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ORIGINAL ARTICLE

Identification of the tannins in traditional Chinese medicine *Paeoniae Radix Alba* by UHPLC-Q-Exactive Orbitrap MS

Pei Xiong [a,1], Shi-han Qin [b,1], Kai-lin Li [a], Ming-juan Liu [a], Lian Zhu [a], Jie Peng [a], Si-lin Shi [a], Su-nv Tang [a], Ai-ping Tian [c], Wei Cai [a,*]

[a] *School of Pharmaceutical Sciences, Hunan Province Key Laboratory for Antibody-Based Drug and Intelligent Delivery System, Hunan University of Medicine, Huaihua 418000, China*
[b] *School of Pharmacy, Weifang Medical University, Weifang 261000, China*
[c] *Department of Traditional Chinese Medicine, National Cancer Center, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China*

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KEYWORDS

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Abstract *Paeoniae Radix Alba* (PRA, dried root of *Paeonia lactiflora Pall.*) is a widely used as traditional Chinese medicine and tannins are one of their main bioactive ingredients. However, there are rarely systematically investigated in this study. This study aimed to establish a rapid, high selective, high sensitive and effective method based on UHPLC-Q-Exactive Orbitrap MS for simultaneous identification the tannins in PRA. Separation was performed on Thermo Scientific Hypersil GOLD™ aQ (100 mm × 2.1 mm, 1.9 μm) using gradient elution consist of 0.1% formic acid acetonitrile and 0.1% formic acid water as the mobile phase at a flow rate of 0.3 mL/min. The mass spectrometer was operated with Q-Exactive Orbitrap spectrometer in negative ion mode. Finally, a total of 106 constituents were identified in PRA by UHPLC-Q-Exactive Orbitrap MS, 75 of those were reported from PRA for the first time. This result laid the foundation for in-depth research on the material basis efficacy and provided scientific basis for the selection of quality marker of PRA.

1. Introduction

Paeoniae radix alba (PRA, Bai Shao in Chinese), the dried root of plant *Paeonia lactiflora Pall.* (Family Ranunculaceae), is a famous Traditional Chinese medicines (TCM) in China. RPA is produced by boiling the fresh root of the whole *Paeonia lactiflora Pall.* in water and peeling off the bark. PRA plays an important role in contribute to most biological activities that including subduing hyperactivity of the liver, relieving pain, reducing sweat, nourishing blood and regulating menstruation (State Pharmacopoeia Commission, 2020). So far, many

* Corresponding author.
E-mail address: 20120941161@bucm.edu.cn (W. Cai).
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compounds have been reported in PRA, including flavonoids, terpenes and volatile oils and so on. Its main active ingredients are paeoniflorin, albiflorin, and gallic acid, which have pharmacological effects such as liver protection, anti-inflammatory, anti-rheumatic, immune-regulating functions, etc (Ou et al., 2013., Wang et al., 2010). However, the research on chemical constituents of PRA is still insufficient.

Tannins, a class of polyphenolic compounds with complex structures, are widely exist in plants. According to the structure of tannins, tannins can be divided into three types: hydrolyzed tannins, condensed tannins and combined tannins of these two types (Xing et al., 2011). Glucogallins, a kind of hydrolyzed tanins, are a class of esters composed of gallic acids or their derivatives and polyols such as glucose, rhamnose and quinic acid, which generate gallic acids or polyols after hydrolysis (Chen, 2000). Most biological activities of glucogallins come from the hydrolysate. It is reported that gallic acids have multiple pharmacological activities including anti-inflammatory, antioxidant, anti-tumor, etc (Li et al., 2004., Ohno et al., 2001), and provide treatment for hypertension, myocardial infarction and diabetes among others (Dianat et al., 2014; Lee et al., 2015; Li et al., 2004; Punithavathi et al., 2011). Condensed tannins are mainly composed of catechin as the precursor through C—C bonds or C—O bonds condensation between each unit (Zhu and Jin, 2015), which have anti-oxidation, anti-tumor, anti-pathogen, anti-HIV and other pharmacological effects (Wang et al., 2013).

Although there are a lot of studies on the chemical components of PRA, they are mainly concentrated on monoterpene glycosides, and less on tannins. UHPLC-MS/MS is a powerful analytical technique used in detection and identification of chemical constituents in traditional Chinese medicine, drug, or biological samples (Cai et al., 2020; Hu et al., 2020; Ren et al., 2020). The characteristics such as the high separation efficiency of UHPLC and the high sensitivity and molecular structure elucidation ability by providing accurate mass measurement and abundant MS^n fragment information of MS were integrated by UHPLC-MS/MS (Liao et al., 2020). Recently, UHPLC-Q-Exactive Orbitrap MS was widely used in the identification of chemical constitutions for its high selectivity, high sensitivity and effectivity. Therefore, it is necessary to systematically identify the type of tannins in PRA using UHPLC-Q-Exactive Orbitrap MS in the negative ion mode, Finally, a total of 106 compounds were found in PRA, 75 of which were reported for the first time. The result will benefit for the in-depth understanding of the pharmacological action of PRA and lay a foundation for the quality control of the drug in clinical use in the future.

2. Experimental

2.1. Chemicals and reagents

The chromatographic grade methanol (MeOH) and acetonitrile (ACN) were purchased from MACKIN company, the MS grade formic acid was purchased from Thermo Fisher Scientific Co., Ltd. (USA), purified water was obtained from Guangzhou Watsons Food & Beverage Co., Ltd. (China). Other solvents were of an analytical grade. Gallic acid (purity $\geq$ 98%), methyl gallate (purity $\geq$ 98%), (–)-epicatechin gallate (purity $\geq$ 98%), corilagin (galloy-HHDP-hexoside) (purity $\geq$ 98%) were purchased from Chengdu Purechem-Standard Co. Ltd. PRA samples were provide by Sun Ten Pharmaceutical Co., Ltd and turned into powder after being crushed and stored in vacuum packages.

2.2. Instruments and LC-MS/MS conditions

LC-MS/MS analyses, which performed on an Dionex Ultimate 3000 UHPLC (a quaternary pump, an LPG-3400SD vacuum degasser unit) and the UHPLC-Q-Exactive Orbitrap MS mass spectrometer equipped with an electrospray ionization (ESI) source, were used for simultaneous determination of tannins in PRA. The liquid chromatographic separations of all analyzed samples were achieved on a Thermo Scientific Hypersil GOLD™ aQ (100 mm × 2.1 mm, 1.9 μm) at 40 °C with a flow rate of 0.3 mL/min. The mobile phase consisted of (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid. The gradient program was as follows: 0–2 min, 95% A; 2–5 min, 95–85% A; 5–20 min, 85–65% A; 20–22 min, 65–95% A; 22–25 min, 95% A. The sample injection volume was 1 μL.

The mass spectrometer analysis was operated in the negative electrospray ionization mode. High resolution mass spectrum was collected in the full scan mode in the mass range m/z 100–1500 at a resolution of 70, 000. The MS^2 data at a resolution of 17, 500 was obtained by data-dependent MS^2 scanning or parallel reaction monitoring (PRM) mode triggered by inclusion ions list, which was built by molecule predicted. The other conditions of MS analysis were as follows: The spray voltage was set to 3.2 kV; the sheath gas flow rate and the aux gas flow rate were set to 35 arb and 10 arb, respectively; the capillary temperature and the heater temperature were set to 320 °C and 350 °C, respectively; the S-lens RF level was 60.

2.3. Preparation of control and standard samples

Dried and pulverised PRA (1 g) was accurately weighed by electronic analytical balance. After 20 mL of 70% aqueous methanol was added, an extract was obtained by sonication for 1 h. The supernatants were removed using a syringe and filtered through a 0.22 μL nylon millipore filter and added to the liquid vial for further analysis.

The standard solutions including gallic acid, methyl gallate, (−)-epicatechin gallate, corilagin (galloy-HHDP-hexoside) were dissolved in methanol, respectively, to get reference standards solutions (0.1 mg/mL). All the standard solutions were stored at 4 °C before analysis.

2.4. Data processing and analysis

The Xcalibur software version 4.2 (Thermo Fisher Scientific, California, USA) was used to obtain the raw data including the full-scan MS and MS^2 data. The peaks detected with intensity over 10,000 were selected for identifications. The chemical formulas for all parent and fragment ions of the selected peaks were calculated from the accurate mass using a formula predictor by setting the parameters as follows: C [0–50], H [0–60], O [0–40], S [0–5], the mass tolerance of MS and MS^2 was within 5 ppm, respectively.

3. Results and discussion

3.1. Establishment of analytical strategy

In order to screen and identify tannins systematically in PRA, an analytical strategy based on UHPLC-Q-Exactive Orbitrap MS was established in this study. Firstly, tannins in RPA were extracted and enriched by ultrasonic extraction with 70%

methanol. Secondly, the sample contained tannins was injected into UHPLC-Q-Exactive Orbitrap MS to gain the high resolution mass data acquired by full MS scan with data dependence MS^2 (Full Mass-ddMS^2), which was processed by Compound Discover version 3.0 using high resolution extracted ion chromatography (HREIC) and expected compounds predicted. Thirdly, for the trace constituents precursor ions with relatively low content in the mass analyzer, especially when they co-eluted with higher content constituents, the subsequent fragments can be obtained by PRM mode triggered by inclusion ions list to make the tannins identification more sufficient in RPA. Fourth, diagnosis fragmentation ions (DFIs) data-mining techniques were adopted for the selective clarification of tannins that possessed similar mass fragmentation behaviors to those of reference standard. Finally, the compounds were identified based on the full scan MS, MS^2 data, retention time and bibliography.

