similarly, 5-(3′,4′-hydroxyphenyl)-γ-valerolactone glucuronide (M₂₁, C₁₇H₁₉O₁₀⁻) was tentatively identified in spite of no fragment ions obtained in the MS² spectrum [36]. M₁₆ (tₚ = 5.50 min) produced a [M – H]⁻ ion at m/z 242.9966 (C₈H₄O₆S⁻). In the MS² spectrum, the fragment ions at m/z 163.0392 ([M – H – SO₃⁻]), 135.0443 ([M – H – SO₃ – CO⁻]) and 119.0491 ([M – H – SO₃ – CO₂]⁻) were consistent with the fragment pathway of m-coumaric acid or p-coumaric acid [39]. M₁₆ was tentatively assigned as m-coumaric acid sulfate or p-coumaric acid sulfate. Based on the above data, the possible metabolic pathways of (epi)catechin in hyperuricemia rats administered orally with P. capitatum extract are shown in Figure 6A.

2.3.3. Characterization of Phenylpropanoid-Related Metabolites

A total of 8 phenylpropanoid-related metabolites were detected in rat plasma, mainly from the products ofisoricresinol and 5,7-dihydroxycromone. M₃₁ (tₚ = 8.39 min), M₃₂ (tₚ = 8.55 min), M₃₅ (tₚ = 9.65 min) and M₃₆ (tₚ = 9.86 min) showed the same quasi-molecular ion [M – H]⁻ at m/z 535.1823 (C₂₆H₃₁O₁₂⁻) and gave the fragment ions at m/z 359.1497 ([M – H – glucuronyl unit]⁻), 344.1266 ([M – H – glucuronyl unit – CH₃]⁻) and 329.1036 ([M – H – glucuronyl unit – 2 × CH₃]⁻) in their MS² spectra. The ion at m/z 241.0507 was formed from the ion at m/z 329.1036 through an RDA fragmentation pathway. M₃₁, M₃₂, M₃₅ and M₃₆ were preliminarily assigned as glucuronidation of isoricresinol. The conjugation sites of the four metabolites were speculated by ClogP values. M₃₁ (ClogP = 1.3851) and M₃₂ (ClogP = 1.3851) were tentatively identified as isoricresinol-4 (or 4')-O-glucuronide. M₃₅ (ClogP = 0.7404) and M₃₆ (ClogP = 0.7404) were tentatively identified as isoricsiresinol-9 (or 9')-O-glucuronide.
Figure 6. The possible metabolic pathways of (epi)catechin (A) and ellagitannins (B) in hyperuricemia rats administered orally with *P. capitatum* extract.

2.3.4. Characterization of Tannis-Related Metabolites

According to the reported literature, although ellagitannins were not absorbed in vivo, ellagitannins located at the distal segment of the gastrointestinal tract could be bio-transformed by the intestinal bacteria into dibenzo-α-pyrones derivatives [40]. Compared to the base
peak chromatography (BPC) of the plasma from the hyperuricemia group, a peak with high intensity at 10.13 min (M38) was detected in the BPC of dosed rat plasma. M38 exhibited an [M − H]⁻ ion at m/z 403.0669 (C₁₀H₁₅O₁₀⁻) and lost a dehydrated glucuronic acid residue to form the ions at m/z 227.0345 ([M − H − glucuronyl unit]⁻) and 175.0239 (glucuronyl unit), suggesting the M38 was a glucuronic conjugate. The aglycone ion at m/z 227.0345 furtherly lost a CO group and a CO₂ unit to generate the fragment ions at m/z 199.0388 and 183.0446, respectively. The ion at m/z 155.0491 was formed through the combined loss of CO and CO₂ from the aglycone ion. The fragment pattern of the aglycone ion was consistent with that of urolithin A [41]. M38 was tentatively identified as urolithin A glucuronide. M42 (m/z 306.9917, [M − H]⁻, C₁₃H₂₃O₇S⁻) lost an SO₃ group to yield the aglycone ion at m/z 227.0345 assigned to urolithin A. M42 was identified as urolithin A sulfate. Similarly, M23 (tᵣ = 6.74 min) lost an SO₃ group and a dehydrated glucuronic acid moiety to form the aglycone fragment of urolithin A. M23 was speculated as glucuronidation and sulfation product of urolithin A. Compared to the quasi-molecular ions and the corresponding fragment ions of M38 and M42, M34 and M40 showed an additional oxygen atom (15.9951 Da). M34 and M40 were identified as urolithin C glucuronide and urolithin C sulfate, respectively. The possible metabolic pathways of ellagitannins in hyperuricemia rats orally administered with P. capitatum were shown in Figure 6B.

2.3.5. Characterization of Alkaloid-Related Metabolites

M52 (tᵣ = 20.68 min) showed an [M + H]⁺ ion at m/z 323.1024 (C₁₈H₁₅O₄N₂⁺) that was more 14.0161 Da (CH₂ moiety) than that of flazin. The MS² spectrum of M52 in positive ion mode showed the fragment ions at m/z 263.0814, 206.0837 and 180.0806 assigned to flazin as discussed above. Furthermore, M52 detected at m/z 321.0880 ([M − H]⁻) in negative ion mode yielded the fragment ions at 291.0775 and 277.0612 by losing CH₂O and C₂H₄O₂, suggesting the existence of a methoxy group in M52. The ion at m/z 277.0612 furtherly lost a CO₂ group and a molecule of H₂O to form the ion at m/z 233.0719 and 259.0511. M52 was tentatively identified as flazin methyl ether.

