Hindawi
Journal of Food Quality
Volume 2021, Article ID 6621811, 12 pages
https://doi.org/10.1155/2021/6621811

Research Article

Comprehensive Mass Spectrometric Analysis of Snake Fruit: Salak (*Salacca zalacca*)

Petra Hlásná Čepková,[1] **Michal Jágr**,[2] **Dagmar Janovská**,[1] **Václav Dvořáček**,[2] **Anna Kotrbová Kozak**,[2] **and Iva Viehmannová**[3]

[1]*Gene Bank, Crop Research Institute, Drnovská 507/73, 161 06 Prague 6, Ruzyně, Czech Republic*
[2]*Quality of Plant Products, Crop Research Institute, Drnovská 507/73, 161 06 Prague 6, Ruzyně, Czech Republic*
[3]*Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague, Czech Republic*

Correspondence should be addressed to Michal Jágr; michaljagr@centrum.cz

Received 11 November 2020; Revised 26 February 2021; Accepted 10 March 2021; Published 23 March 2021

Academic Editor: Francisca Hernández

Copyright © 2021 Petra Hlásná Čepková et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Snake fruit (*Salacca zalacca* (Gaert.) Voss) is a fruit species traditionally cultivated in Indonesia and other Southeast Asian countries. The edible parts of the fruits contain a certain amount of total phenolic, flavonoid, and monoterpenoid compounds, proving them to be their perfect sources. The main goal of this work was to detect, quantify, and identify various phenolic compounds present in snake fruit pulp. Ultrahigh performance liquid chromatography coupled to a Q-Orbitrap tandem mass spectrometer was able to detect 19 phenolic compounds in the salak pulp, including 5 flavanols, 6 phenolic acids, 2 flavonols, 1 flavone, and also 5 presumably new phenolic compounds. Among the detected compounds, 11 were reported and quantified for the first time in salak pulp. Chlorogenic acid was by far the most predominant phenolic compound. The next relatively abundant compounds in snake fruit were epicatechin, isoquercetin, neochlorogenic acid, ferulic acid, gallic acid and procyanidine B2 (levels at ca 5–10 μg/g in MeOH extract), syringic acid, and caffeic acid (levels at ca 1 μg/g in H_2O extract). A significant total phenolic content (257.17 μL/mL) and antioxidant activities (10.56 μM TE/g of fruit pulp) were determined. In conclusion, *S. zalacca* fruit has potential to serve as a natural source of phenolic compounds with antioxidative activities which may be associated with their health benefits.

1. Introduction

Fruit consumption is nowadays associated with life quality improvement due to their high content of vitamins, minerals, and antioxidant compounds [1]. Fruits and vegetables in particular are key sources of antioxidants in the human diet. A lot of clinical and epidemiological research supports the hypothesis that consuming fruits is beneficial to age-related diseases, several cancers, heart diseases, etc. [2]. The most known and consumed fruits are the ones cultivated on a large scale, industrialized, and sold worldwide. Exotic fruits are usually local fruits known and consumed according to cultural practices [1]. Many other tropical fruits, already well known in the tropics, are now appearing in larger temperate city markets [3].

One of the exotic fruits is snake fruit—salak (*Salacca zalacca* (Gaert.) Voss). Salak is a tropical palm from the family Arecaceae, a palm tree family. Salak has been known as snake fruit and originally it came from Indonesia and other Southeast Asian countries [4]. The palm thrives under humid tropical lowland conditions with rainfall of 1,700–3,100 mm per year and a temperature range of 22–32°C. The dioecious nature of many cultivars leads to wide phenotypic variations, so vegetative propagation is used to produce a suitable clone [3] with the desired fruit quality [5, 6].

Salak is cultivated for its fruits, which are globose to ellipsoid drupes [7]. The most noticeable feature is the numerous yellow to brown united scales that end in a small

spine and cover the skin [3]. The edible part is nonfibrous and has a sweet taste and crisp texture with a colour from yellowish white to brown [8]. The unripe pulps are sour and have a sharp taste due to the presence of tannin acids. However, the ripe pulps are crunchy soft with sweet taste added to a distinct and pleasant aroma [9]. The fruit has been eaten as fresh fruit in Southeast Asia for a long time. It can be also candied, pickled, and dried, with fresh unripe fruits made into salad [8].

The content of basic nutritional compounds (fibres, crude protein, and crude fats) in snake fruit is higher than in kiwi fruit and a high content of minerals was also confirmed in salak [10]. Snake fruit is one of the tropical fruits with the highest level of total sugars (17.4 g/100 g of edible portion) [11]. Previous studies confirmed that salak fruits had shown high antioxidative activity 217 mg/100 g and 260 mg/100 g of edible portion [4, 12], which is even higher than in kiwi fruit with 8.42 to 10.24 μM TE/g [10]. The compounds responsible for its antioxidant property were identified as chlorogenic acid, catechin, (−)-epicatechin, and various proanthocyanidins [13]. Thus, 60% ethanolic extract of salak fruit was used for 6 weeks to treat and monitor metabolic changes in obese-diabetic rats. The authors observed significant declination of the blood glucose level and normalization of the blood lipid profile in these animals. Extract of salak fruit also was able to decrease ketone bodies and to improve energy metabolism. Findings described in this study support the traditional claims of salak fruits in management of diabetes [14].

The aim of this study was to comprehensively investigate an interesting group of biologically active phenolic compounds present in salak fruit pulp. Ultrahigh performance liquid chromatography (UHPLC) coupled to Q-Orbitrap was used to analyze methanolic and/or aqueous extracts of snake fruit pulp and to detect, identify, and quantify compounds present in the samples. Application of this highly effective and sensitive method facilitated the identification of several possible new compounds in salak fruit.

2. Materials and Methods

2.1. Standards Preparation and Sample Isolation. Mature salak fruit (*Salacca zalacca*) was purchased at a common Indonesian market. The fruit at the ripeness stage just suitable for consumption was peeled, the pulp was cut into small portions (of ca 100 g each) which were frozen, then lyophilized, and finally ground to obtain a yellowish powder. The powder was stored in the dark at −18°C prior to further processing.

To prepare reference stock solutions, individual reference standards of phenolic compounds (apigenin; caffeic acid; catechin; chlorogenic acid; epicatechin; ferulic acid; gallic acid; isoquercetin; naringenin; neochlorogenic acid; procyanidines B1, B2, and B3; syringic acid) were dissolved in MeOH to obtain a stock solution of 0.5 mg/mL and stored at −18°C. The stocks were further diluted with methanol to 1–500,000 ng/mL to create calibration curves for phenolic compound quantification. Probenecid was dissolved in MeOH at 0.5 mg/mL to prepare a stock solution of the internal standard. Probenecid was then added to individual reference standard solutions or test samples to the final concentration of 100 ng/mL.

Samples of lyophilized mature pulp salak fruit (0.1 g) in 2 mL centrifuge tubes were extracted in triplicate by 1 mL of extraction solvent with added probenecid as an internal standard at $c = 100$ ng/mL. Three various manners of extraction were tested: (1) pure MeOH; (2) 80% aqueous MeOH; and (3) water. Extraction was performed twice for each sample for 30 min at room temperature using an ultrasonic bath. Then, the samples were centrifuged (15 min; 13,500 rpm; 25°C) and corresponding supernatants were combined and filtered through 0.2 μm nylon syringe filters (Thermo Scientific, Rockwood, TN, USA). Extracts were stored at −18°C prior to ultrahigh performance liquid chromatography-electrospray ionization-tandem mass spectroscopy (UHPLC-ESI-MS/MS) analysis.