3.2. Optimization of UHPLC-Q-Exactive Orbitrap MS condition

In order to obtain better chromatographic peak type and separation, variables factors were investigated in detection and identification process, including extraction solvent ranging from 60% to 100% methanol, the kind of mobile phase (acetonitrile/water, and acetonitrile containing 0.1% formic acid/water), the kind and content of acid (formic acid and acetic acid, 0.05, 0.1, and 0.2%), column (Thermo Scientific Hypersil GOLD™ aQ 100 mm × 2.1 mm, 1.9 μm and Waters ACQUITY BEH C18 column, 100 mm × 2.1 mm, 1.7 μm), flow rate of mobile phase (0.2, 0.3, and 0.4 mL/min), column temperature (30, 35, 40, 45 °C) and the mobile phase gradient. The MS parameters including the flow rate of sheath gas and auxiliary gas, the temperature of capillary and heater, spray voltage, et al. were examined. In the optimization condition of UHPLC-Q-Exactive Orbitrap MS, most of the tannins have shown efficient separation and parent/daughter ion pairs with high responses.

3.3. Establishment of DFIs

There are three types of tannins including hydrolyzed tannins, condensed tannins and compound tannins. It is easily understood that tannins with the same carbon skeletons will generate the similar fragmentations, which can be use as DFIs for the distinguish and characterization of tannins. The fragmentation patterns of 4 reference standards were investigated by UHPLC-Q-Exactive Orbitrap MS in negative mode to establish the DFIs, such as 169.0133 ([gallic acid-H]$^-$), 125.0232 ([gallic acid-CO_2-H]$^-$) generated from gallic acid moiety, 300.9984 ([ellagic acid-H]$^-$), 283.0457 ([ellagic acid-H-H_2O]$^-$) yielded by ellagic acid. Furthermore, there are fragment ions at *m*/*z* 321.0262 ([digallic acid–H]$^-$), 331.0672 ([galloylglucose-H]$^-$), 313.0580 ($C_{13}H_{14}O_9$), 211.0426 ($C_{13}H_7O_3$), 275.0192 ($C_{13}H_7O_7$) and 183.0427 ($C_{12}H_7O_2$). All the above can be used as DFIs of hydrolytic tannins. Fragment ions at *m*/*z* 289.0717 ([catechin acid-H]$^-$) emerged from catechin moiety and can be used as DFIs of condensed tannins.

3.4. Characterization of the tannins in PRA

Under the LC-MS conditions of "2.3", the extracted ion chromatogram (EIC) in negative ion mode was obtained as shown in Fig. 1 and the selected fragmentation pattern of components identified from PRA were shown in Fig. 2. As listed in Table 1, the chromatographic and mass data of those detected components are summarized though Xcalibur software version 4.2, which including retention times (t_R), experimental Mass (negative-ion mode), molecular formula, error in ppm (between the theoretical mass and the experimental mass) of each tannins, as well as the MS/MS fragment ions. Eventually, a total of 106 tannins (75 first report) was accuracy or tentatively identified in PRA though UHPLC-Q-Exactive Orbitrap MS.

3.4.1. Identification of hydrolyzed tannins

3.4.1.1. Identification of gallotannins. Gallic acid derivatives and gallotannins, composed of monomer galloyl moiety and multiple galloyl moieties linked to polyols were identified based on the DFIs at *m*/*z* 169.0133 ([gallic acid-H]$^-$) and 125.0232 ([gallic acid-CO_2-H]$^-$) as well as the neutral loss of a dehydrated galloyl moiety (152 Da) (Erşan et al., 2016).

Compound 1, 3 and 6 were found at 0.85, 1.24, 1.70 min, which show common precursor ion at [M−H]$^-$ *m*/*z* 493.119. The major fragment at *m*/*z* 331.0672 due to loss of a glucose reside and *m*/*z* 313.0566 attributed to neutral loss of a glucose moiety, which further gave rise to the product ions at *m*/*z* 169.0133 and 125.0232. Then they were tentatively characterized as 1′-O-galloylsucrose, 6′-O-galloylsucrose and 6-O-galloylsucrose, respectively (Li et al., 2009). Compounds 2 and 7 appeared at a retention time (t_R) of 1.15 min and 1.72 min respectively, which tentatively identified as galloylquinic acid isomers. The parent ions at *m*/*z* 343.066 due to the loss of galloyl moieties (152 Da) and further generated the characteristic fragments (*m*/*z* 169.0133 and 125.0232) of gallic acid moiety and characteristic fragments (*m*/*z* 191.0553) of quinic acid moiety (Erşan et al., 2016). Similarly, compound 15 was observed at 4.13 min, appeared similar losses to galloylquinic acid isomers with an extra loss of galloyl moiety (152 Da) and deduced as di-O-galloyl quinic acid.

Compound 5 was found at 1.53 min, possessing the quasi-molecular ion [M−H]$^-$ at *m*/*z* 331.0668 and tentatively identified as galloylglucose. The daughter ion at *m*/*z* 169.0132 attributed to the loss of a hexose moiety and further generated the ion at *m*/*z* 125.0232 by loss of CO_2 (Erşan et al., 2016). The proposed fragmentation pathway of galloylglucose was shown in Fig. 2(A). The compound 24, 29, 30 with the molecular formula $C_{20}H_{20}O_{14}$ were found at 5.91, 6.19, 6.34 min and having the quasi-molecular ions at *m*/*z* 483.078 in the ESI-mode, which were been tentatively proposed as isomers of digalloylglucose. (Erşan et al., 2016). Compounds 27, 41, and 44 yielded a quasi-molecular ion [M−H]$^-$ at *m*/*z* 635.089 and were eluted at 6.14, 7.13 and 7.75 min, respectively. All of those compounds showed the fragment ions at *m*/*z* 169.0133 ($C_7H_5O_5$), 125.0232 ($C_6H_5O_3$), 313.0565 ($C_{13}H_{14}O_9$), 123.0075 ($C_5H_3O_3$) and might be considered as isomer of trigalloylglucose. Compounds 49, 53, and 56 were eluted at 8.38, 8.66 and 9.04 min and shared the same empirical