3. Materials and Methods

3.1. Material and Reagents

The herb of P. capitatum was collected from Qianxi county, Guizhou province, China and was identified by Qingde Long (Guizhou Medical University) as the whole plant of Polygonum capitatum Buch.-Ham. ex D. Don. The voucher specimen of P. capitatum (No.: PC20201103) was deposited in the Herbarium of Guizhou Medical University.

The reference standards of gallic acid, protocatechuic acid, (+)-catechin, rutin, quercitrin, quercetin and emodin were purchased from Chengdu Chroma-Biotechnology Co., Ltd. (Chengdu, China). The pure materials of myricitrin, quercetin-3-O-(2′″-O-galloyl)-β-D-glucopyranoside, quercetin-3-O-β-D-glucopyranoside, cis-N-caffeoyltaramine and 3″-O-galloylquercetin were obtained in our laboratory. The purities of the reference standards were determined to be more than 98% by HPLC (+DAD). Hypoxanthine and potassium oxonate were purchased from the Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). Urate assay kits were purchased from Nanjing Jiansheng Bioengineering Institute (Nanjing, China).

HPLC-grade methanol and acetonitrile were acquired from Honeywell Burdick & Jackson Company (Morristown, NJ, USA). Formic acid (MS grade) was obtained from Fisher Scientific (Madrid, Spain). Deionized water for HPLC analysis was prepared using a Milli-Q water purification system (Millipore, Milford, MA, USA). All other reagents were of analytical grade.

3.2. Preparation of Mixed Standard Solutions

The stock solutions of standards were prepared by weighting appropriate amounts of 13 reference substances individually and dissolving them in methanol at a concentration of 1.0 mg/mL. The final mixed standard solution (200 ng/mL) was obtained by mixing
3.3. Preparation of *P. capitatum* Samples

The dried raw herb of *P. capitatum* (1453 g) was weighed and crushed into powder. The obtained powder was immersed in a ten-fold volume of distilled water for 30 min and decocted three times by boiling for 1 h. The decoctions were filtered to remove the herbal residue. The supernatants were merged together and concentrated to yield the extract residue (291.1 g, the extraction rate 20.03%).

10 mg of the obtained extract residue was dissolved in 1 mL of 60% \((v/v)\) methanol and ultrasonicated for 30 min at 100 kHz. After centrifuging at 12,000 rpm for 10 min, 10 \(\mu L\) of the supernatant was used for UHPLC-Q-Orbitrap HRMS analysis.

3.4. Animal Treatment and Drug Administration

A total of 18 male Sprague-Dawley (SD) rats (weighing 200 \(±\) 20 g) were obtained from Changsha Tianqin Biotechnology Company (Hunan, China). The rats were housed under a standard 12-h light-dark cycle at 25 \(±\) 2 °C and 60 \(±\) 5% humidity with free access to water and a normal diet for 7 days. All of the experiments were approved by the Animal Care Welfare Committee of Guizhou Medical University (approval number 2100138) and performed according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

Rats were randomly divided into three groups with six animals per group (control, hyperuricemia and drug-treated groups). Hyperuricemia was induced in the rats according to the described method previously [42]. Briefly, except for the control group, intragastric hypoxanthine (500 mg/kg/day) and intraperitoneal injection of potassium oxonate (100 mg/kg/day) were given to the rats for 7 days. The animals in the control group received physiological saline in a similar fashion. On the 4th day of hyperuricemia induction, the *P. capitatum* extract (5 g/kg/day) was administered orally to the rats in the drug-treated group at 1 h after dosing of the modeling agents for 3 days. The serum urate levels in the control and hyperuricemia rat groups were detected by urate assay kits during the experimental period. The serum urate levels in the hyperuricemia rat group significantly increased compared to those of the control rats \((p < 0.05)\), indicating the successful establishment of the hyperuricemia model.

3.5. Collection and Preparation of Plasma Sample

Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after the last administration from the retro-orbital plexus into heparinized tubes. Plasma was obtained through centrifugation at 3000 rpm for 10 min. All plasma samples at different time points from each group of rats were combined to produce the pooled sample for eliminating the individual variability. A 500 \(\mu L\) volume of the pooled plasma sample was added with 1.5 mL of acetonitrile and vortexed for 1.0 min to precipitate protein. The sample was centrifuged at 12,000 rpm and 4 °C for 10 min. The supernatant was evaporated to dryness under a gentle flow of nitrogen at room temperature. The residue was redissolved with 200 \(\mu L\) of 60% methanol in water and centrifuged at 12,000 rpm for 10 min. The supernatant was injected into the UHPLC-Q-Orbitrap HRMS system for analysis.

