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UPLC-Q-TOF/MS analysis of chemical constituents of methanolic extract from cassava tender stems

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Abstract

In the processing and utilisation of cassava, stems and leaves usually become waste. The tender stems of cassava were analysed by electrospray ionisation high-performance liquid chromatography-tandem mass spectrometry. Using UPLC BEH C_{18} column (50 × 2.1 mm; i.d.: 1.7 µm), the mobile phases were H_2O (0.1% FA) and CH_3CN (0.1% FA), the column temperature was 30°C, and the flow rate was 0.30 mL/min. Mass spectrometry was performed by the Waters SYNAPT G2 HDMS system. This gave excellent reproducibility and avoided the interference of some similar substances. A total of 33 compounds were identified in Bzhe samples of cassava tender stems by this method, mainly cyanosides, catechins, flavonoids, glycosides, glycosides, phospholipids, and other components. The present work provided reference value for the development and utilisation of cassava tender stems.

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Introduction

Cassava is the fifth-largest food crop in the world, and widely planted in tropical areas (Eyinla *et al*., 2021). During the processing and utilisation of cassava, stems and leaves usually become waste (Khempaka *et al*., 2022). Cassava is rich in many substances and functions, for example, proteins and enriched proteins (Vuong *et al*., 2021). Alamu *et al*. (2020) found that cassava roots contain a large number of micro- and macro-elements, and the water extract of cassava buds can be used as a protective agent against acetaminophen-induced liver injury (Elshamy *et al*., 2021). At present, cassava tender stems are simply used as raw materials for raising chickens, fish, and pigs, among other livestock. Tesfaye *et al*. (2014) used cassava stem slices and *Moringa oleifera* leaf powder as alternative feed components in the diet of laying hens; Okrathok *et al*. (2018) used cassava residue to make feed; and Khempaka *et al*. (2022) used cassava pulp to make feed. At present, there are many reports, conducted both at home and abroad, on using cassava stalk as animal feed (Alamu *et al*., 2020; Kurnianingsih *et al*., 2021). Only by studying the chemical composition of cassava tender stem can it be further developed and utilised. The composition of cassava tender stem is the material basis for its function. However, at present, there is a lack of reports on cassava tender stem, and the related characteristic components of cassava tender stem are still unclear (Alamu *et al*., 2020; Elshamy *et al*., 2021). This leads to the difficulty to effectively utilise cassava tender stem.

A fingerprint is based on the inherent quality characteristics of food, which can reflect the internal characteristics of the substance detected by modern instruments such as spectrum and chromatography (Benavides *et al*., 2006). Fingerprint has the characteristics of integrity, systematicness, and stability. Kurnianingsih *et al*. (2021) used high-performance liquid chromatography to study amino acids and anthocyanins in purple sweet potato. Tao *et al*. (2009) used mass spectrometry to study mineral elements and heavy metals in cassava products, while

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He *et al*. (2021) used mass spectrometry to study the effects of metabolic differences in purple sweet potato roots on the flavonoid pathway. The qualitative ability of mass spectrometry is somewhat deficient, but the combination of liquid chromatography and mass spectrometry can improve its own defects. It is often used to test various components such as *Artemisia annua* extract (Protti *et al*., 2019), antibiotics and pesticides in poultry feathers (Wu *et al*., 2021), as well as the identification of components (Kurnianingsih *et al*., 2021) in liquor and other fields such as the components of cassava tender stems. However, there is no literature reporting on the fingerprint of cassava tender stems.

In the present work, the components in the tender stem of cassava were studied by liquid chromatography and mass spectrometry. Through the retention behaviour of chromatographic peaks, accurate molecular weight, and the MS^E fragment information of the compounds and comparison with the literatures, 33 compounds were identified in the cassava tender stem Bzhe sample which mainly included cyanosides, catechins, flavonoids, glycosides, glycosides, and phospholipids. This provided reference value for the development and utilisation of cassava tender stem.

Materials and methods

Preparation of test solution

A 25 mg tender cassava stem (Bzhe) were put into a 5-mL centrifugal tube. Then, 2 mL methanol was added, ultrasonic treatment was performed for 20 min, centrifuged, and the supernatant was taken and passed through a 0.22 μm microporous filter membrane to obtain a sample solution with a concentration of about 12.5 mg/mL to be used for further testing.

Analysis of test conditions

Liquid phase conditions: Waters ACQUITY UPLC system (PDA detector), binary solvent delivery system, and automatic sampler module were used; column: UPLC BEH C_{18} column (50 × 2.1 mm, i.d.: 1.7 μm); mobile phase: A = H_2O (0.1% FA) and B = CH_3CN (0.1% FA); column temperature: 30°C; sample room temperature: 10°C; flow rate: 0.30 mL/min; sampling volume: 2 μL/2 μL (positive/negative ion mode). Table 1 shows the flow gradient of the mobile phases.

MS conditions: Waters SYNAPT G2 HDMS system; the atomisation and cone gas were used as the nitrogen mass spectrometry ion source; electrospray ionisation positive and negative ion modes; capillary voltage: 3.0 KV (positive ion mode)/2.5 KV (negative ion mode); taper hole voltage: 40 V; extraction cone voltage: 3 V; ion source temperature: 100°C; desolvation gas temperature: 400°C (positive ion mode)/350°C (negative ion mode); reverse taper hole airflow: 50 L/h; the flow rate of desolvation gas: 600 L/h (positive ion mode)/600 L/h (negative ion mode); collision gas velocity: 0.5 mL/min; scanning time: 0.5 s; scanning time interval: 0.02 s; mass-charge ratio range: 50 - 1200 *m/z*; data acquisition form: continuum; sensitivity: normal; dynamic range: extended; number of locked masses: $[M+H]^+$ = 556.2771 and $[M-H]^-$ = 554.2615.