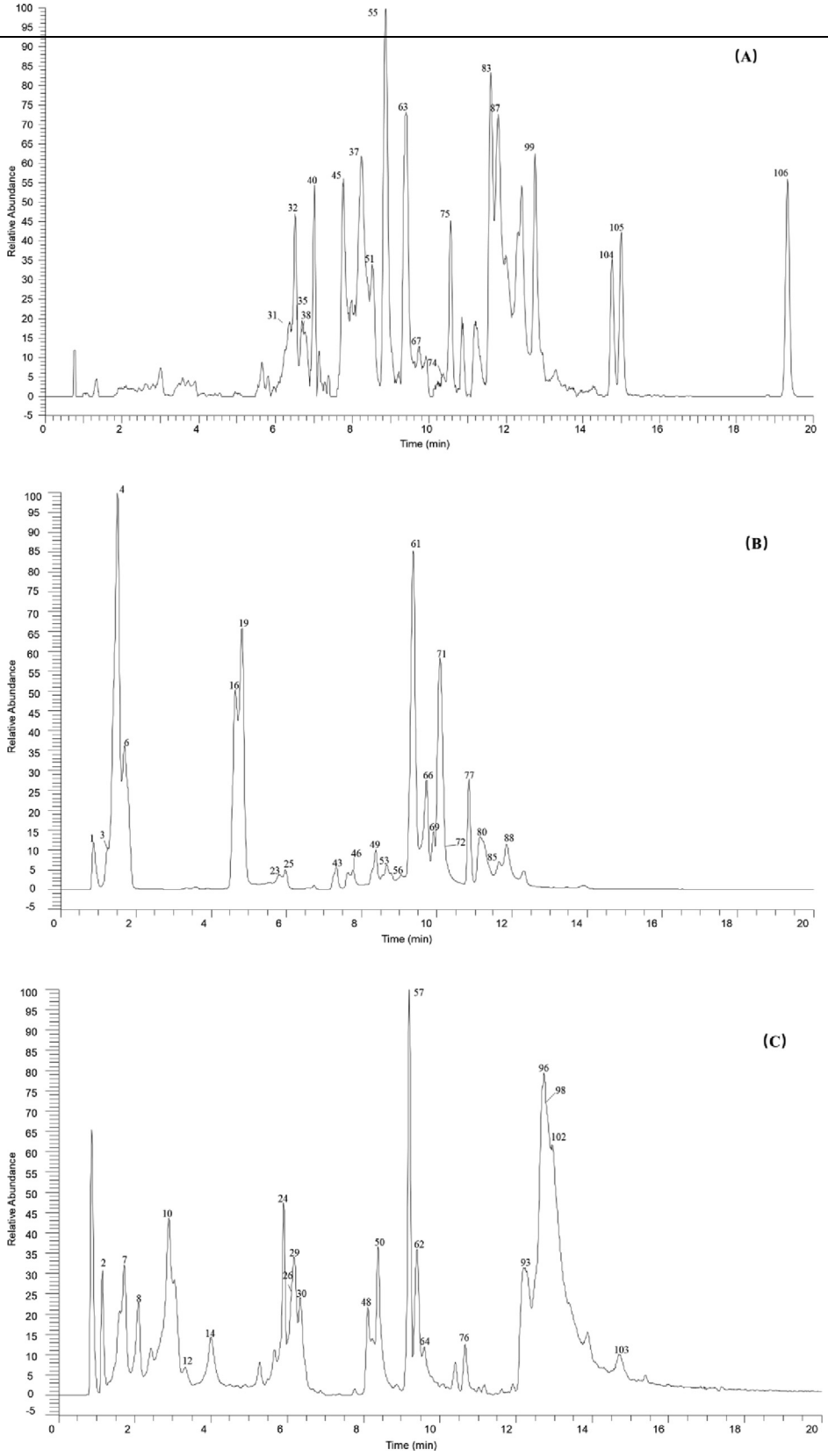

Fig. 1 The high-resolution extracted ion chromatogram (HREIC) in 5 ppm for the multiple compounds in PRA. (A) *m*/*z* 481.09876, 315.01464, 783.10503, 873.15198, 343.04594, 935.07960, 703.16684, 599.10424, 329.03029, 694.12091, 801.0792, 461.07254, 473.03616; (B) *m*/*z* 463.05181, 321.02520, 787.09994, 545.05729, 335.04085, 493.11989, 939.11090, 183.02989, 631.16684, 169.01424; (C) *m*/*z* 477.06746, 621.05808, 483.07802, 937.09525, 721.14102, 315.07215, 343.06706, 801.11559, 461.10893, 633.07333; (D) 445.13514, 197.04554, 785.08429, 1243.13282, 345.08271, 487.05181, 495.07802, 715.13045, 491.08311; (E) 300.99899, 441.08271, 1091.12186, 783.17780, 635.08898, 451.12458, 527.14062.

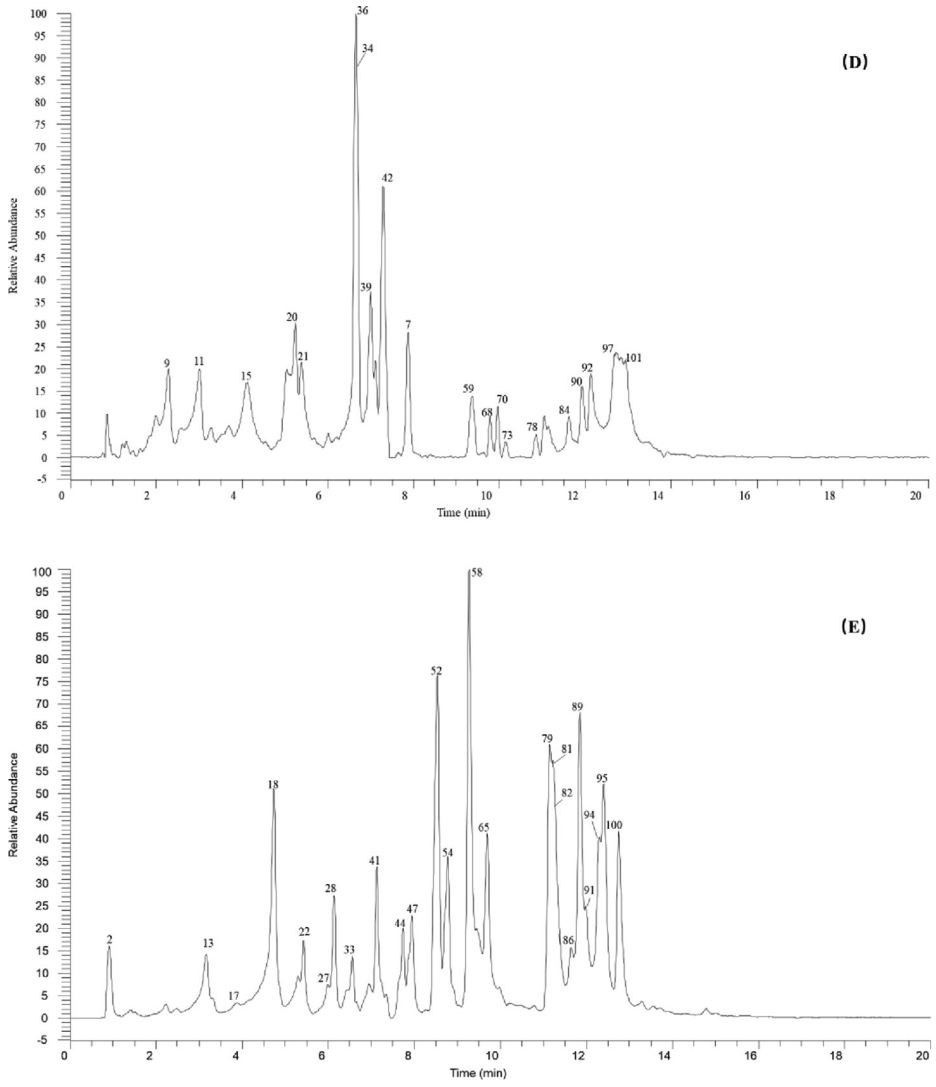

Fig. 1 (*continued*)

molecular formula $C_{34}H_{28}O_{22}$ at *m*/*z* 787.100, matched to that of tetragalloylglucose isomers. The fragments of [M−gallic acid]$^-$, [M−3gallic acids-108]$^-$, [M−3gallic acids-152]$^-$ were found at *m*/*z* 617.0789, 169.0133, 125.0232 in tetragalloylglucose isomers (Erşan et al., 2016). Similarly, compound 71 was eluted at 10.08 min, yielded a deprotonated ion [M−H]$^-$ *m*/*z* 939.1114 and deduced as pentagalloylglucose. Compounds 79, 82, 86 and 89 at *m*/*z* 1091.122 having the same molecular formula $C_{48}H_{36}O_{30}$ and appeared at a retention time (t_R) of 11.13, 11.25, 11.65 and 11.85 min, which tentatively identified as hexagalloyl glucose isomers. Compound 97 was observed at 12.71 min, yielded a precursor ion at *m*/*z* 1243.133 have been characterized as heptagalloy glucose. The typical fragment ions of [M−H−galloyl]$^-$, [M−H−2galloyl]$^-$, [M−H−2galloyl−Glu]$^-$ were found at *m*/*z* 1091.1224, 939.1108 and 769.0879. In short, The mono-, di-, tri-, tetra-, penta-, hexa- and hepta- exhibited sequential losses of galloyl moieties (152 Da) from their parent ions at *m*/*z* 331.0668, 483.078, 635.089, 787.100, 939.1114, 1091.122 and 1243.133, respectively.

Compound 8, 10 and 12 were found at 2.10, 2.89, 3.10 min, possessing the same quasi-molecular ions [M−H]$^-$ at *m*/*z* 315.072 and tentatively identified as galloylrhamnose isomers. The main product ions at *m*/*z* 169.0133 were obtained by the loss of a rhamnose residue (146 Da) at [M−H]$^-$ and then obtain product ions at *m*/*z* 125.0232 by the loss of two CO_2 (Sobeh et al., 2019).

Compound 9 and 11 exhibited the quasi-molecular ions at *m*/*z* 345.082 and appeared similar losses to galloylglucose with an extra loss of methyl moiety (14 Da). Based on major fragment ions at *m*/*z* 183.0290 ([M−H−hexose]$^-$), 139.0390 ([M−H−hexose−CO_2]$^-$), these compounds were tentatively assigned to methyl galloylglucose isomers. Similarly, The compound 76 with the molecular formula $C_{35}H_{30}O_{22}$ and having the deprotonated ions at *m*/*z* 801.1162 in the ESI-mode, which were been tentatively proposed as methyl tetragalloylglucose. It showed the presence of tetragalloylglucose fragment ions like 125.0232 and 169.0133. The fragment ion at *m*/*z* 183.0290 indicated the presence of a methyl gallate.

Compound 17 was found at 4.74 min, yielded parent ion [M−H]$^-$ *m*/*z* 451.1244, consisted of a galloyl moiety (152 Da) and a salidroside moiety (299 Da) and deduced as galloylsalidroside according to the MS and MS/MS spectra (Liu et al., 2017).