3.6. UHPLC-Q-Orbitrap HRMS Conditions

A Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) consisted of a quaternary solvent delivery system, a column compartment and a refrigerated auto-sampler. The sample separation was performed on an ACQUITY UPLC® BEH C18 column (2.1 mm \(×\) 100 mm, 1.7 \(\mu m\)) eluted with acetonitrile (A) and 0.1% aqueous formic acid (B). The flow rate was set at 0.3 mL/min with an initial mobile phase of 5% (A). The chromatographic elution program was set: 5–5% A at 0–1.0 min, 5–10% A at 1.0–4.0 min, 10–12% A at 4.0–9.0 min, 12–20% A at 9.0–14.0 min, 20–45% A at 14.0–19.0 min,
45–70% A at 19.0–20.0 min, 70–100% A at 20.0–22.0 min, 100–5% A at 22.0–22.1 min, 5–5% A at 22.1–25.0 min. The injection volume was 10 µL.

A Q-Exactive™ Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with a heated electrospray ionization source (HESI) was used for qualitative analysis. The analysis was carried out both in positive and negative ion modes. The collision and nebulizing gases were ultra-high purity helium (He) and high purity nitrogen (N₂). The parameters were set as follows: ion spray voltage: +3.0 kV and −2.5 kV, capillary temperature: 320°C, S-lens RF level: 60%. The flow rates of sheath gas and auxiliary gas were set to 35 and 10 arbitrary units, respectively. A full MS/dd-MS² acquisition program was executed with resolutions of 70,000 and 17,500 FWHM. For the full MS experiments, the scan range was from 80 to 1200 m/z, the automatic gain control (AGC) target was defined as 1e⁶ and the maximum injection time (IT) was set as auto. For the dd-MS² experiments, AGC target: 2e⁵, maximum IT: auto, loop count: 1, the isolation window was 3.0 m/z. The stepped normalized collision energies (NCE) were 20, 40, and 60 eV.

3.7. Data Analysis

The information of chemical constituents from P. capitatum, including CAS number, molecular formula and molecular weight, were obtained by retrieving SciFinder Scholar and Dictionary of Natural Product databases. An in-house library containing potential compounds from P. capitatum extract was established. Data analysis was performed through Xcalibur 3.0 software (Thermo Fisher Scientific, Waltham, MA, USA) and Compound Discoverer 2.0 software coupled to mzCloud© and ChemSpider© databases. The data processing workflow for the identification of chemical ingredients from P. capitatum and its metabolites was shown in Figure 7.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Chemical constituents identified and characterized in P. capitatum by UHPLC-Q-Orbitrap HRMS in negative and positive modes. Figure S1: The chemical structures of the constituents from P. capitatum analyzed by UHPLC-Q-Orbitrap HRMS.

Author Contributions: S.L. and H.G. conceived and designed the research. H.G., P.L., Q.W., F.Z., D.W., M.Z. (Mei Zhou), M.Z. (Meng Zhou) and X.H. participated in the experiment operation. H.G. performed the experiments. H.G. and P.L. analyzed the data. H.G. wrote the paper. P.L. supervised the paper. All authors have read and agreed to the published version of the manuscript.

Figure 7. The data processing workflow for identification of chemical ingredients from P. capitatum and its absorbed constituents in hyperuricemia rat plasma.
4. Conclusions

In this study, a sensitive and accurate UHPLC-Q-Orbitrap HRMS method was utilized to systematically analyze the chemical constituents of *P. capitatum* and its absorbed components in hyperuricemia rats. A total of 114 compounds including phenolic acids, flavonoids, phenylpropanoids, tannins, phenolics, amino acids, amides and others were identified or characterized. At the same time, 68 *P. capitatum*-related xenobiotics were found in the hyperuricemia rats’ plasma. These exogenous components in hyperuricemia rats might be the potential active constituents of *P. capitatum* for anti-hyperuricemia and anti-gouty arthritis. The detected metabolic pathway of *P. capitatum* in hyperuricemia rats included ring fission, hydrolysis, decarboxylation, dehydroxylation, methylation, glucuronidation and sulfation. This study not only supplied a basis for the further investigation of the active components and pharmacokinetics of *P. capitatum*, but also provided insight into the anti-hyperuricemia mechanism and quality control of *P. capitatum*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27113521/s1, Table S1: Chemical constituents identified and characterized in *P. capitatum* by UHPLC-Q-Orbitrap HRMS in negative and positive ion modes. Figure S1: The chemical structures of the constituents from *P. capitatum* analyzed by UHPLC-Q-Orbitrap HRMS.

Author Contributions: S.L. and H.G. conceived and designed the research. H.G., P.L., Q.W., F.Z., D.W., M.Z. (Mei Zhou), M.Z. (Meng Zhou) and X.H. participated in the experiment operation. H.G. and P.L. analyzed the data. W.P. and S.L. supervise the research. H.G. and P.L. wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Animal Care Welfare Committee of Guizhou Medical University (approval number: 2100138 and approval date: 5 March 2021).

Informed Consent Statement: Not applicable.

Conflicts of Interest: There are no conflicts of interest to declare.

Sample Availability: Samples of the compounds are not available from the authors.

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