Table 1. UPLC mobile phase gradient program of cassava tender stem sample.

No.	Time (min)	A (%)	B (%)	Curve
1	0.0	99.0	1.0	
2	10.0	85.0	15.0	6
3	20.0	50.0	50.0	6
4	25.0	1.0	99.0	6

Statistical analyses

The experiment was replicated at least twice. The data were expressed as mean ± SD of triplicate experiments. Calculations were carried out using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA), and the data were analysed using IBM SPSS statistical software, version 21.0 (SPSS Inc., Chicago, IL, USA). Differences were assessed using Duncan's test and ANOVA, with significance accepted at $p < 0.05$.

Results and discussion

Comprehensive results

The optimised UPLC-Q-TOF/MS analysis method was used to detect the Bzhe extract samples of cassava tender stems. Figures 1 and 2 show the UPLC and base peak chromatogram in the positive and negative ion modes. Based on the chromatographic peak retention behaviour, the accurate molecular weight and MS^E fragment information of the compounds were obtained, and

compared with the literature. Here, 33 compounds were identified from the Bzhe samples of cassava tender stems (Table 2), which mainly included cyanoside, catechin, flavone, glycoside, glycoside, and phospholipid, as well as other components. All of these 33 components were flavonoids, which are one of the important secondary metabolites in cassava leaves with high content.

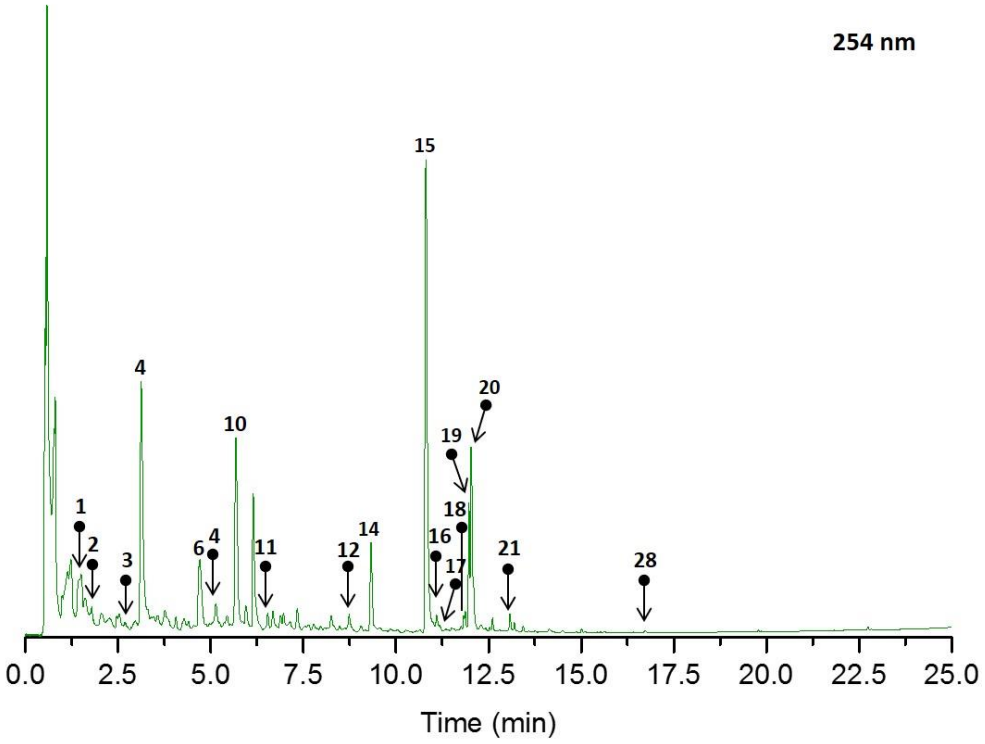

Figure 1. UPLC fingerprint of cassava tender stem (Bzhe) sample.