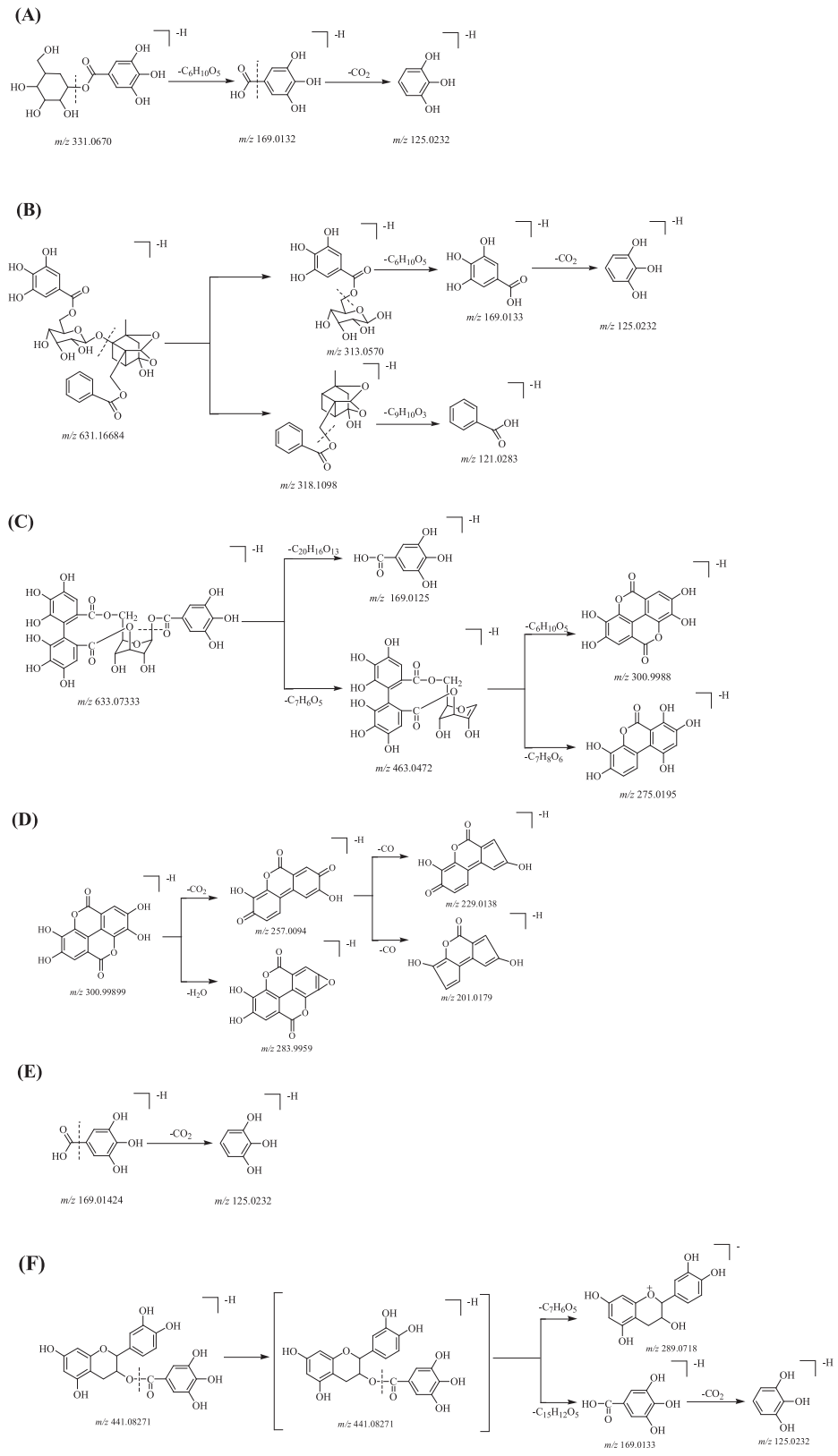

Fig. 2 Proposed selected fragmentation pattern of components identified from PRA: Galloylglucose (A); Galloylpaeoniflorin(B); Corilagin (Galloy-HHDP-hexoside) (C); Ellagic acid (D); Gallic acid (E); (–)-Epicatechin gallate (F).

Compounds 18 was observed at 4.76 min, yielded a deprotonated ion $[M-H]^-$ m/z 527.1403 and tentatively identified as 6′-O-Galloyl-desbenzoylpaeoniforin (Li et al., 2016b). The MS/MS spectrum presented $[M-H-HCHO]^-$, $[M-H-HCHO-H_2O]^-$, $[M-H-HCHO-H_2O-gallic-2HCHO-CO_2]^-$ ion at m/z 497.1295, 479.1187 and 271.0455.

Table 1 The chromatographic and mass data of detected components from PRA though UHPLC-Q-Exactive Orbitrap MS.

Peak	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula	MS/MS fragment	Identification/ Reactions
1	0.85	493.11989	493.11972	−0.351	$C_{19}H_{26}O_{15}$	MS^2[493]: 169.0133(100), 125.0233(82), 313.0657(31), 123.0076(19), 151.0027(14)	1′-O-galloylsucrose
2#	1.15	343.06706	343.06683	0.857	$C_{14}H_{16}O_{10}$	MS^2[343]: 169.0132(100), 191.0553(76), 125.0231(68), 107.0125(36), 109.0282(16), 85.0281(11)	galloylquinic acid isomer
3	1.24	493.11989	493.11963	−0.534	$C_{19}H_{26}O_{15}$	MS^2[493]: 169.0133(100), 125.0232(70), 331.0671(47), 123.075(10), 313.0564(10)	6′-O-galloylsucrose
4*	1.51	169.01424	169.01418	−0.394	$C_7H_6O_5$	MS^2[169]: 125.0232(100)	gallic acid
5#	1.53.	331.06706	331.06689	−0.543	$C_{13}H_{16}O_{10}$	MS^2[331]: 125.0232(100), 169.0132(86), 241.0349(19), 149.9947(17), 271.0457(16)	galloylglucose
6	1.70	493.11989	493.11954	−0.716	$C_{19}H_{26}O_{15}$	MS^2[493]: 169.0132(100), 129.0231(80), 313.0565(27), 283.0457(14)	6-O-galloylsucrose
7#	1.72	343.06706	343.06677	0.797	$C_{14}H_{16}O_{10}$	MS^2[343]: 169.0132(100), 125.0232(65), 173.0445(25), 191.0553(23), 93.0332(16)	galloylquinic acid isomer
8#	2.10	315.07215	315.07236	0.650	$C_{13}H_{16}O_9$	MS^2[315]: 108.0203(100), 152.0104(59), 109.0282(28), 153.0182(21)	galloylrhamnose isomer
9#	2.29	345.08271	345.08289	0.493	$C_{14}H_{18}O_{10}$	MS^2[345]: 183.0291(100), 139.0390(28), 107.0126(27), 225.0399(25)	methyl galloylglucose isomer
10#	2.89	315.07215	315.07227	0.364	$C_{13}H_{16}O_9$	MS^2[315]:109.0282(100), 153.0183(66), 169.0133(19), 123.0076(15), 125.0232(13)	galloylrhamnose isomer
11#	3.01	345.08271	345.08273	0.029	$C_{14}H_{18}O_{10}$	MS^2[345]: 183.0290(100), 124.0154(48), 139.0390(14)	methyl galloylglucose isomer
12#	3.10	315.07215	315.07233	0.555	$C_{13}H_{16}O_9$	MS^2[315]: 169.0133(100)., 123.0075(66), 125.0232(55), 151.0026(54), 107.0126(11), 139.0021(11)	galloylrhamnose isomer
13	3.17	527.14062	527.140691	−3.297	$C_{23}H_{28}O_{14}$	MS^2[527]: 169.0219(100), 165.0545(20), 313.0567(18), 61.09859(16), 125.0230(10)	6′-O-Galloyl-desbenzoylpaeoniforin isomer
14#	4.01	633.07333	633.07385	0.811	$C_{27}H_{22}O_{18}$	MS^2[633]: 300.9984(100), 275.0192(21), 169.0128(12), 125.0230(10)	gorilagin(Galloy-HHDP-hexoside) isomer
15#	4.13	495.07802	495.07822	0.387	$C_{21}H_{20}O_{14}$	MS^2[495]:109.0122(100), 137.0232(83), 169.0131(30), 125.0232(25), 313.0558(17)	di-O-galloylquinic acid
16*	4.64	183.02989	183.02980	−0.528	$C_8H_8O_5$	MS^2[183]: 168.0054(100), 124.0153(92), 140.0103(76), 111.0075(45), 139.0025(41)	methyl gallate
17#	4.74	451.12458	451.12445	0.962	$C_{21}H_{24}O_{11}$	MS^2[451]: 289.0717(100), 109.0282(27), 125.0232(19), 245.0817(19), 123.0439(18)	galloylsalidroside
18	4.76	527.14062	527.14032	−0.585	$C_{23}H_{28}O_{14}$	MS^2[527]: 479.1187(100), 271.0455(84), 497.1295(52), 313.0563(49), 169.0129(44)	6′-O-Galloyl-desbenzoylpaeoniforin
19	4.83	183.02989	183.02985	−0.255	$C_8H_8O_5$	MS^2[183]: 168.0055(100), 124.0153(94), 140.0104(79), 111.0074(50), 139.0025(38)	methyl gallate isomer
20	5.24	445.13514	445.13495	−0.448	$C_{19}H_{26}O_{12}$	MS^2[445]: 121.0283(100), 59.0125(12)	benzoylsucrose isomer
21#	5.40	785.08429	785.08490	0.770	$C_{34}H_{26}O_{22}$	MS2[785]: 300.9988(100), 275.0201(44), 249.0401(37), 125.0233(27), 169.0137(23), 137.0233(18), 231.0286(15)	digalloyl-HHDP-glucose isomer
22	5.43	527.14062	527.14026	−0.699	$C_{23}H_{28}O_{14}$	MS^2[527]: 169.0130(100), 313.0559(60), 167.0336(30), 345.1181(26), 151.0022(26)	6′-O-Galloyl-desbenzoylpaeoniforin isomer
23#	5.82	321.02520	321.02509	−0.358	$C_{14}H_{10}O_9$	MS^2[321]: 169.0132(100), 125.0232(74)	digallic acid isomer
24#	5.91	483.07802	483.07797	−0.121	$C_{20}H_{20}O_{14}$	MS^2[483]: 169.0133(100), 125.0232(78), 211.0242(42), 271.0459(40), 313.0566(10)	Isomer of digalloylglucose
25#	5.98	321.02520	321.02512	−0.265	$C_{14}H_{10}O_9$	MS^2[321]: 169.0132(100), 125.0232(78)	digallic acid isomer
26*#	6.11	633.07333	633.07367	0.526	$C_{27}H_{22}O_{18}$	MS^2[633]: 300.9988(100), 275.0195(17), 125.0231(7), 169.0125(6)	Corilagin(Galloy-HHDP-hexoside)
27#	6.14	635.08898	635.08929	0.477	$C_{27}H_{24}O_{18}$	MS^2[635]: 169.0132(100), 125.0232(78), 465.0673(30), 313.0566(27), 123.0075(17)	Isomer of trigalloylglucose
28	6.15	527.14062	527.14056	−0.130	$C_{23}H_{28}O_{14}$	MS^2[527]: 169.0133(100), 125.0232(85), 123.0075(41), 151.0024(23), 107.0126(13)	6′-O-Galloyl-desbenzoylpaeoniforin isomer
29#	6.19	483.07802	483.07809	0.128	$C_{20}H_{20}O_{14}$	MS^2[483]: 125.0232(100), 169.0133(70), 151.0026(42), 439.0882(18), 107.0125(15)	Isomer of digalloylglucose
30#	6.34	483.07802	483.07813	0.210	$C_{20}H_{20}O_{14}$	MS^2[483]: 125.0232(100), 169.0133(43),	Isomer of