2.2. Chemicals and Plant Material. Standards of the phenolic compounds apigenin, catechin, chlorogenic acid, epicatechin, ferulic acid, gallic acid, isoquercetin, naringenin, neochlorogenic acid, procyanidines (B1, B2, and B3), syringic acid, rutin, and the internal standard probenecidverapamil hydrochloride were purchased from Sigma Aldrich (St. Louis, MO, USA). Methanol (LC-MS grade, ≥99.9%) was obtained from Riedel de Haën (Seelze, Germany). Formic acid (LC-MS grade, 99%) was purchased from VWR (Leuven, Belgium). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.3. UHPLC-ESI-MS/MS Instrumentation. The chromatographic system (Dionex UltiMate 3000 UHPLC system, Dionex Softron GmbH, Germany) consisted of a binary pump (HPG-3400RS), an autosampler (WPS-3000RS), a degasser (SRD-3400), and a column oven (TCC-3000RS). Detection was performed on a quadrupole/orbital ion trap Q exactive mass spectrometer (Themo Fisher Scientific, San Jose, CA, USA). Analytes were separated on a reversed phase C18 Titan column (2.1 × 100 mm, 1.9 μm) from Supelco (Bellefonte, PA, USA). The LC-MS system was equipped with a heated elecrospray ionization source (HESI-II) and Xcalibur software, version 4.0.

2.4. UHPLC-ESI-MS/MS Analysis. Chromatographic separation was performed using gradient elution with 0.2% formic acid in water as solvent A, and methanol with 0.2% formic acid as solvent B. The LC gradient was as follows: 0 min: 98% of solvent A + 2% of solvent B; 2 min 98% A + 2% B; 11 min 5% A + 95% B; 12 min 100% of B; 14 min 98% A + 2% B. The column was maintained at 40°C at a flow rate of 0.35 mL/min and injection volume was 1 μL.

MS/MS conditions were similar to those used in our previous study [15]. Briefly, ionization was run in the positive or negative electrospray ionization (ESI) mode. Spray voltage was maintained at 3.5 kV in the positive ion mode and at −2.5 kV in the negative ion mode. Sheath gas

flow was 49 arbitrary units, auxiliary gas flow rate was kept at 12 arbitrary units, and sweep gas flow was 2 arbitrary units. Capillary temperature was 259°C. Nitrogen was used as sheath, auxiliary, and sweep gas. Heater temperature was kept at 419°C. S-lens RF level was 50 for positive ion mode and 30 for negative ion mode. The mass spectrometer was generally operated in parallel reaction monitoring (PRM) mode. The precursor ions in the inclusion list were isolated within the retention time window ±20 s, filtered in the quadrupole at isolation window (target $m/z \pm 1.0$ amu), and fragmented in an HCD collision cell. Product ions were collected in the C-trap at resolution 17,500 FWHM, an AGC target value of 2×10^5 and maximum injection time of 100 ms. The normalized collision energy (NCE) was optimized for each compound. Precursor and daughter ions monitored, retention times, and NCE values are shown in Table 1. The accuracy and calibration of the *Q* Exactive Orbitrap LC-MS/MS were checked using a reference standard mixture obtained from Thermo Fisher Scientific. Data were evaluated by the Quan/Qual Browser Xcalibur software, v 4.0.

2.5. Determination of Phenolic Compound Concentration in Salak Samples and Statistical Analysis. Identification of phenolic compounds in salak pulp samples was based on their retention times relative to the authentic standards and on mass spectral data (accurate mass determination generating elemental composition and fragmentation patterns of a molecular ion) obtained by LC-MS, which were compared with those described in previous studies [9, 13]. Calibration curves for compound quantification were constructed by plotting the peak area (adjusted by probenecid as an internal standard) versus concentration of relevant reference standards.

2.6. Chemical Analyses

2.6.1. Folin Assay. Total phenolic content (TPC) was determined spectrophotometrically with Folin-Ciocalteau reagent as was shortly mentioned in our previous study [16]. A modified method of Holasova et al. [17] was used. Two grams of lyophilized sample was extracted with 20 mL of 80% MeOH for 60 min in a centrifugation tube. The tubes were protected from sunlight by aluminium foil. Resulting extract (0.5 mL) was pipetted into 50 mL volumetric flask and diluted with distilled water. Then, 2.5 mL Folin-Ciocalteau reagent (PENTA, Czech Republic) and 7.5 mL 20% sodium carbonate solution were added after agitation. After 2 h standing in the dark at laboratory temperature, absorbance at wave length $\lambda = 765$ nm was measured against blank on the spectrophotometer Genesys 10 UV (Thermo Scientific, USA). The results were quantified using gallic acid standard (Merck, Germany) and expressed as gallic acid equivalents (GAE). DPPH assay: the radical scavenging capacity (RSC) was determined on microtiter plates in MeOH extracts using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, 20 mL of MeOH was added to 1 g of sample and shaken for 90 min while it was protected from light by aluminium foil. Twenty μL of extract reacted for 10 min with 150 μL DPPH solution with initial absorbance $A = 0.6$ at 550 nm. The reaction occurred in the dark, and the absorbance at 550 nm was read afterward using a spectrophotometer (Sunrise absorbance reader, Tecan, Switzerland). The ability to scavenge the DPPH radical was determined using the standard curve obtained with Trolox (Sigma Aldrich, Germany) in a range from 0.0 to 0.2 mmol/L. The results were expressed as Trolox equivalent (TE) antioxidant activity (AA).

2.7. Statistical Analyses. All samples were analyzed in triplicate and expressed as mean ± standard deviation. Data analysis was performed in Statistica 12 software (TIBCO software, Palo Alto, CA, USA) and tested for outliers (Grubb's test). One-way analysis of variation (ANOVA) was applied to compare means followed by post hoc Tukey's test at a significance level of $P \leq 0.05$.

3. Results and Discussion

3.1. Chemical Analyses. There is some available information about high total phenolic content (TPC) influenced by maturity stage of snake fruit [15] which is accompanied by higher antioxidative activity in comparison with mangosteen, mango, and rambai in previous studies [4].

In our study, a high content of total phenolic 257.17 ± 1.62 mg/100 g GAE was determined in *S. zalacca*. Similar levels were also previously reported in *S. zalacca* with TPC that were higher than other fruit species [18, 19] and it is comparable with fruits of sea buckthorn (*Hippophaë rhamnoides* L.) [18]. The sample of salak pulp in our study showed antioxidant activity (AA) at the level of 10.56 ± 4.20 μmol TE/g, which corresponds with previously published results [20] and it is comparable with AA of black mulberry (*Morus nigra* L.) [21].

3.2. Extraction of Phenolic Compounds from Salak Pulp. Many researchers used water or alcohol-based solvents (mainly aqueous MeOH or EtOH at various organic concentrations) for phenolic compound extraction [9, 13, 14, 22]. Ethylacetate was also used in the extraction protocol [23]. Based on all these previous studies, we decided to use three extraction solvents with different lipophilicity (MeOH, 80% MeOH, and water) to reveal as many various phenolic compounds as possible. Extraction recovery was enhanced by using an ultrasonic bath. Triple extraction for 30 min at 25°C finally appeared to be the best compromise choice to obtain the highest phenolic compounds yields in the extraction solution.