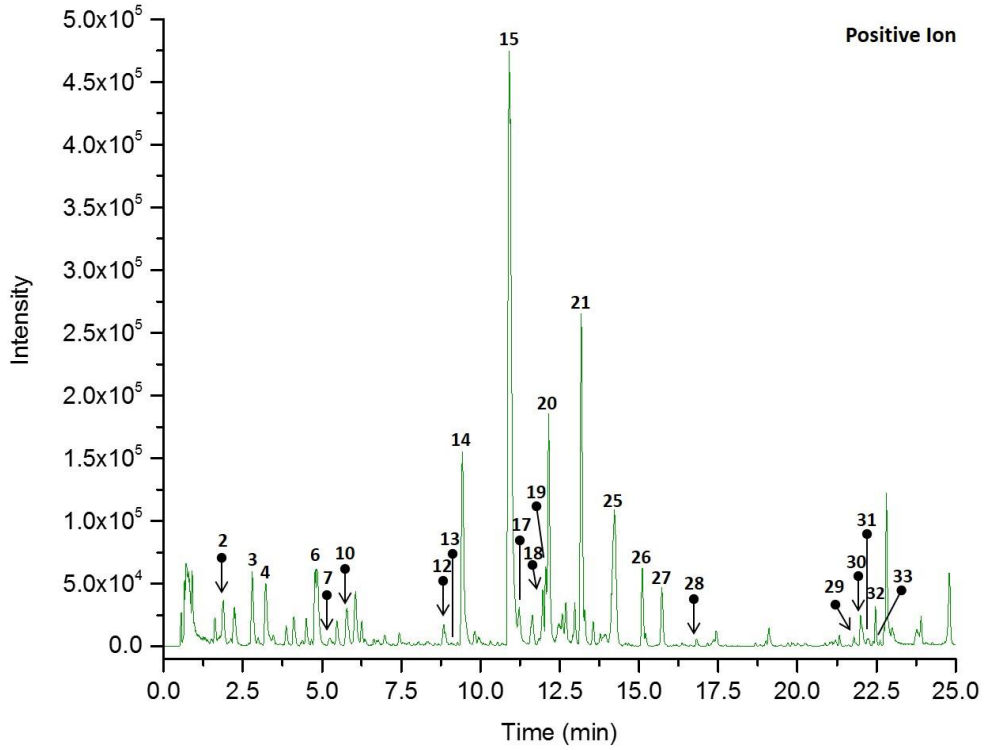

Figure 2. Ion diagram of mass spectrum base peak of cassava tender stem (Bzhe) sample in UPLC-Q-TOF/MS positive ion mode.

Table 2. List of components identified by UPLC-Q-TOF/MS of cassava tender stem (Bzhe) extract.

No.	t_R (min)	Compound	Formula	Experimental *m/z*
1	1.58	6-O-galloylglucose	$C_{13}H_{16}O_{10}$	331.067
2	1.89	Guanosine	$C_{10}H_{13}N_5O_5$	284.0978
3	2.8	N-(1-Deoxy-1-fructosyl)phenylalanine	$C_{15}H_{21}NO_7$	328.1384
4	3.23	(+)-Gallocatechin	$C_{15}H_{14}O_7$	307.0814/305.0669
5	3.46	(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)propane	$C_{14}H_{26}O_{10}$	353.1452/707.2969
6	4.87	Subaphylline	$C_{14}H_{20}N_2O_3$	265.1548
7	5.28	Procyanidin B2	$C_{30}H_{26}O_{12}$	579.1500/577.1352
8	5.39	Procyanidin B1	$C_{30}H_{26}O_{12}$	579.1500/577.1352
9	5.42	(2S)-((6-O-(β-D-apio-furanosyl)-β-D-glucopyranosyl)oxy)butane	$C_{15}H_{28}O_{10}$	367.1602
10	5.79	(+)-Catechin	$C_{15}H_{14}O_6$	291.0862/289.0791
11	6.64	Quercetin-3-O-(4-O-crotonyl)-β-D-glucopyranoside	$C_{26}H_{26}O_{13}$	547.1461/545.1298
12	8.82	Glucosylrutin	$C_{33}H_{40}O_{21}$	773.2101/771.1977
13	9.18	Procyanidin B3	$C_{30}H_{26}O_{12}$	579.1500/577.1352
14	9.43	Quercetin 3-O-sophoroside	$C_{27}H_{30}O_{17}$	627.1553/625.1402
15	10.93	Rutin	$C_{27}H_{30}O_{16}$	611.1616/609.1459
16	11.19	Quercetin glycoside	$C_{21}H_{20}O_{12}$	463.0878
17	11.23	Quercetin	$C_{15}H_{10}O_7$	303.0508
18	11.94	Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	595.1670/593.1504
19	11.96	Kaempferol	$C_{15}H_{10}O_6$	287.0553
20	12.16	Isorhamnetin-3-O-rutinoside	$C_{28}H_{32}O_{16}$	625.1787/623.1625
21	13.16	Forsythin	$C_{27}H_{34}O_{11}$	535.2196/533.2037
22	13.44	Lauroside B	$C_{19}H_{32}O_9$	403.1964
23	13.59	Lauroside C	$C_{19}H_{32}O_9$	403.1967
24	13.81	Lauroside E	$C_{19}H_{32}O_9$	403.1967
25	14.22	Unidentified	—	666.3132/664.2980
26	15.11	Unidentified	—	666.2771/664.2625
27	15.73	Unidentified	—	777.3442/775.3302
28	16.82	Amentoflavone	$C_{30}H_{18}O_{10}$	539.0986/537.0820
29	21.1	LysoPC(18:3(6Z,9Z,12Z)/0:0)	$C_{26}H_{48}NO_7P$	518.3254
30	21.36	LysoPC(18:3(9Z,12Z,15Z)/0:0)	$C_{26}H_{48}NO_7P$	518.3257
31	21.8	LysoPC(0:0/18:2(9Z,12Z))	$C_{26}H_{50}NO_7P$	520.3405
32	22.01	LysoPC(18:2(9Z,12Z)/0:0)	$C_{26}H_{50}NO_7P$	520.3412
33	22.48	LPC(16:0)	$C_{24}H_{50}NO_7P$	496.3400