(*continued on next page*)

Table 1 (*continued*)

Peak	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula	MS/MS fragment	Identification/ Reactions
						331.0672(33), 149.9948(31), 151.0027(16)	digalloylglucose
31#	6.38	703.16684	703.16718	0.479	$C_{36}H_{32}O_{15}$	MS^2[703]: 61.9869(100), 289.0714(98), 251.0556(76), 125.0230(62), 287.0556(26),	theaflavin 3′-gallate isomer
32#	6.50	703.16684	703.16699	0.209	$C_{36}H_{32}O_{15}$	MS^2[703]: 61.9869(100), 289.0714(12), 251.0556(11), 125.0230(7)	theaflavin 3′-gallate isomer
33	6.57	527.14062	527.14056	−0.130	$C_{23}H_{28}O_{14}$	MS^2[527]: 347.0765(100), 345.1185(25), 169.0130(37), 125.0229(13)	6′-O-Galloyl-desbenzoylpaeoniforin isomer
34#	6.64	785.08429	785.08472	0.541	$C_{34}H_{26}O_{22}$	MS^2[785]: 300.9991(100), 275.0198(45), 249.0399(34), 125.0232(32), 169.0132(23)	digalloyl-HHDP-glucose isomer
35#	6.67	703.16684	703.16742	0.820	$C_{36}H_{32}O_{15}$	MS^2[703]: 61.9869(100), 289.0714(10), 125.0230(8)	theaflavin 3′-gallate isomer
36	6.68	445.13514	445.13501	−0.313	$C_{19}H_{26}O_{12}$	MS^2[445]: 121.0282(100), 135.0440(15)	benzoylsucrose isomer
37#	6.69	801.07920	801.07983	0.774	$C_{34}H_{26}O_{23}$	MS^2[801]: 125.0231(100), 289.0714(99), 121.0281(81), 96.9587(57), 151.0389(30), 169.0131(22)	punigluconin
38#	6.84	703.16684	703.16858	2.470	$C_{36}H_{32}O_{15}$	MS^2[703]: 61.9869(100), 289.0714(16), 251.0555(15), 125.0230(10)	theaflavin 3′-gallate isomer
39	7.00	445.13514	445.13489	−0.583	$C_{19}H_{26}O_{12}$	MS^2[445]: 121.02823(100)	benzoylsucrose isomer
40#	7.02	481.09876	481.09830	−0.964	$C_{21}H_{22}O_{13}$	MS^2[481]: 121.0280(100), 122.0314(39), 313.0559(27), 169.0129(24)	galloyvanilloy glucose isomer
41#	7.13	635.08898	635.08911	0.194	$C_{27}H_{24}O_{18}$	MS^2[635]: 169.0133(100), 125.0232(80), 465.0673(52),313.0565(20), 123.0075(13)	isomer of trigalloylglucose
42	7.29	197.04554	197.04495	−3.028	$C_9H_{10}O_5$	MS^2[197]: 169.0133(100), 125.0233(90), 140.0104(21), 111.0075(13)	ethyl gallate
43#	7.62	463.05181	463.05188	0.143	$C_{20}H_{16}O_{13}$	MS^2[463]: 300.9991(100), 89.0231(14), 101.0230(12), 59.0125(20)	ellagic acid hexose
44#	7.75	635.08898	635.08917	0.288	$C_{27}H_{24}O_{18}$	MS^2[635]: 169.0133(100), 125.0232(92), 313.0565(13),	Isomer of trigalloylglucose
45#	7.76	935.07960	935.08142	1.942	$C_{41}H_{28}O_{26}$	MS^2 [935]: 300.9985(100), 633.0729(78), 302.0016(14), 275.0195(11), 125.0230(10), 169.0129(8)	galloyl-bis-HHDP-D-glucopyranose
46#	7.78	335.04085	335.04089	0.104	$C_{15}H_{12}O_9$	MS^2[335]: 183.0291(100), 168.0056(8)	methyl digallate isomer
47#	7.95	441.08271	441.08270	−0.045	$C_{22}H_{18}O_{10}$	MS^2[441]: 289.0718(100), 109.0282(35), 125.0232(27), 123.0440(23), 245.0817(23), 203.0707(22), 137.0233(20)	(–)-Epicatechin gallate isomer
48#	8.11	477.06746	477.06760	0.286	$C_{21}H_{18}O_{13}$	MS^2[477]: 298.9834(100), 314.0070(93), 270.9885(67), 312.9991(24), 285.0047(18)	methylellagic acid glucopyranoside isomer
49#	8.38	787.09994	787.10040	0.578	$C_{34}H_{28}O_{22}$	MS^2[787]: 169.0133(100), 125.0232(76)	tetragalloylglucose isomer
50#	8.39	937.09525	937.09589	0.679	$C_{41}H_{30}O_{26}$	MS^2[937]:300.9989(100), 275.0196(27), 169.0134(47), 125.0232(24), 249.0403(14)	trigalloyl-HHDP-glucose isomer
51#	8.52	473.03616	473.03589	−0.578	$C_{21}H_{14}O_{13}$	MS^2[473]: 59.0125(100), 71.0125(53), 125.0233(50), 169.0133(39), 101.0231(27)	trigallic acid
52#	8.54	441.08271	441.08273	0.023	$C_{22}H_{18}O_{10}$	MS^2[441]: 289.0716(100), 125.0232(41), 137.0232(39), 109.0282(37), 245.0817(24)	(–)-Epicatechin gallate isomer
53#	8.66	787.09994	787.10059	0.819	$C_{34}H_{28}O_{22}$	MS^2[787]: 169.0133(100), 125.0232(76), 123.0076(17)	tetragalloylglucose isomer
54#	8.78	441.08271	441.08289	0.386	$C_{22}H_{18}O_{10}$	MS^2[441]: 169.0133(100), 125.0232(87), 289.0716(35), 109.0283(13), 245.0815(7), 203.0706(7)	(–)-Epicatechin gallate isomer
55#	8.87	481.09876	481.09906	0.616	$C_{21}H_{22}O_{13}$	MS^2[481]: 121.0283(100), 168.0550(53), 125.0232(43), 122.0316(39), 149.9948(30), 59.0125(15)	Galloyvanilloy glucose isomer
56#	9.04	787.09994	787.10065	0.895	$C_{34}H_{28}O_{22}$	MS^2[787]: 169.0133(100), 125.0232(86), 123.076(16), 617.0789(13)	tetragalloylglucose isomer
57#	9.20	477.06746	477.06747	0.013	$C_{21}H_{18}O_{13}$	MS^2[477]: 229.9911(100), 315.0147(76), 298.9832(28), 270.9883(20),	methylellagic acid glucopyranoside isomer
58	9.28	300.99899	300.99884	−0.500	$C_{14}H_6O_8$	MS^2[300]: 229.0138(100), 247.0085(84), 185.0235(39), 283.9959(37), 201.0179(30)	Ellagic acid

Table 1 (*continued*)