3.3. Separation and Identification of Bioactive Phenolic Compounds in Salak Pulp by LC-MS/MS. Recently, separations of compounds isolated from salak fruit were most often achieved by HPLC or LC-MS equipped with a C18 column from various manufacturers [13, 23, 24]. Also, GC-MS analyses were performed to analyze derivatized or naturally volatile compounds from snake fruit [9, 25, 26]. We successfully used a

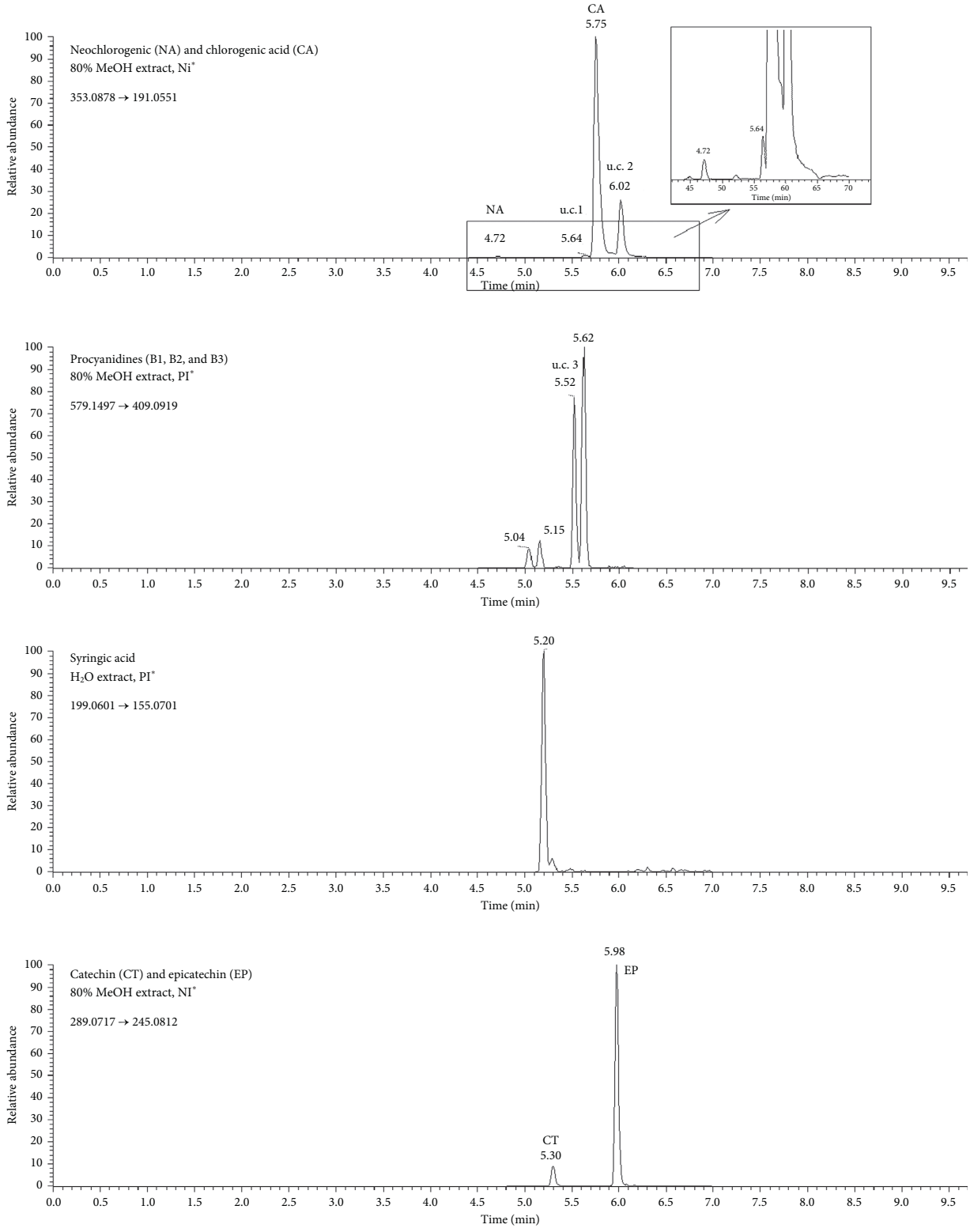
Table 1: Mass spectrometric data (positive and negative ionization) for phenolic compounds detected in *Salacca zalacca* fruit pulp by UHPLC-HRMS/MS analysis.

Phenolic compound	Elemental composition	Rt (min)	[M + H]+ (m/z) measured	NCE*	Typical fragment ions (positive mode, m/z), elemental composition of fragment, mass accuracy (ppm)
Procyanidine B1	C30H26O12	5.15	579.1497	20	427.10242 C22H19O9 (0.14 ppm); 409.09189 C22H17O8 (0.23 ppm); 291.08594 C15H15O6 (–1.30 ppm); 127.03896 C6H7O3 (–0.06 ppm)
Procyanidine B2	C30H26O12	5.62	579.1496	20	427.10220 C22H19O9 (–0.36 ppm); 409.09128 C22H17O8 (–1.26 ppm); 291.08597 C15H15O6 (–1.19 ppm); 127.03893 C6H7O3 (–0.36 ppm)
u.c. 3: presumable procyanidine isomer	C30H26O12	5.04	579.1499	20	427.10225 C22H19O9 (–0.41 ppm); 409.09130 C22H17O8 (–1.14 ppm); 127.03895 C6H7O3 (–0.10 ppm)
Procyanidine B3	C30H26O12	5.04	579.1499	20	427.10214 C22H19O9 (–0.51 ppm); 409.09137 C22H17O8 (–1.04 ppm); 127.03892 C6H7O3 (–0.46 ppm)
Syringic acid	C9H10O5	5.20	199.0601	25	155.07005 C8H11O3 (–1.40 ppm); 123.04406 C7H7O2 (0.03 ppm); 95.04945 C6H7O (3.29 ppm)
Rutin isomer (presumable)	C27H30O16	5.73	611.1606	20	300.02673 C15H8O7 (0.54 ppm); 271.02441 C14H7O6 (0.98 ppm); 255.02959 C14H7O5 (1.54 ppm)
Phenolic compound	Elemental composition	Rt (min)	$[M-H]^-$ (m/z) measured	NCE*	Typical fragment ions (negative mode, m/z), elemental composition of fragment, mass accuracy (ppm)
Gallic acid	C7H6O5	1.80	169.01425	45	125.02322 C6H5O3 (–0.10 ppm); 97.02815 C5H5O2 (–0.25 ppm)
Neochlorogenic acid	C16H18NO9	4.72	353.0878	40	191.05516 C7H11O6 (0.76 ppm)
Catechin	C15H14NO6	5.30	289.0717	30	245.08122 C14H13O4 (1.58 ppm); 203.07039 C12H11O3 (0.58 ppm); 125.02337 C6H5O3 (0.38 ppm); 109.02823 C6H5O2 (–1.63 ppm)
Ferulic acid	C10H10O4	5.40	193.05063	45	134.03600 C8H6O2 (0.23 ppm); 178.02608 C9H6O4 (0.01 ppm); 137.02319 C7H5O3 (–0.12 ppm)
u.c. 1: presumable chlorogenic acid isomer	C16H18NO9	5.64	353.0878	40	191.05506 C7H11O6 (0.25 ppm)
Chlorogenic acid	C16H18NO9	5.75	353.0878	40	191.05504 C7H11O6 (0.13 ppm)
u.c. 2: presumable chlorogenic acid isomer	C16H18NO9	6.02	353.0878	40	191.05514 C7H11O6 (0.64 ppm)
Caffeic acid	C9H8NO4	5.80	179.0350	20	135.04391 C8H7O2 (–1.04 ppm)
Epicatechin	C15H14NO6	5.98	289.0719	30	245.08121 C14H13O4 (1.51 ppm); 203.07028 C12H11O3 (0.06 ppm); 125.02325 C6H5O3 (–0.59 ppm); 109.02833 C6H5O2 (–0.72 ppm)
Isoquercetin	C21H20NO12	7.49	463.0882	30	301.03427 C15H9O7 (–0.03 ppm); 300.02667 C15H8O7 (0.73 ppm); 255.02887 C14H7O5 (0.27 ppm); 151.00255 C7H3O4 (–0.24 ppm)
Naringenin	C15H12NO5	8.33	271.0612	40	151.00249 C7H3O4 (–0.65 ppm); 119.04897 C8H7O (–1.41 ppm)
u.c. 4: presumable naringenin isomer	C15H12NO5	8.33	271.0612	40	151.00250 C7H3O4 (–0.60 ppm); 119.04894 C8H7O (–1.35 ppm)
Apigenin	C15H10NO5	9.02	269.0456	50	151.00266 C7H3O4 (0.46 ppm); 11.03336 C8H5O (–1.09 ppm)