Identification of flavonoids and glycosides

Identification of rutin

Rutin has the basic structure of 2-benzolone. It has a high degree of super delocalisation, and a complete conjugated system. It is easy for strong coordination oxygen atoms and suitable spatial configurations to form metal complexes with metal ions (Ye *et al*., 2021). The positive and negative ion scanning modes of the electrospray ionisation source were selected for detection and analysis. For the positive and negative ion modes, as shown in Figure 3, the peaks with a retention time of t_R = 10.91 and 10.90 min gave 611.1616 and 609.1459 *m/z*, respectively, in the primary mass spectrum, which were the excimer ion peaks $[M+H]^+$ and $[M-H]^-$ ($C_{27}H_{30}O_{16}$ Δ*m/z*; 0.3 ppm). In the secondary mass spectrum, there were abundant fragment ion peaks of 465, 303, 257, 229, 201, 153, and 137 *m/z*, and 301, 300, 271, 255, 178, and 151 *m/z*. In addition, there were characteristic peaks of 229, 256, and 352 nm in the ultraviolet spectrum of this chromatographic peak. By using the study conducted by Li *et al*. (2015) as a comparison, it was preliminarily inferred that compound 15 (Bzhe) might be rutin, where part of its mass spectrum cleavage pathway is shown in Figure 4.

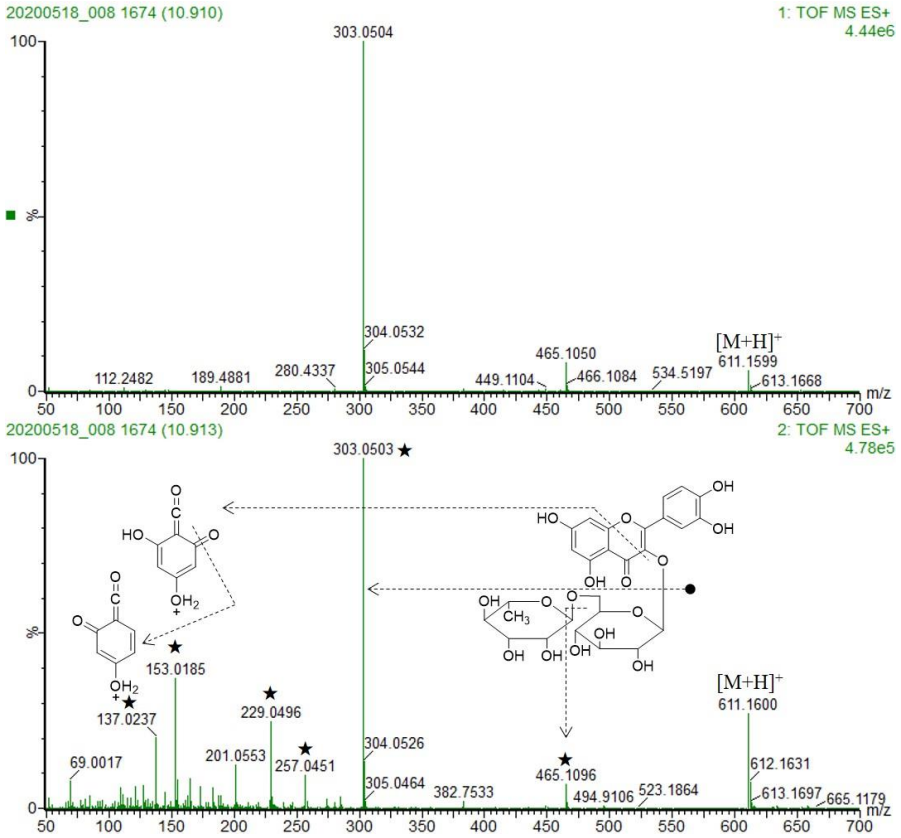

Figure 3. Mass spectrogram of rutin in UPLC-Q-TOF/MS positive ion mode.

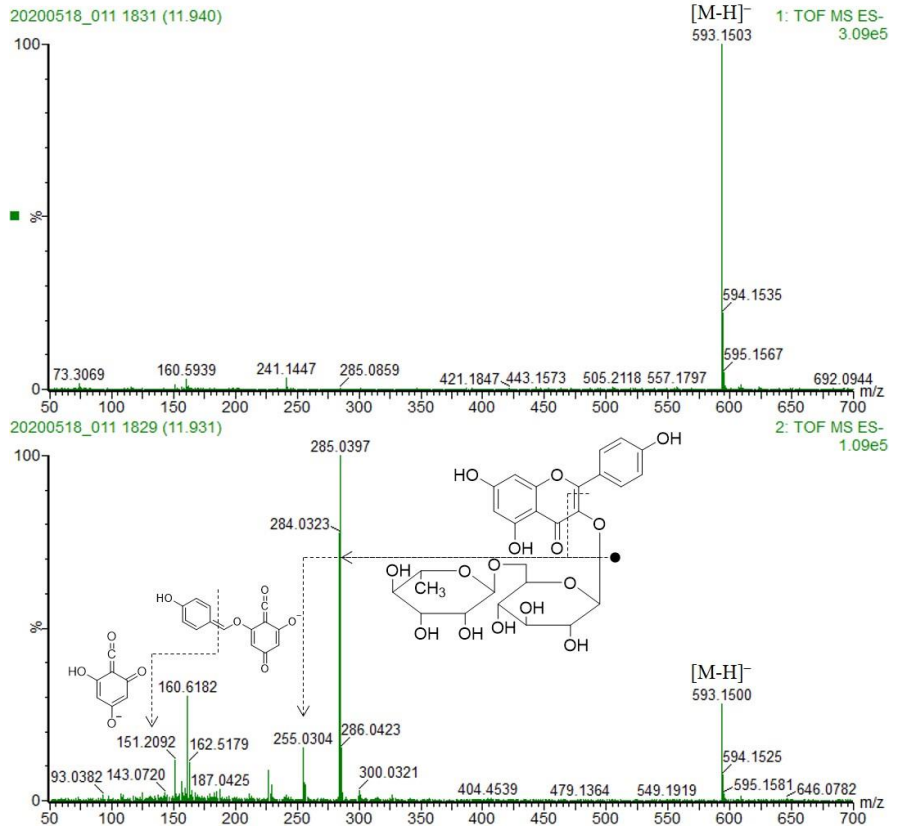

Figure 4. Mass spectrum of kaempferol-3-O-rutinoside in UPLC-Q-TOF/MS negative ion mode.