Peak	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula	MS/MS fragment	Identification/ Reactions
59#	9.36	715.13045	715.12921	−1.745	$C_{36}H_{28}O_{16}$	MS^2[715]: 82.9528(100), 169.0132(22), 121.0282(14), 125.0233(13)	theaflavin-3-Gallate isomer
60	9.37	631.16684	631.16681	−0.053	$C_{30}H_{32}O_{15}$	MS^2[631]: 169.01329(100), 125.0232(78), 121.0283(45), 123.0075(23), 211.0240(21), 313.0563(17), 271.0462(17)	galloylpaeoniflorin isomers
61#	9.38	335.04085	335.04068	−0.523	$C_{15}H_{12}O_9$	MS^2[335]: 183.0290(100), 168.0056(7)	methyl digallate isomer
62#	9.40	721.14102	721.13950	−2.111	$C_{35}H_{30}O_{17}$	MS^2[721]:169.0.0133(100), 125.0233(70), 461.1089(36), 211.0241(27), 313.0565(23), 123.0075(16)	thonningianin B
63#	9.42	783.10503	783.10773	3.447	$C_{35}H_{28}O_{21}$	MS^2[783]: 169.0133(100), 125.0232(92), 121.0283(72), 631.1673(34), 151.0027(22)	myricetin 3-O-(2′′,3′′-digalloyl)-β-D-galactopyranoside
64#	9.60	937.09525	937.09595	0.743	$C_{41}H_{30}O_{26}$	MS^2[937]: 300.9990(100), 275.0199(22), 169.0133(19), 125.0231(18), 229.0129(11)	trigalloyl-HHDP-glucose isomer
65*#	9.69	441.08271	441.08255	−0.385	$C_{22}H_{18}O_{10}$	MS^2[441]: 289.0717(100), 125.0232(62), 169.0132(45), 137.0232(25), 203.0706(22), 245.0818(22)	(−)-Epicatechin gallate
66#	9.72	335.04085	335.04080	−0.165	$C_{15}H_{12}O_9$	MS^2[335]: 183.0290(100), 168.0055(7)	methyl digallate isomer
67#	9.72	461.07254	461.07260	0.110	$C_{21}H_{18}O_{12}$	MS^2[461]: 271.9177(100), 182.0210(93), 285.0400(82), 183.0287(50), 273.9148(33), 89.0229(31)	3,3′-Di-O-methyl-4-O-((β-D-xylopyranosyl) ellagic acid)
68#	9.79	491.08311	491.08307	−0.089	$C_{22}H_{20}O_{13}$	MS^2[491]: 328.0220(100), 312.9987(49), 169.0130(3), 125.0231(1)	dimethylellagic acid glucoside
69	9.91	631.16684	631.16693	0.137	$C_{30}H_{32}O_{15}$	MS^2[631]: 169.0133(100), 125.0232(89), 121.0283(55), 123.0075(38), 107.0126(21), 151.0026(18), 313.0569(13)	galloylpaeoniflorin isomers
70#	9.96	491.08311	491.08316	0.094	$C_{22}H_{20}O_{13}$	MS^2[491]: 169.0133(100), 125.0232(73)	dimethylellagic acid glucoside isomer
71	10.08	939.11090	939.11145	0.581	$C_{41}H_{32}O_{26}$	MS^2[939]: 169.0133(100), 125.0232(800), 123.0075(38), 95.0125(11)	pentagalloylglucose
72	10.15	631.16684	631.16705	0.327	$C_{30}H_{32}O_{15}$	MS^2[631]: 169.0133(100), 125.0232(93), 121.0283(56), 123.0075(37), 107.0125(23), 151.0025(15), 313.0570(12)	galloylpaeoniflorin isomers
73#	10.16	715.13045	715.12903	−1.997	$C_{36}H_{28}O_{16}$	MS^2[715]: 82.9528(100), 631.1674(67), 169.0134(7)	Theaflavin-3-Gallate isomer
74#	10.35	694.12091	694.12047	−0.646	$C_{30}H_{31}O_{17}S$	MS^2[694]: 169.0133(100), 125.0232(73), 121.0283(45), 123.0075(23), 631.1670(22)	Galloypaeoniforin sulfonate
75#	10.56	599.10424	599.10474	0.829	$C_{28}H_{24}O_{15}$	MS^2[599]: 169.0130(100), 313.0562(74), 285.0400(46), 284.0324(43), 241.0358(12)	kaempferol-3-O-(2′′-O-galloyl)-β-D-glucopyranoside
76#	10.66	801.11559	801.11627	0.842	$C_{35}H_{30}O_{22}$	MS^2[801]: 125.0232(100), 169.0133(98), 183.0290(62), 123.0076(30), 139.0390(20), 107.0125(15)	methyl tetragalloylglucose
77	10.85	631.16684	631.16711	0.423	$C_{30}H_{32}O_{15}$	MS^2[631]: 125.0232(100), 121.0283(97), 169.0133(79), 123.0076(59), 631.1675(39)	galloylpaeoniflorin isomers
78#	10.85	715.13045	715.12988	−0.808	$C_{36}H_{28}O_{16}$	MS^2[715]: 82.9528(100), 631.1674(46), 169.0133(8)	Theaflavin-3-Gallate isomer
79	11.13	1091.12186	1091.12244	0.529	$C_{48}H_{36}O_{30}$	MS^2[1091]: 939.1113(100), 169.0133(32), 769.0895(24)	hexagalloyl glucose isomer
80	11.15	545.05729	545.05743	0.137	$C_{24}H_{18}O_{15}$	MS^2[545]: 169.0133(100), 125.0232(76), 469.0517(65), 123.0075(11), 393.0457(8)	Dihydroxybenzoic acetate-digallate derivative
81#	11.20	783.17780	783.17847	0.853	$C_{37}H_{36}O_{19}$	MS^2[783]: 169.0134(100), 125.0232(84), 121.0283(48), 631.1669(29), 123.0076(26), 211.0243(22)313.0565(18)	digalloylpaeoniflorin isomer
82	11.25	1091.12186	1091.12244	0.529	$C_{48}H_{36}O_{30}$	MS^2[1091]: 939.1112(100), 169.0133(34), 769.0895(23)	hexagalloyl glucose isomer
83#	11.60	315.01464	315.01480	0.507	C15H8O8	MS^2[315]: 299.9912(100)	methylellagic acid isomer
84#	11.61	487.05181	487.05090	−0.914	$C_{22}H_{16}O_{13}$	MS^2[487]: 169.0129(100), 125.0229(21), 183.0285(5)	methyl trigallate isomer
85	11.65	545.05729	545.05762	0.327	$C_{24}H_{18}O_{15}$	MS^2[545]: 169.0133(100), 125.0232(77),	Dihydroxybenzoic

(*continued on next page*)

Table 1 (*continued*)

Peak	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula	MS/MS fragment	Identification/ Reactions
						469.0517(63), 123.0075(11), 393.0462(9)	acetate-digallate derivative
86	11.65	1091.12186	1091.12268	0.749	$C_{48}H_{36}O_{30}$	MS^2[1091]: 169.0133(100), 939.1123(27), 769.0891(17)	hexagalloyl glucose isomer
87#	11.80	315.01464	315.01471	0.221	C15H8O8	MS^2[315]: 299.9912(100)	methylellagic acid isomer
88	11.85	545.05729	545.05756	0.267	$C_{24}H_{18}O_{15}$	MS^2[545]: 169.0133(100), 125.0232(79), 469.0517(69), 123.0075(11), 393.0460(10)	Dihydroxybenzoic acetate-digallate derivative
89	11.85	1091.12186	1091.12256	0.639	$C_{48}H_{36}O_{30}$	MS^2[1091]: 169.0133(100), 939.1115(31), 769.0903(19)	hexagalloyl glucose isomer
90#	11.93	487.05181	487.05106	−0.754	$C_{22}H_{16}O_{13}$	MS^2[487]: 183.0290(100), 169.0133(81), 125.0232(66), 395.0323(21), 123.0075(20)	methyl trigallate isomer
91#	12.01	783.17780	783.17865	1.083	$C_{37}H_{36}O_{19}$	MS^2[783]: 169.0133(100), 125.0232(87), 121.0283(25), 123.0076(17), 151.0024(11), 107.0126(14)	digalloylpaeoniflorin isomer
92#	12.13	487.05181	487.05127	−0.544	$C_{22}H_{16}O_{13}$	MS^2[487]: 169.0133(100), 125.0232(76), 183.0290(72), 125.0232(76)	methyl trigallate isomer
93	12.23	621.05808	621.05758	−0.806	$C_{22}H_{22}O_{21}$	MS^2[621]: 469.0516(100), 169.0133(98), 125.0232(69)	galloy-valoneic acid bilactone isomer
94#	12.30	783.17780	783.17834	0.687	$C_{37}H_{36}O_{19}$	MS^2[783]: 169.0134(100), 125.0233(98), 121.0283(45), 123.0075(24), 107.0126(16),	digalloylpaeoniflorin isomer
95#	12.41	783.17780	783.17853	0.930	$C_{37}H_{36}O_{19}$	MS^2[783]: 169.0133(100), 125.0232(90), 121.0283(23), 123.0076(19), 107.0126(13)	digalloylpaeoniflorin isomer
96	12.71	621.05808	621.05763	−0.726	$C_{22}H_{22}O_{21}$	MS^2[621]: 469.0517(100), 169.0133(93), 125.0232(67)	galloy-valoneic acid bilactone isomer
97	12.71	1243.13282	1243.13391	0.876	$C_{55}H_{40}O_{34}$	MS^2[1243]: 939.1108(100), 769.0879(11), 1091.1224(8)	heptagalloy glucose
98#	12.74	461.10893	461.10910	1.262	$C_{22}H_{22}O_{11}$	MS^2[461]: 169.0133(100), 125.0232(98)	cinnamoylgalloy glucose isomers
99#	12.75	873.15198	873.15129	−0.791	$C_{42}H_{34}O_{21}$	MS^2[873]: 125.0232(100), 169.0133(83), 123.0074(24), 121.0284(16), 211.0240(12)	thonningianin A
100#	12.76	783.17780	783.17859	1.006	$C_{37}H_{36}O_{19}$	MS^2[783]: 125.0232(100), 169.0133(94), 121.0283(37), 123.0075(36),151.0026(14), 631.1684(11)	digalloylpaeoniflorin isomer
101#	12.96	487.05181	487.05194	0.126	$C_{22}H_{16}O_{13}$	MS^2[487]: 183.0290(100), 335.0411(13)	methyl trigallate isomer
102#	12.97	461.10893	461.10922	1.382	$C_{22}H_{22}O_{11}$	MS^2[461]: 151.0026(100), 125.0232(62), 169.0134(29), 83.0125(23), 107.0125(23)	cinnamoylgalloy glucose isomers
103#	14.70	461.10893	461.10913	1.292	$C_{22}H_{22}O_{11}$	MS^2[461]: 125.0232(100), 169.0055(94), 149.9949(64), 89.0231(14)	cinnamoylgalloy glucose isomers
104#	14.76	329.03029	329.03058	0.880	$C_{16}H_{10}O_8$	MS^2[329]: 314.0065(100), 298.9833(20)	dimethylellagic acid isomer
105#	15.00	329.03029	329.03046	0.515	$C_{16}H_{10}O_8$	MS^2[329]: 314.0068(100), 298.9834(80), 270.9883(76)	dimethylellagic acid isomer
106#	19.33	343.04594	343.04608	0.407	$C_{17}H_{12}O_8$	MS^2[343]: 312.9995(100), 328.0221(94), 297.9757(55)	trimethyl ellagic acid