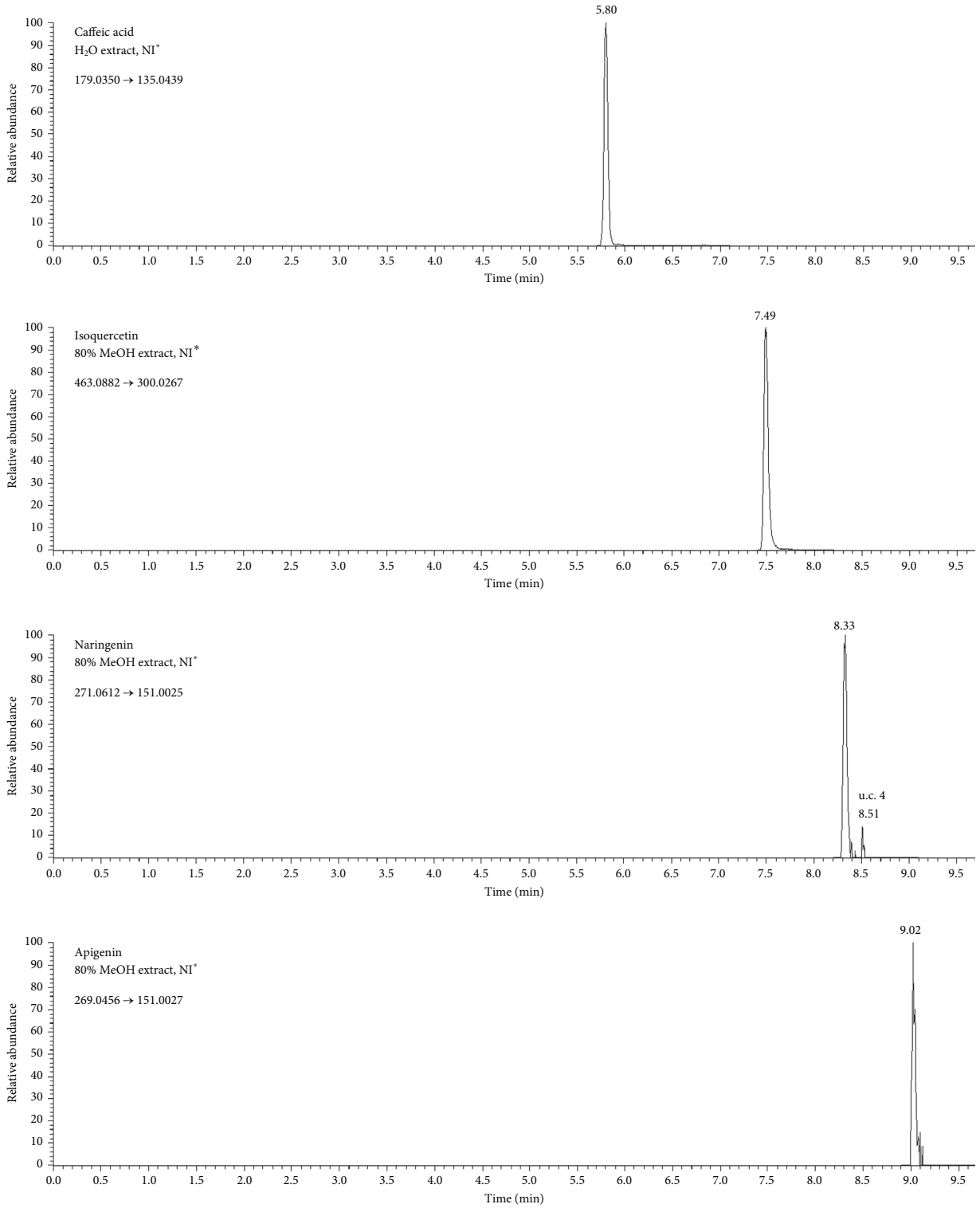
NCE*: normalized collision energy.

Titan C18 10 cm long column for sufficient separation of various scales of phenolic compounds, in total only a 14 min long analysis (Figure 1). Well resolved PRM chromatograms were obtained for all phenolic compounds investigated on this column: from early eluted gallic acid ($t_R = 1.80$ min), through neochlorogenic acid ($t_R = 4.72$ min) and isoquercetin ($t_R = 7.49$ min), to the latest apigenin with $t_R = 9.02$ min. The identities of the compounds discovered (Figure 1 and Supplementary Figure 1) were confirmed by comparing their retention times and MS/MS fragmentation behaviour to the standard compounds.

We analyzed methanolic, 80% MeOH and aqueous extracts of salak pulp fruit by UHPLC followed by positive or negative ionization (PI or NI) and HRMS/MS (Figures 1 and 2


Neochlorogenic (NA) and chlorogenic acid (CA)
80% MeOH extract, Ni*
353.0878 → 191.0551
CA
5.75
u.c. 2
6.02
NA
4.72
u.c.1
5.64
Relative abundance
Time (min)
Procyanidines (B1, B2, and B3)
80% MeOH extract, PI*
579.1497 → 409.0919
5.62
u.c. 3
5.52
5.04
5.15
Syringic acid
H2O extract, PI*
199.0601 → 155.0701
5.20
Catechin (CT) and epicatechin (EP)
80% MeOH extract, NI*
289.0717 → 245.0812
5.98
EP
CT
5.30

(a)



(b)

Figure 1: Analysis of phenolic compounds in *Salacca zalacca* fruit pulp by UHPLC-HRMS/MS with MS/MS spectrometer operating in PRM (parallel reaction monitoring) mode. Note: each PRM transition is described by name of corresponding compound and characterized by its retention time, and *m/z* values of respective precursor ion and product ion. u.c.—unknown compound. PI*—positive ionization; NI*—negative ionization.