Identification of kaempferol-3-O-rutinoside

Kaempferol-3-O-rutinoside is a light-yellow crystal with a unique structure of flavonoids. It has C_6-C_3-C_6 skeleton and 2-phenylchromogen ketone as the basic structure (Li *et al*., 2019). The two benzene rings are connected through the three central carbon atoms. Its hydroxyl were different from the rutin structure. Therefore, the negative ion test was selected. In the negative ion mode, as shown in Figure 4, the peak with the retention time of t_R = 11.93 min gave 593.1504 *m/z* in the primary mass spectrum, which was the excimer ion peak $[M-H]^-$ ($C_{27}H_{30}O_{15}$ Δ*m/z*; 0.3 ppm). In the secondary mass spectrum, there were abundant fragment ion peaks at 285, 255, 227, 160, and 151 *m/z*. In addition, there were characteristic peaks of 226 and 281 nm in the ultraviolet spectrum of this chromatographic peak. Based on the comparison of the study conducted by Blagbrough *et al*. (2011), it was preliminarily inferred that compound 18 (Bzhe) might be kaempferol-3-O-rutinoside, where its partial mass spectrum cleavage pathway is presented in Figure 5.

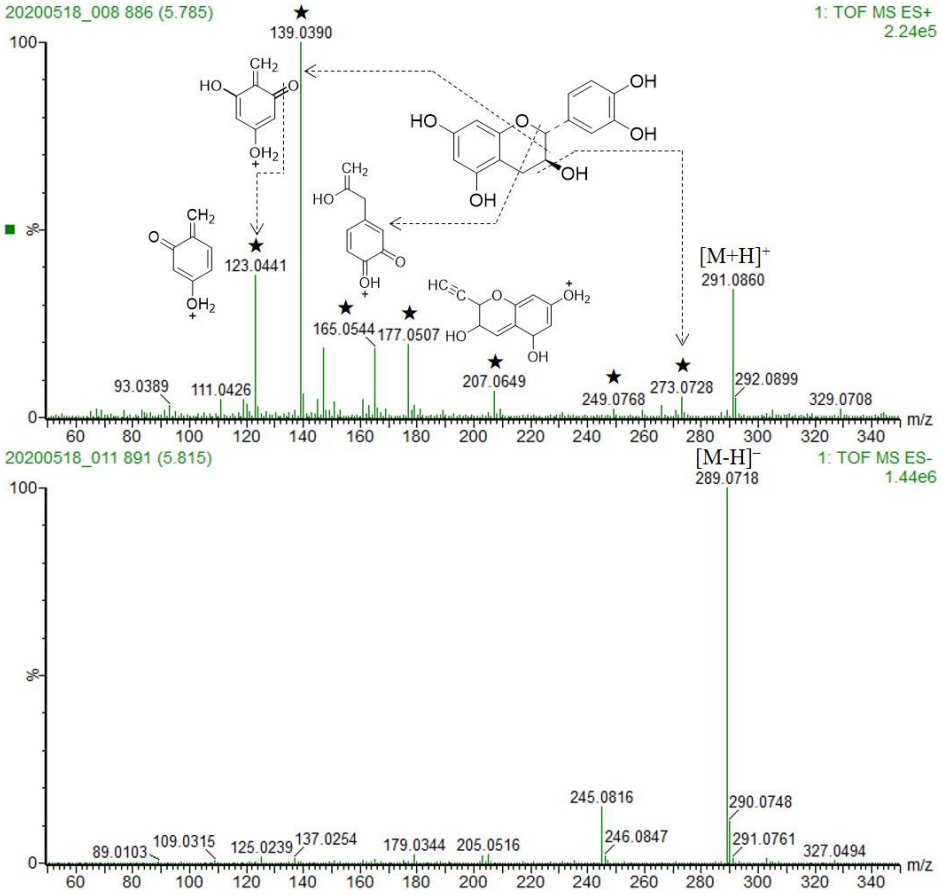

Figure 5. Mass spectrum of (+)-catechin in positive (top) and negative (bottom) ion modes of UPLC-Q-TOF/MS.

Identification of catechins

Identification of gallocatechin ((+)-gallocatechin)

Catechins have a 2-phenylbenzodihydropyran structure, and belong to flavanols. They have a variety of monomer modes. Therefore, the positive ion mode of mass spectrometry was selected. In the positive ion mode, as shown in Figure 6, the peak with a retention time of t_R = 3.23 min gave 307.0814 *m/z* in the first-order mass spectrum, which was the excimer ion peak $[M+H]^+$ ($C_{15}H_{14}O_7$ Δ*m/z*; 2.6 ppm). In the secondary mass spectrum, there were abundant fragment ion peaks at 289, 227, 163, and 139 *m/z*. In addition, there were characteristic peaks of 229 and 270 nm in the ultraviolet spectrum of this chromatographic peak. Based on the comparison with the study conducted by Sun *et al*. (2019), it was preliminarily inferred that compound 4 (Bzhe) might be (+)-gallocatechin, where its partial mass spectrum cleavage pathway is shown in Figure 7.