* Identified by comparison with standards.
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Compounds 13, 22, 28 and 33 were observed at 3.17, 5.43, 6.15 and 6.57 min, which appeared same precursor ions at *m/z* 527.140 with compounds 18 and yielded fragment ion at *m/z* 169.0130, 313.0559, 125.0232. The fragment ion at *m/z* 313.0563 and 169.0129 indicated the presence of a galloyl glucose. These compounds were tentatively proposed as isomers of 6′-O-Galloyl-desbenzoylpaeoniforin.

Compound 20, 36, and 39, were detected at 5.24, 6.68 and 7.00 min, with the same empirical molecular formula $C_{19}H_{25}O_{15}$, matched to that of benzoylsucrose isomers and consistent with reference (Li et al., 2009).

Compound 40 and 55 were observed at 7.02 and 8.87 min, possessing the same quasi-molecular ions $[M-H]^-$ at *m/z* 481.099, matched to that of galloyvanilloy glucose isomers. The fragment at *m/z* 313.0559 generated by the loss of a vanilloy group, which further gave rise to the product ions at *m/z* 169.0129 and 125.0232. Similarly, compounds 98, 102 and 103 were observed at 12.74, 12.97 and 14.70 min, possessing

the same quasi-molecular ions $[M-H]^-$ at *m/z* 461.109, matched to that of cinnamoylgalloy glucose isomers (Wang et al., 2020). The main daughter ion at *m/z* 151.0026 may be due to loss of a galloyl glucose (313 Da). The DFIs 169.0133 ([gallic acid-H]$^-$) and 125.0232 ([gallic acid-CO_2-H]$^-$) were found in the UHPLC-Q-Exactive Orbitrap MS analysis. Compound 63 was eluted at 9.42 min, yielded parent ion $[M-H]^-$ *m/z* 783.1077 and deduced as myricetin 3-O-(2′′,3′′-digalloyl)-β-D-galactopyranoside and had an MS/MS fragment ion at *m/z* 631.1673 caused by loss of a galloyl moiety (152 Da), showed characteristic fragments (*m/z* 169.0133 and 125.0232) of gallic acid moiety and characteristic fragments (*m/z* 151.0027) of myricetin moiety (Abu-Reidah et al., 2015). Compound 75 was detected at 10.56 min, yielded a parent ion $[M-H]^-$ *m/z* 599.1047 in the ESI-mode, which produced typical daughter ions at *m/z* 313.0562 and 285.0400 indicated the presence of a galloyl glucose and kaempferol, respectively. So it was tentatively identified as kaempferol-3-O-(2′′-O-galloyl)-β-D-glucopyranoside (Zehl et al., 2011).

Compound 60, 69, 72 and 77 were detected at 9.37, 9.91, 10.15 and 10.85 min, possessing the same quasi-molecular ions $[M-H]^-$ at *m/z* 631.166, matched to that of galloylpaeoniflorin isomers. MS/MS fragment ion at *m/z* 313.0570 and 121.0283 indicated the presence of a galloyl glucose and a benzoyl group, respectively (Xu et al., 2006) . Then the fragment at *m/z* 313.0570 gave rise to 169.0133, 125.0232 by successive loss of a glucose reside and a CO_2. The proposed fragmentation pathway of galloylpaeoniflorin was shown in Fig. 2 (B). Similarly, compounds 81, 91, 94, 95 and 100 were detected at 11.20, 12.01, 12.30, 12.41 and 12.76 min, possessing the same precursor ions $[M-H]^-$ at *m/z* 783.178, matched to that of digalloylpaeoniflorin isomers and had one more dehydrated galloyl moiety (152 Da) than galloylpaeoniflorin. Therefore, their MS/MS fragmentation pattern were very similar to galloylpaeoniflorin. Besides, compounds 74 was eluted at 10.35 min, with the molecular formula $C_{30}H_{31}O_{17}S$ at *m/z* 694.1204. Among them, its fragment ion *m/z* 631.1670 proved the existence of a galloylpaeoniflorin. Therefore, this compound was determined as galloypaeoniforin sulfonate.

3.4.1.2. Identification of ellagitannins. Compound 26 with quasi-molecular ions $[M-H]^-$ at *m/z* 633.073 was eluted at 6.11 min and unambiguously identified as corilagin (galloy-HHDP-hexoside), which was affirmed by comparison to commercial reference standard. MS/MS fragment ion at *m/z* 300.9988 due to sequential loss of a gallic acid (170 Da) and a glucose residue (162 Da). The proposed fragmentation pathway of corilagin was shown in Fig. 2(C). Compound 14 also had the same $[M-H]^-$ *m/z* 633.073 and characteristic fragment *m/z* 300.9984, 275.0193, 169.0128 with compound 26, which was tentatively inferred as corilagin (galloy-HHDP-hexoside) isomer. Similarly, compound 21, 34 had quasi-molecular ions at *m/z* 785.084 and compound 50, 64 yielded same deprotonated molecule $[M-H]^-$ *m/z* 937.095. These compounds appeared similar losses to galloy-HHDP-hexoside isomers with an extra loss of galloyl moiety (152 Da) or two extra loss of galloyl moieties (304 Da), and all generated the characteristic fragment ions at *m/z* 300.9989 ($C_{14}H_5O_8$), 275.0198 ($C_{13}H_7O_7$), 125.0230 ($C_6H_5O_3$), 169.0132 ($C_7H_5O_5$), which been tentatively characterized as digalloyl-HHDP-glucose isomers and trigalloyl-HHDP-glucose isomers, respectively (Khaled et al., 2019). Compound 45 was found at 7.76 min and possessing the deprotonated ion $[M-H]^-$ at *m/z* 935.0798. The one of main fragments at 633.0724 *m/z* shows the loss of one HHDP group and fragment ions at *m/z* 300.9985 and 275.0195 indicated the presence of a HHDP group. The characteristic fragments *m/z* 169.0129 ([gallic acid-H]$^-$), 125.0230 ([gallic acid-CO_2-H]$^-$) also can be observed. Based on these MS data, compound 45 was deduced as galloyl-bis-HHDP-D-glucopyranose and consistent with reference (Glasenapp et al., 2019).

Compound 37 was eluted at 6.69 min, yielded a deprotonated ion $[M-H]^-$ *m/z* 801.0798 and fragment ions at *m/z* 125.0231, 289.0714, 121.0281, 96.9587, 151.0389, 169.0131. Based on these MS data, compound 37 was suggested as punigluconin and consistent with reference (Chan et al., 2018).

Compounds 48 and 57 were eluted at 8.11 and 9.20 min, possessing the same quasi-molecular ions $[M-H]^-$ at *m/z* 477.067 and deduced as methylellagic acid glucopyranoside isomers (Chen et al., 2017). The main daughter ion at *m/z* 315.0147 was attributed to the loss of a glucose residue (162 Da), which further gave rise to the product ions at *m/z* 298.9832 due to loss of a methyl radical (15 Da).

Compound 58 was eluted at 9.28 min, yielded parent ion $[M-H]^-$ *m/z* 300.9988 and deduced as ellagic acid according to the MS and MS/MS spectra. The typical daughter ions at *m/z* 229.0138 $[M-2H]^-$ and 283 $[M-H-H_2O]^-$ were attributed to loss of a H^- or a H_2O and then gave rise to the ions at *m/z* 169.0134 and 125.0232 (Abu-Reidah et al., 2015). The proposed fragmentation pathway of ellagic acid was shown in Fig. 2(D). Similarly, compound 43, which was eluted at 7.62 min, exhibited quasi-molecular ions $[M-H]^-$ at *m/z* 463.0518, generated main fragments at *m/z* 300.9991 by loss of a hexose (162 Da) and identified as ellagic acid hexose (Yang et al., 2012).

Compounds 67 appeared at a retention time (t_R) of 9.72 min, possessing the quasi-molecular ions $[M-H]^-$ at *m/z* 461.0726 and tentatively identified as 3,3′-Di-O-methyl-4-O-(β-D-xylopyranosyl) ellagic acid. The MS/MS spectrum presented $[M-H-189\ Da]^-$, $[M-H-187\ Da]^-$, [methyl gallate–H]$^-$, [methyl gallate–2H]$^-$ ion at *m/z* 273.9148, 271.9177, 183.0287 and 182.0210 (Singh et al., 2016).