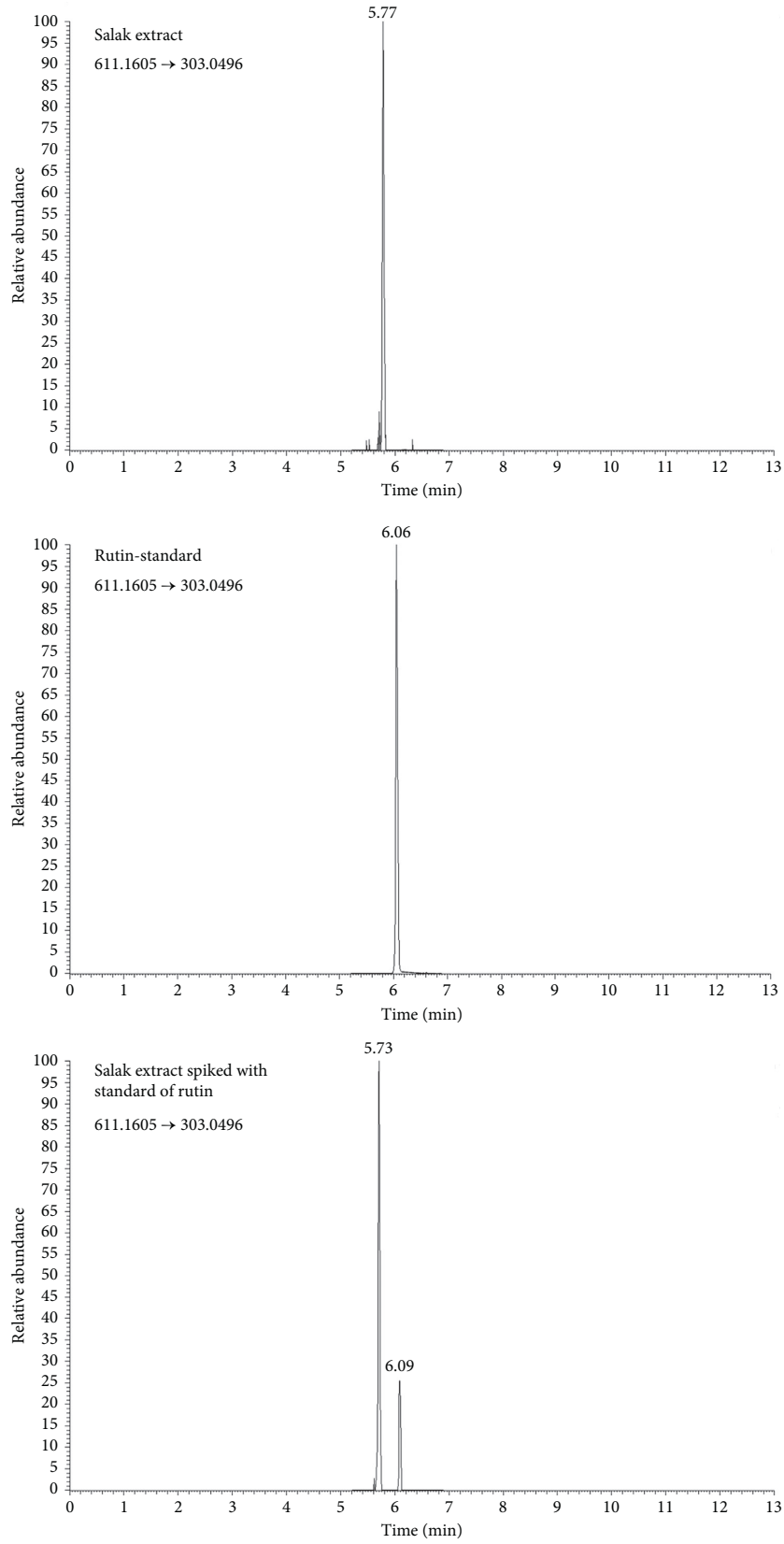


Figure 2: Detection of rutin-like compound (Rt = 5.73 min) in *Salacca zalacca* fruit pulp and rutin standard (Rt = 6.09 min) by UHPLC-HRMS/MS with MS/MS spectrometer operating in PRM (parallel reaction monitoring) mode with positive ionization. PRM transition is described by name of corresponding compound and characterized by its retention time, and *m/z* values of respective precursor ion and product ion.

Table 2: Contents of individual phenolic compounds determined in *Salacca zalacca* fruit pulp. n.d.: not determined.

Group of polyphenols	Compound[a]	Polarity	Phenolic compounds in Salacca zalacca fruit[b], HESI ionization		
			MeOH extract	80% MeOH extract	H_2O extract
Flavanols	Epicatechin	−	13.77 ± 0.46	12.82 ± 0.20	0.41 ± 0.02
	Catechin	−	$(2.96 \pm 0.11).10^{-1}$	$(2.97 \pm 0.14).10^{-1}$	n.d.
	Procyanidine B1	+	$(5.81 \pm 0.17).10^{-1}$	$(4.71 \pm 0.08).10^{-1}$	$(1.29 \pm 0.08).10^{-2}$
	Procyanidine B2	+	9.64 ± 0.31	11.51 ± 0.23	$(2.26 \pm 0.32).10^{-2}$
	Procyanidine B3	+	$(4.42 \pm 0.19).10^{-1}$	$(4.92 \pm 0.11).10^{-1}$	$(1.20 \pm 0.09).10^{-2}$
Phenolic acids	Caffeic acid	−	$(1.51 \pm 0.03).10^{-2}$	$(1.49 \pm 0.03).10^{-2}$	1.20 ± 0.16
	Chlorogenic acid	−	476.94 ± 8.39	450.08 ± 6.65	32.69 ± 1.73
	Ferulic acid	−	11.72 ± 0.36	10.61 ± 0.40	0.38 ± 0.04
	Gallic acid	−	7.45 ± 0.15	7.17 ± 0.18	0.18 ± 0.02
	Neochlorogenic acid	−	9.39 ± 0.29	7.07 ± 0.15	0.13 ± 0.01
	Syringic acid	+	$(0.86 \pm 0.02).10^{-1}$	$(0.84 \pm 0.03).10^{-1}$	1.25 ± 0.07
Flavonols	Naringenin	−	$(8.54 \pm 0.90).10^{-2}$	$(9.64 \pm 0.40).10^{-2}$	$(0.43 \pm 0.04).10^{-2}$
	Isoquercetin	−	6.85 ± 0.18	6.59 ± 0.11	0.20 ± 0.01
Flavons	Apigenin	−	$(1.32 \pm 0.66).10^{-1}$	$(0.31 \pm 0.01).10^{-1}$	$(0.05 \pm 0.01).10^{-1}$

[a]Compounds are aligned alphabetically. [b]Concentration of active compound. All values are expressed as μg/g dry weight. The phenolic compound levels are reported and expressed as mean ± standard deviation.

and Supplementary Figure 1). The mode of ionization used was optimized for all investigated compounds. Some compounds afforded higher signals (higher sensitivity) after PI. On the other hand, other compounds were preferably ionized after NI. Therefore, PI was preferentially used for analysis of procyanidines and also for syringic acid. NI was used for analysis of other compounds because it provided a slightly higher total ion signal for these compounds. Tables 1 and 2 summarize the HRMS/MS data essential for evaluation of all compounds analyzed in this study which are suitable for their identification after PI or NI modes.