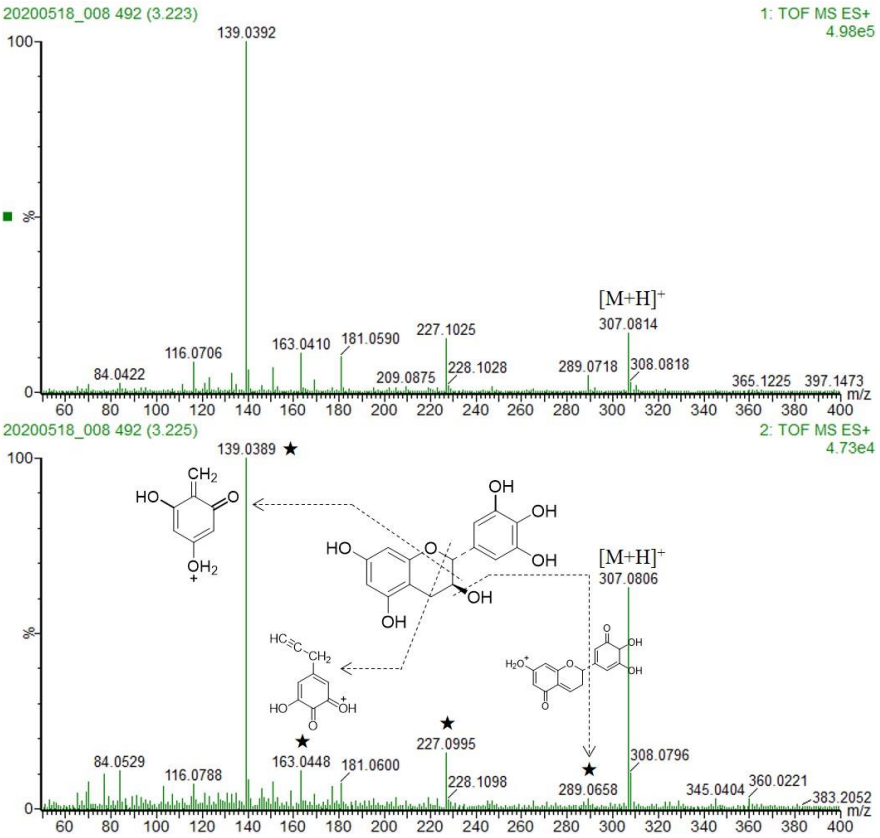

Figure 6. Mass spectrum of (+)-gallocatechin in UPLC-Q-TOF/MS positive ion mode.

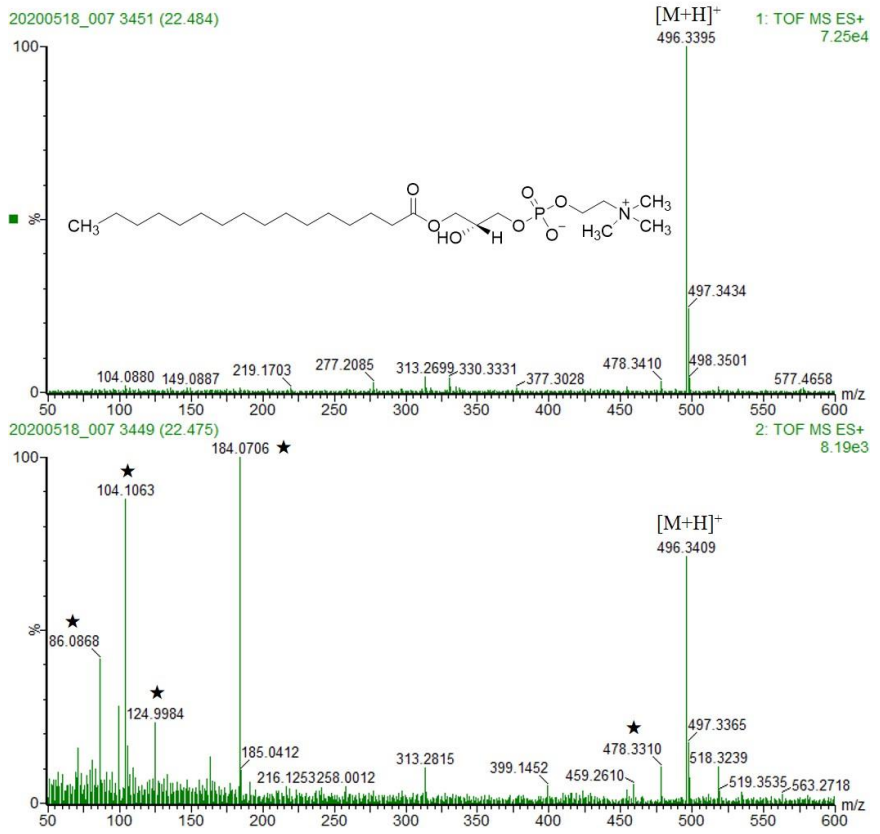

Figure 7. Mass spectrogram of LPC (16:0) in UPLC-Q-TOF/MS positive ion mode.