Compounds 68 was eluted at 9.79 min, with the molecular formula $C_{22}H_{20}O_{13}$ at *m/z* 491.083. The fragment ions *m/z* 328.0220 and 312.9987 indicated the presence of a dimethyl ellagic acid and methyl ellagic acid, respectively. Based on these MS data, compound 70 are suggested as dimethylellagic acid glucoside. Compounds 70 had same MS/MS spectra date with compounds 68 and characterized as the isomer of dimethylellagic acid glucoside.

Compounds 83 and 87 were eluted at 11.60 and 11.80 min, possessing the same quasi-molecular ions $[M-H]^-$ at *m/z* 315.014 and deduced as methylellagic acid isomers. The main daughter ion at *m/z* 299.9912 was attributed to the loss of a methyl radical (15 Da). Similarly, compound 104, 105 with the same precursor ions at *m/z* 329.030 have been characterized as dimethylellagic acid isomers and had an MS/MS fragment ion at *m/z* 314.0065, 298.9833 due to sequential loss of a methyl radical (15 Da) in MS^2 (Zehl et al., 2011). Compounds 106 was eluted at 19.33 min, and possessing the deprotonated ion $[M-H]^-$ at *m/z* 343.046 and deduced as trimethyl ellagic acid. The product ions at *m/z* 328.0221, 312.9995 and 297.9757 were attributed to the loss of successive loss of a methyl radical (15 Da).

3.4.1.3. Identification of gallic acid derivatives. Gallic acid (4, t_R 1.51 min) was the major tannins of PRA and exhibited quasi-molecular ion $[M-H]^-$ at m/z 169.0141, generated main characteristic fragments at m/z 125.0232 ([gallic acid-CO_2-H]$^-$), which was identified by comparison with reference substances. The proposed fragmentation pathway of gallic acid was shown in Fig. 2(E). Therefore, multiple galloyl moieties were found at m/z 321.025 (23, t_R 5.82 min and 25, t_R 5.98 min) and m/z 473.035 (51, t_R 8.52 min), which were deduced as digallic acid isomers and trigallic acid, respectively. The product ions at m/z 321.0262 and 169.0147 were obtained by successive loss of gallic acid moieties from a main ion at m/z 473.0358 in the UHPLC-Q-Exactive Orbitrap MS analysis.

Compounds 16 was eluted at 4.64 min, possessing quasi-molecular ion $[M-H]^-$ at m/z 183.0298, showed characteristic fragments at (m/z 168.0056) of a demethylated product ion, and was accurately characterized as methyl gallate, which was identified by comparison with reference substances. Compounds 19 had the same mass spectrum information and identified as methyl gallate isomer. Similarly, compounds (46, t_R 7.78 min, 61, t_R 9.38 min and 66, t_R 9.72 min) and compounds (84, t_R 11.61 min, 90, t_R 11.93 min, 92, t_R 12.13 min, and 101, t_R 12.96 min) were tentatively characterized as methyl digallate isomers and methyl trigallate isomers due to sequential loss of galloyl moieties (152 Da) from their parent ions at $[M-H]^-$ m/z 335.0408 and $[M-H]^-$ m/z 487.051, respectively, showing that the characteristic fragments m/z 169.0133 ([gallic acid-H]$^-$), 125.0232 ([gallic acid-CO_2-H]$^-$). One of major fragment at m/z 183.0291 could be yielded by the loss of a galloyl moiety (152 Da). Compound 42 was eluted at 7.29 min, yielded a deprotonated ion $[M-H]^-$ m/z 197.044 and deduced as ethyl gallate.

Compounds 80, 85 and 88 were observed at 11.15, 11.65, and 11.85 min, possessing the some quasi-molecular ions $[M-H]^-$ at m/z 545.057, matched to that of Dihydroxybenzoic acetate-digallate derivative. MS/MS fragment ion at m/z 469.0517 ($[M-H-72]^-$) and 393.0462 ($[M-H-152]^-$) were obtained owing to neutral losses of acetyl + H_2O and galloyl moieties from the precursor ion (m/z 545), respectively. In addition, The product ions at m/z 169.0133, 125.0232 and 123.0075 indicated the presence of a gallic acid (Dienaitė et al., 2019).

Compound 93 and 96 were detected at 12.23 and 12.71 min, possessing the same quasi-molecular ions $[M-H]^-$ at m/z 621.057, matched to that of galloy-valoneic acid bilactone isomers. The dominant product ions at m/z 469.0516 attributed to neutral loss of galloyl moiety (152 Da), indicating valoneic acid bilactone in structure (Dienaitė et al., 2019). The characteristic fragments m/z 169.0133 ([gallic acid-H]$^-$), 125.0232 ([gallic acid-CO_2-H]$^-$) also can be observed.

3.4.2. Identification of condensed tannins

Compound 65 was eluted at 9.69 min, yielded deprotonated ion $[M-H]^-$ m/z 441.082 and unambiguously identifed as (–)-Epicatechin gallate, which was affirmed by comparison to commercial reference standard. There compounds (47, t_R 7.95 min, 52, t_R 8.54 min and 54, t_R 8.78 min) had the same $[M-H]^-$ m/z 441.082 and characteristic fragment m/z 289.0716 ($C_{15}H_{13}O_6$), 169.0133 ($C_7H_5O_5$), 125.0232 ($C_6H_5O_3$), 245.0817 ($C_{14}H_{13}O_4$) with compounds 65. So, they were tentatively assigned as (–)-Epicatechin gallate isomers. The proposed fragmentation pathway of (–)-Epicatechin gallate was shown in Fig. 2(F).

Compound 31, 32, 35 and 38 were found at 6.38, 6.50, 6.67 and 6.83 min, possessing the same parent ions $[M-H]^-$ at m/z 703.167 and tentatively deduced as theaflavin 3′-gallate isomers. One of main fragment at m/z 125.0230 probably produced by sequential two loss of catechin moiety (289 Da). The product ions at m/z 61.9869, 289.0714 emerged from catechin moiety (Poon, 1998).

Compounds 59, 73 and 78 at m/z 715.129 having the same molecular formula $C_{36}H_{28}O_{16}$ and appeared at a retention time (t_R) of 9.36, 10.16, and 10.85 min. Based on these MS^2 data, compound 61, 75 and 79 are suggested as theaflavin-3-gallate isomers and consistent with reference (Kuhnert et al., 2010).

Compound 62 (t_R 9.40 min) had quasi-molecular ion at m/z 721.1395 and compound 99 (t_R 12.75 min) yielded deprotonated ion $[M-H]^-$ at m/z 873.1512. The molecular weight of Compound 62 is 152 Da less than that of compound 99, whereby the difference lies in the different substituents present at the C-3 position of glucose, whereby one is the H and the other is the galloyl, and all generated the characteristic fragment ions at m/z 169.0133 ($C_7H_5O_5$), 125.0233 ($C_7H_5O_5$), 211.0241 ($C_{13}H_7O_3$), which been tentatively characterized as thonningianin B and thonningianin A, respectively (Wong et al., 2020).

3.5. Pharmacological activity of tannins in PRA

According to reports, tannins have antiviral, antitumour, antihypertensive, uraemic toxin decreasing and renal failure-improving actions, and have great potential as a new pharmaceutical resource (Adderson et al., 1961; Nishioka, 1983).

Gallotannins represent the simplest class of hydrolyzable tannins, containing gallic acid substituents esterified with a polyol residue. As highmolecular-weight tannins, penta-, hexa- and heptagalloylglucose, have greatest potential to reduce blood glucose in an insulin resistant state (Juan et al., 2011). Moreover, there are many studies on the biological activity of pentagalloylglucose , which have seen traditional use in the treatment of diseases related to skin barrier disruption (Kim et al., 2020) Furthermore, pentagalloylglucose has other main physiological activities including anti-inflammatory, anti-allergic, antitumor, antiviral, and antibacterial effects (Parker et al.,2016), and recently been highlighted as the most important and is currently being developed into a therapeutic for cancer and diabetes (He et al., 2010).

Ellagitannins have antibacterial and antioxidant properties, and can be used as chemical defense barriers due to the unique particularities of their structure and the remarkable selectivity of their embedded chemical reactivity, sush as galloy-HHDP-hexoside (Quideau et al., 2010., Li et al., 2016a).

Gallic acid, an abundant hydrolyzable tannin found in RPA, has been extensively studied for its antioxidant and antiviral activity (Parker et al.,2016).

Condensed tannins, also referred to as proanthocyanidins, have been shown to have the potential beneficial effects on human health, including immunomodulatory, anti-inflammatory, anticancer, antioxidant, cardioprotective and antithrombotic properties (Sieniawska, 2015., Smeriglio et al., 2014). In particular, theaflavin 3′-gallate and

theaflavin-3-gallate would be potential compounds for treating platinum-resistant ovarian cancer (Pan et al., 2017).

4. Conclusion

In this research, an efficient strategy based on UHPLC Q-Exactive Orbitrap MS in the negative ion mode was established to detect tannins components in PRA. Finally, a total of 106 constituents, among them, 75 compounds were first reported in PRA, including gallotannins, ellagitannins, gallic acid derivatives and condensed tannins were detected and identified based on their chromatographic retention, MS and MS^2, and bibliography data. According to previous studies, gallic acids are the main component of RPA, which have multiple pharmacological activities including anti-inflammatory, antioxidant, antibacterial, antitumor, and provide treatment for hypertension, myocardial infarction and diabetes. Overall, the result laid the foundation for in-depth research on the Pharmacodynamic material basis of PRA.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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