Interestingly, in some cases we detected several peaks at distinct m/z values for a relevant molecular ion which afforded identical fragments of the same m/z values in their MS/MS spectra. We observed this fact in particular in the case of procyanidines (4 peaks), chlorogenic and neochlorogenic acid (4 peaks), catechin, and epicatechin (2 peaks), and also for naringenin (2 peaks) (Figure 1). Neochlorogenic ($t_R = 4.72$ min) and chlorogenic acid ($t_R = 5.75$ min) could be easily detected by PRM transition from molecular ion $[M - H]^-$ at $m/z = 353.0878$ to fragment at $m/z = 191.0551$ (Table 1). But another two peaks were also detected for this PRM transition at $t_R = 5.64$ and 6.02 min. Both peaks (named "u.c.1" and "u.c.2" in Figure 1) did not match any commercially available standard related to chlorogenic acid (e.g., neochlorogenic acid or cryptochlorogenic acid). Therefore, we can assume that both unknown compounds could be some new positional isomers of chlorogenic acid. Compound "u.c.2" was more abundant than compound neochlorogenic acid and the area of its peak was on the average about 20% of the area for chlorogenic acid. Unfortunately, any corresponding standards for these novel compounds are not available at this time; thus, their structure cannot be fully confirmed. Procyanidines afforded a molecular ion $[M + H]^+$ at $m/z = 579.1497$, which formed specific fragments at $m/z = 427.102$, 409.091, and 127.038 in relevant MS/MS spectra. Four peaks were observed for the PRM transition from $m/z = 579.1497$ to 409.0919 (or to 427.102 and 127.038, respectively). Three of the peaks were easily identified, by comparing their analysis data and behaviour with the corresponding synthetic standards, as procyanidine B1 ($t_R = 5.15$ min), procyanidine B2 ($t_R = 5.62$ min), and procyanidine B3 ($t_R = 5.04$). The remaining fourth peak at $t_R = 5.52$ (compound "u.c.3" in Figure 1) also afforded the same MS/MS spectral characteristics as the previously mentioned procyanidines. Therefore, we can speculate that compound "u.c3" could be a positional isomer of some procyanidine compound. Moreover, the peak of compound "u.c3" was relatively high, its area was repeatedly about 80% of the area of the most abundant procyanidine B2, and it was much larger than the area of less abundant procyanidines B1 and B3. A similar situation was also observed for naringenin, where a small additional peak was observed at $t_R = 8.51$ min. This unknown compound ("u.c4") has the same MS/MS spectral characteristics as naringenin ($t_R = 8.33$ min); thus, we can assume that it is most probably some novel positional isomer of flavonoid naringenin. Interestingly, we also detected a new unknown compound at $t_R = 5.77$ min with molecular ion and MS/MS spectral characteristics identical to rutin standard ($t_R = 6.09$ min) (Figure 2). Both these compounds could be clearly distinguished, even if salak extract was spiked with rutin standard (Figure 2, bottom part). This might be explained by the fact that this novel compound present in salak extract could be a novel positional isomer of rutin. By means of our HRMS/MS analytical method, we cannot precisely distinguish the structure of all of the novel isomers; that is why future NMR studies on the newly prepared relevant phenolic acid and flavonoid standards will have to solve this issue.

3.4. Quantitative Analysis of Individual Phenolic Compounds in Salak Fruit by UHPLC-ESI-MS/MS. Saleh et al. [9, 14] revealed the presence of flavonoids, phenolics, glycosides, and some volatile and aromatic compounds in the ripe salak fruits. However, to date, there is little published information about content of the following compounds in snake fruit. In this

study, the identified phenolic compounds and flavonoids were quantified by PRM of UHPLC-HRMS/MS analysis, as shown in Figure 1 and Table 2. The amounts of analytes were calculated on basis of calibration curves generated by analysis of the corresponding external standards. The highest levels for most of analyzed compounds were observed in pure MeOH extract, followed by 80% MeOH extract (Table 2). Chlorogenic acid in salak was reported for the first time as a major antioxidant, together with (−)-epicatechin, and proanthocanindines [13]. Chlorogenic acid was also detected in salak peel in a high amount [23]. Chlorogenic acid together with caffeic acids exhibited high antioxidant properties and, in addition, they are reported as an inhibitor of α-amylase and α-glucosidase enzymes. This activity could be part of the possible mechanism by which the phenolic acids exert their antidiabetic effects [27]. Here, chlorogenic acid was the most abundant phenolic compound of fruit flesh found with a level in the range of about 450 μg/g in MeOH extract and 480 μg/g in 80% MeOH extract. Also, another phenolic acid—neochlorogenic acid—was present in the high concentration 9.39 μg/g of fw. Both phenolic acids were found in higher concentration than identified in *Actinidia arguta* fruit [28]. Chlorogenic acid is also present in high amounts in the juices of other less common fruits, such as acai berry (*Euterpe oleracea* Engel) (12.6 mg/kg) and elderberry (*Sambucus nigra* L.) (100.9 mg/kg) [29]. Chlorogenic acid was also detected in wild mulberry (*Morus nigra* L.) and tree tomato (*Cyphomandra betacea* Cav.) by capillary electrophoresis but in significantly lower levels in comparison with our study (5.21 μg/g and 5.28 μg/g) [30]. Chlorogenic acid was detected in eight fruit species at outstanding levels, together with caffeic acid, and they also indicated the beneficial effects of these compounds on glucose metabolism, lipids, and tumour inhibition [31]. Syringic acid and caffeic acid are among the major flavonoids detected in the four kiwifruit cultivars [19] and they are also commonly present in many other fruit species [19, 32, 33]. Here, these two phenolic acids were detected in lower amounts, 1.25 and 1.20 μg/g (aqueous extract), in comparison with previously mentioned studies. In another exotic fruit, mutamba (*Guazuma ulmifoli* Lam.), other members of phenolic acids were detected than in salak fruit. The exception was caffeic acid, which was present in mutamba at a higher amount (5.44 μg/g of fresh weight (fw)) in comparison with salak fruit [34]. In particular, concentrations of chlorogenic acid and their derivates may be important in fruit processing and could affect oxidation and colour development processes during technological processing [28]. Ferulic and gallic acids were also assessed in salak fruit [9]. Their levels in 60% ethanolic extract varied at the level of about 8.5 μg/100 mg of their extract. In our study, we detected both phenolic acids at the level of about 7–11 μg/g of dry weight (dw).

The next relatively abundant flavonoid compounds in salak pulp were procyanidines, epicatechin, and isoquercetin (ca. 5–10 μg/g in MeOH extract). Observation of these compounds is in close accordance with data from previous studies [22, 23, 35]. This study showed procyanidin dimer B1 at the level of about 5.81 μg/g of dw and the procyanidin B2 was detected at 11.51 μg/g of dw. However, levels of this group of polyphenols detected in apples cv. Jonagold were higher in the case of procyanidin B1 [36] than in salak. Likewise, in mutamba the observed levels of procyanidin dimer B2 and B1 were significantly higher than in salak fruit, including procyanidin trimer C1 [34]. In the group of flavanols, other compounds responsible for antioxidant property of this fruit were identified, such as (−)-epicatechin, that mainly existed as dimer through hexamers of catechin or epicatechin [13]. Catechin was also detected in other exotic fruit species, such as feijoa (*Acca sellowiana* (O. Berg) Burret) [37] and kainth (*Pyrus pashia* Buch.-Ham. Ex D.Don) [38]. Similarly, as in the case of procyanidines B1 and B2, the detected amount of epicatechin (13.77 μg/g fw) and catechin (0.29 μg/g fw) in salak in this study was significantly lower than presented in the studies of [34, 36].