Identification of catecholic acid ((+)-catechin)

Catechuic acid is a phenolic substance which can be easily confused with tannins, anthocyanins, and other substances. Additionally, catechin compounds are diverse. Catechuic acid and gallic catechin have only simple hydroxyl groups, so the positive and negative ion modes were selected. In the positive and negative ion modes, as shown in Figure 5, the peaks with a retention time of t_R = 5.79 and 5.81 min gave 291.0862 and 289.0791 *m/z*, respectively, in the primary mass spectrogram, which were the excimer ion peaks $[M+H]^+$ and $[M-H]^-$ ($C_{15}H_{14}O_6$ Δ*m/z*; 2.4 ppm). In the secondary mass spectrum, there were abundant fragment ion peaks at 273, 249, 207, 177, 165, 139, and 123 *m/z*, as well as 245, 205, 179, 137, and 125 *m/z*. In addition, there were characteristic peaks of 228 and 279 nm in the ultraviolet spectrum of this chromatographic peak. Based on the comparison with the study conducted by Benavides *et al*. (2006), it was preliminarily inferred that compound 10 (Bzhe) might be (+)-catechin, where part of its mass spectrum cleavage pathway is shown in Figure 8.

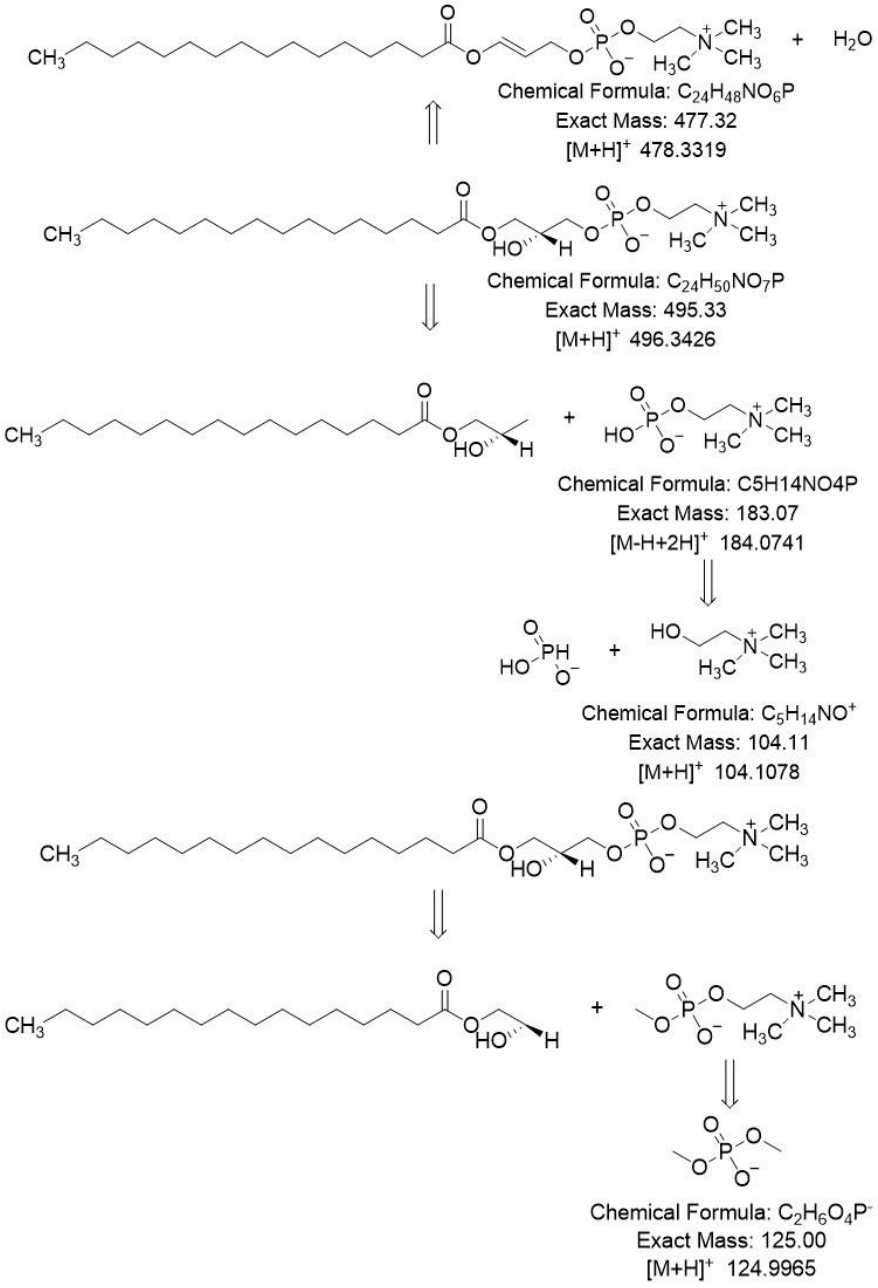

Figure 8. The process diagram of LPC (16:0) mass spectrum pyrolysis deduction.