We also observed naringenin in salak fruit. Naringenin is present in citrus fruits, such as grapefruit, as "naringin" in an inactive form and is broken down into an active aglycone form "naringenin" by the action of intestinal bacterial enzymes [39], and it was confirmed as one of the major flavanones [40, 41]. Here, the detected amount in snake fruit was incomparably at lower concentration (0.09 μg/g of dw). Low concentrations of naringenin are also found in tomatoes and their products [42]. As far as we are aware, this compound has not yet been observed in salak fruit pulp.

Here, isoquercetin was also detected in salak fruit for the first time. Similarly, it was identified in feijoa juice [37]. Isoquercetin, together with rutin, is a well-known major phenolic compound in common and Tartary buckwheat with a wide range of diversity in content, depending on the genotype and area of collection [43]. In the case of salak, rutin is not present, but the concentration of quercetin in fruit flesh was high [36], 6.85 μg/g of dry weight (dw). Interestingly, as it was described in the text above, we detected a new rutin-like compound in salak extract.

Another compound observed with level sub 1 μg/g dry weight was apigenin (0.13 μg/g dw). Chemically known as 4′,5,7-trihydroxyflavone, apigeninn belongs to the flavone class, that is, the aglycone of several naturally occurring glycosides. Numerous pharmacological activities, including anti-inflammatory, antitoxicant, and anticancer, are attributed to apigenin [44]. The content of apigenin in fresh foods, especially vegetables and herbs [45], oscillated from 0.10 to 0.54 mg/kg of fw and these values are comparable to the levels observed in this study. The content of apigenin presented in fresh parsley [45] was at the exceptional level of 21.6 mg/kg of fw.

4. Conclusion

Our study confirmed UHPLC-HRMS/MS as a powerful analytical technique for the separation and detection of phenolics compounds and, for the first time, allowed us to reveal and quantitate phenolic acids and flavonoids in salak fruit. This method is fast, sensitive, and accurate with good repeatability and the experimental procedures are simple. From the bioactive compounds, in total 14 phenolic compounds were identified in the pulp of salak fruit which have been known for their extensive biological properties and linked with health promoting features. Eleven compounds were reported for the first time in salak pulp. The most

abundant phenolic compound was chlorogenic acid, followed by epicatechin, procyanidine B2, neochlorogenic acid, and ferulic acid. Total phenolic content (257.17 μL/mL) and antioxidant activities (10.56 μM TE/g of fruit pulp) were detected at significant levels. Large variations in abundance were observed for other phenolic compounds in salak fruit. The obtained data indicate that salak fruit could be regarded as a promising source of bioactive functional food.

Abbreviations

ANOVA:	Analysis of variance
NCE:	Normalized collision energy
PRM:	Parallel reaction monitoring
RSD:	Relative standard deviation
UHPLC-MS/MS:	Ultrahigh performance liquid chromatography tandem mass spectrometry
dw:	Dry weight
fw:	Fresh weight
TE:	Trolox
u.c.:	Unknown compound.

Data Availability

Supplementary Figure S1 will be submitted as a separate file.

Conflicts of Interest

All authors declare that there are no conflicts of interest.

Authors' Contributions

P.H.C. and I.V. contributed to conceptualization; M.J., P.H.C., and I.V. contributed to methodology; M.J., P.H.C., and A.K.K. contributed to formal analysis, validation, and data curation; P.H.C. and M.J. contributed to writing of original draft; D.J. and V.D. contributed to critically reviewing and editing the manuscript; D.J and V.D. contributed to project administration. All authors read and approved the final manuscript and agreed with the published version of the manuscript.

Acknowledgments

This work was financed by project by the Ministry of Agriculture of the Czech Republic (no. RO0418), partially by NAZV QK1810102 of the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic, by the Subsidy Programme-the National Programme for the Conservation and Use of Plant Genetic Resources and Agrobiodiversity (no. 6.2.5/51834/2017-MZE-17253), and by the Internal Grant Agency of Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague IGA (Project No. 20205006).

Supplementary Materials

Supplementary Figure 1: analysis of phenolic compounds in *Salacca zalacca* fruit pulp by UHPLC-HRMS/MS with MS/MS spectrometer operating in PRM (parallel reaction monitoring) mode. Note: each PRM transition is described by name of corresponding compound and characterized by its retention time, and *m/z* values of respective precursor ion and product ion. (*Supplementary Materials*)

References

[1] S. Rodrigues, E. de Oliveira Silva, and E. S. de Brito, *Exotic Fruits Reference Guide*, Academic Press, Oxford, UK, 2018.

[2] M. A. S. Van Duyn and E. Pivonka, "Overview of the health benefits of fruit and vegetable consumption for the dietetics professional," *Journal of the American Dietetic Association*, vol. 100, no. 12, pp. 1511–1521, 2000.

[3] R. E. Paull and O. Duarte, *Tropical Fruits*, CAB International, Oxfordshire, UK, 2nd edition, 2012.

[4] N. A. Ismail and r. M. F. B. Baka, "Salak—Salacca zalacca," in *Exotic Fruits Reference Guide*, S. Rodrigues, E. de Oliveira Silva, and R. S. de Brito, Eds., pp. 383–390, Academic Press, Elsevier, Oxford, UK, 2018.

[5] S. Hadiati, T. Budiyanti, A. Soemargono, and A. Susiloadi, "Characterization of fruit on several salak varieties and their hybrids," *AGRIVITA*, vol. 34, no. 2, pp. 187–192, 2012.

[6] R. Lestari, S. Huyskens Keil, and G. Ebert, "Variation in fruit quality of different salak genotypes (Salacca zalacca (Gaert.) Voss) from Indonesia," in *Proceedings of the 2003 Deutscher Tropentag*, Gottingen, Germany, 2003.

[7] D. L. Schuiling and J. P. Mogea, "Salacca zalacca (gaertner) Voss," 2016, https://uses.plantnet-project.org/en/Salacca_zalacca_(PROSEA.

[8] J. Janick and R. E. Paul, *The Encyclopedia of Fruit & Nuts*, CAB International, Oxfordshire, UK, 2008.

[9] M. S. M. Saleh, M. J. Siddiqui, A. Mediani et al., "Salacca zalacca: a short review of the palm botany, pharmacological uses and phytochemistry," *Asian Pacific Journal of Tropical Medicine*, vol. 11, no. 12, pp. 645–652, 2018.

[10] S. Gorinstein, R. Haruenkit, S. Poovarodom et al., "The comparative characteristics of snake and kiwi fruits," *Food and Chemical Toxicology*, vol. 47, no. 8, pp. 1884–1891, 2009.

[11] R. Chareoansiri and R. Kongkachuichai, "Sugar profiles and soluble and insoluble dietary fiber contents of fruits in Thailand markets," *International Journal of Food Sciences and Nutrition*, vol. 60, no. 4, pp. 126–139, 2009.

[12] H. Leontowicz, M. Leontowicz, J. Drzewiecki et al., "Bioactive properties of snake fruit (Salacca edulis Reinw) and mangosteen (Garcinia mangostana) and their influence on plasma lipid profile and antioxidant activity in rats fed cholesterol," *European Food Research and Technology*, vol. 223, no. 5, pp. 697–703, 2006.

[13] G. Shui and L. P. Leong, "Screening and identification of antioxidants in biological samples using high-performance liquid chromatography-mass spectrometry and its application on Salacca edulis Reinw," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 4, pp. 880–886, 2005.