Identification of phospholipid components

Identification of LPC (16:0)

Lysophosphatidylcholine (LPC) is haemolytic lecithin. It is the main component of oxidised low-density lipoprotein, and one of the components of phospholipids in eukaryotic cells. It is transformed from phosphatidylcholine under the action of phospholipase A_2. Furthermore, LPC contains a choline head group. In the positive mode, choline phosphate fragment ion *m/z* of 184 is the characteristic fragment for identifying LPC compounds. In the positive ion mode, as shown in Figure 7, the peak with a retention time of t_R = 22.48 min gave 496.3400 *m/z* in the primary mass spectrum, which was the excimer ion peak $[M+H]^+$ ($C_{24}H_{50}NO_7P$ Δ*m/z*; 0.6 ppm). In the secondary mass spectrum, abundant fragment ion peaks at 478, 184, 124, 104, and 86 *m/z* were produced. Based on the comparison with a study conducted by Park *et al*. (2019), it was preliminarily inferred that compound 33 (Bzhe) might be LPC (16:0), where part of its mass spectrum cleavage pathway is shown in Figure 8.

Identification of LysoPC (0:0/18:2 (9Z,12Z))

In the positive ion mode, as shown in Figure 9, the peak with a retention time of t_R = 22.01 min gave 520.3409 and 542.3259 *m/z* in the primary mass spectrum, which were excimer ion peaks $[M+H]^+$ and $[M+Na]^+$ ($C_{26}H_{50}NO_7P$ Δ*m/z*; 1.7 ppm). In the secondary mass spectrum, there were abundant fragment ion peaks at 502, 335, 184, 124, 104, and 86 *m/z*. Compared with the LPC (16:0) fragment information, fragment ions with the same characteristics of 184, 124, 104, and 86 *m/z* were found, and it was preliminarily inferred that compound 32 (Bzhe) might be lysoPC (0:0/18:2(9Z,12Z)).

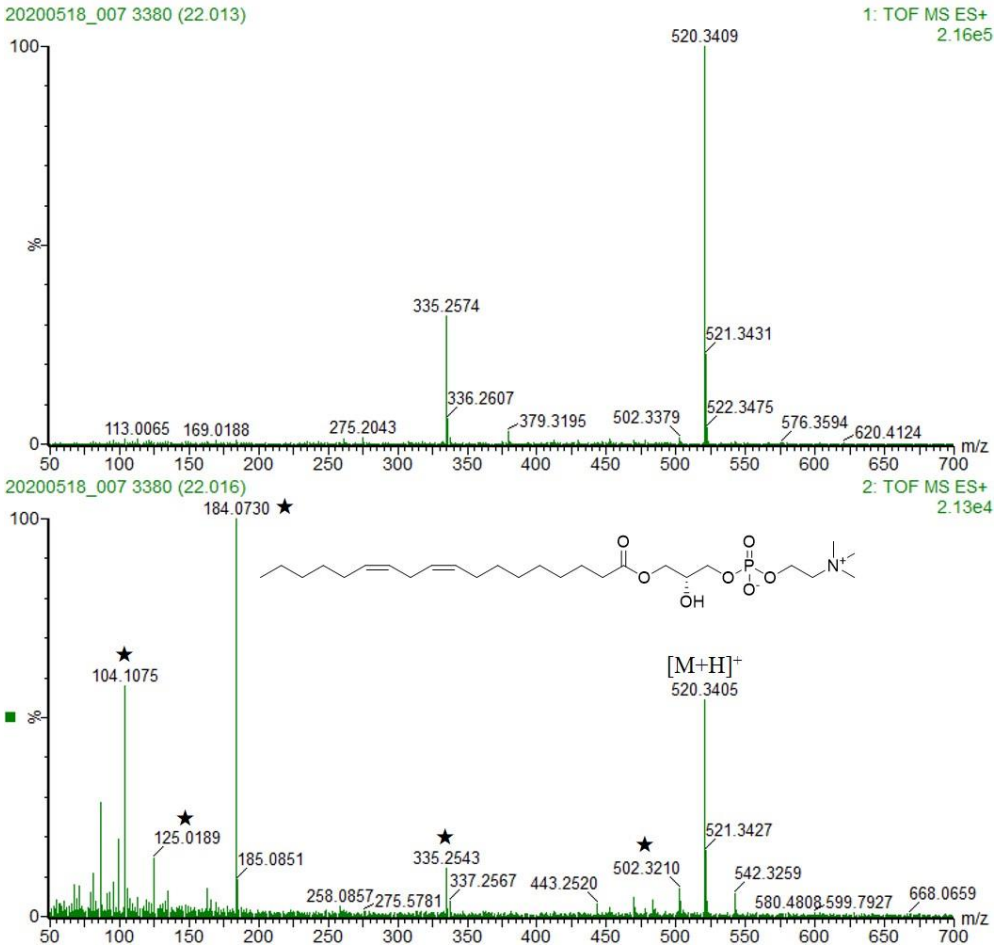

Figure 9. Mass spectrogram of lysoPC (0:0/18:2(9Z,12Z)) in UPLC-Q-TOF/MS positive ion mode.

Conclusion

The chemical components of Bzhe in the tender stem of cassava were successfully identified with 33 compounds observed. The structure of substances in cassava tender stem was analysed by UV-vis spectroscopy and liquid chromatography-mass spectrometry. Using the characteristics of first- and second-order mass spectrometry, Bzhe was compared with the existing literature. It was speculated that rutin, kaempferol-3-O-rutinoside, gallic catechin, catechuic acid LPC (16:0), and lysoPC (0:0/18:2 (9Z,12Z)) were some components in cassava shoots. In the present work, the exploration of the extract from cassava tender stem shed light on the action mechanism and future reference application, thus helping to make efficient use of the tender stem.

Acknowledgement

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