[14] M. S. M. Saleh, M. J. Siddiqui, S. Z. Mat So'ad, S. Murugesu, A. Khatib, and M. M. Rahman, "Antioxidant and α-glucosidase inhibitory activities and gas chromatography-mass spectrometry profile of salak (Salacca zalacca) fruit peel extracts," *Pharmacognosy Research*, vol. 10, no. 4, pp. 385–390, 2018.

[15] M. Jágr, V. Dvořáček, P. Hlásná Čepková, and J. Doležalová, "Comprehensive analysis of oat avenanthramides using hybrid quadrupole-orbitrap mass spectrometry: possible detection of new compounds," *Rapid Communications in Mass Spectrometry*, vol. 34, p. e8718, 2020.

[16] L. Štěrbová, P. Hlásná Čepková, I. Viehmannová, and D. C. Huansi, "Effect of thermal processing on phenolic content, tocopherols and antioxidant activity of sacha inchi kernels," *Journal of Food Processing and Preservation*, vol. 41, Article ID e12848, 2017.

[17] M. Holasova, V. Fiedlerova, H. Smrcinova, M. Orsak, J. Lachman, and S. Vavreinova, "Buckwheat—the source of antioxidant activity in functional foods," *Food Research International*, vol. 35, no. 2-3, pp. 207–211, 2002.

[18] S. I. Mokhtar, P. C. Leong, L. E. Ven, and N. A. A. Aziz, "Total phenolic contents, antioxidant activities and organic acids composition of three selected fruit extracts at different maturity stages," *Journal of Tropical Resources and Sustainable Science*, vol. 2, pp. 40–46, 2014.

[19] K. M. Saeed, L. J. You, C. Chen, Z. G. Zhao, X. Fu, and R. H. Liu, "Comparative assessment of phytochemical profiles and antioxidant and antiproliferative activities of kiwifruit (Actinidia deliciosa) cultivars," *Journal of Food Biochemistry*, vol. 43, no. 11, p. 9, 2019.

[20] I. Sytarova, J. Orsavova, L. Snopek, J. Mlcek, L. Byczynski, and L. Misurcova, "Impact of phenolic compounds and vitamins C and E on antioxidant activity of sea buckthorn (Hippophae rhamnoides L.) berries and leaves of diverse ripening times," *Food Chemistry*, vol. 310, p. 12, 2020.

[21] M. K. Gecer, M. Akin, M. Gundogdu, S. P. Eyduran, S. Ercisli, and E. Eyduran, "Organic acids, sugars, phenolic compounds, and some horticultural characteristics of black and white mulberry accessions from eastern Anatolia," *Canadian Journal of Plant Science*, vol. 96, no. 1, pp. 27–33, 2016.

[22] I. R. Suica-Bunghez, S. Teodorescu, I. D. Dulama, O. C. Voinea, S. Simionescu, and R. M. Ion, "Antioxidant activity and phytochemical compounds of snake fruit (Salacca Zalacca)," in *Proceedings of the International Conference on Innovative Research—ICIR Euroinvent 2016*, vol. 133, Bristol, UK, 2016.

[23] M. Kanlayavattanakul, N. Lourith, D. Ospondpant, U. Ruktanonchai, S. Pongpunyayuen, and C. Chansriniyom, "Salak plum peel extract as a safe and efficient antioxidant appraisal for cosmetics," *Bioscience, Biotechnology, and Biochemistry*, vol. 77, no. 5, pp. 1068–1074, 2013.

[24] N. Khonsarn and S. Lawan, "Level of xanthophyll, lutein and zeaxanthin in selected Thai fruits determined by HPLC," in *Proceedings of the 2012 International Conference on Nutrition and Food Sciences*, vol. 39, Singapore, 2012.

[25] C. H. Wijaya, D. Ulrich, R. Lestari, K. Schippel, and G. Ebert, "Identification of potent odorants in different cultivars of snake fruit [Salacca zalacca (gaert.) Voss] using gas chromatography-olfactometry," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 5, pp. 1637–1641, 2005.

[26] Supriyadi, Suhardi, M. Suzuki et al., "Changes in the volatile compounds and in the chemical and physical properties of snake fruit (Salacca edulis reinw) cv. Pondoh during maturation," *Journal of Agricultural and Food Chemistry*, vol. 19, no. 1, pp. 44–50, 2002.

[27] G. Oboh, O. M. Agunloye, S. A. Adefegha, A. J. Akinyemi, and A. O. Ademiluyi, "Caffeic and chlorogenic acids inhibit key enzymes linked to type 2 diabetes (in vitro): a comparative study," *Journal of Basic and Clinical Physiology and Pharmacology*, vol. 26, no. 2, pp. 165–170, 2015.

[28] A. Wojdylo and P. Nowicka, "Anticholinergic effects of Actinidia arguta fruits and their polyphenol content determined by liquid chromatography-photodiode array detector-quadrupole/time of flight-mass spectrometry (LC-MS-PDA-Q/TOF)," *Food Chemistry*, vol. 271, pp. 216–223, 2019.

[29] D. Nowak, M. Gośliński, K. Przygoński, and E. Wojtowicz, "The antioxidant properties of exotic fruit juices from acai, maqui berry and noni berries," *European Food Research and Technology*, vol. 244, no. 11, pp. 1897–1905, 2018.

[30] T. S. Fukuji, F. G. Tonin, and M. F. M. Tavares, "Optimization of a method for determination of phenolic acids in exotic fruits by capillary electrophoresis," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 51, no. 2, pp. 430–438, 2010.

[31] A. D. Meinhart, F. M. Damin, L. Caldeirão et al., "Chlorogenic and caffeic acids in 64 fruits consumed in Brazil," *Food Chemistry*, vol. 286, pp. 51–63, 2019.

[32] V. Zunjar, D. Mammen, and B. M. Trivedi, "Antioxidant activities and phenolics profiling of different parts of Carica papaya by LCMS-MS," *Natural Product Research*, vol. 29, no. 22, pp. 2097–2099, 2015.

[33] S. P. Eyduran, M. Akin, S. Ercisli, E. Eyduran, and D. Maghradze, "Sugars, organic acids, and phenolic compounds of ancient grape cultivars (Vitis vinifera L.) from Igdir province of eastern Turkey," *Biological Research*, vol. 48, p. 8, 2015.

[34] G. A. Pereira, H. S. Arruda, D. R. de Morais, N. M. P. Araujo, and G. M. Pastore, "Mutamba (Guazuma ulmifolia Lam.) fruit as a novel source of dietary fibre and phenolic compounds," *Food Chemistry*, vol. 310, p. 9, 2020.

[35] N. A. M. Zaini, A. Osman, A. A. Hamid, A. Ebrahimpour, and N. Saari, "Purification and characterization of membrane-bound polyphenoloxidase (mPPO) from snake fruit [Salacca zalacca (Gaertn.) Voss]," *Food Chemistry*, vol. 136, no. 2, pp. 407–414, 2013.

[36] G. P. Lysiak, A. Michalska-Ciechanowska, and A. Wojdylo, "Postharvest changes in phenolic compounds and antioxidant capacity of apples cv. Jonagold growing in different locations in Europe," *Food Chemistry*, vol. 310, p. 7, 2020.

[37] Y. Y. Peng, K. S. Bishop, J. Y. Zhang, D. L. Chen, and S. Y. Quek, "Characterization of phenolic compounds and aroma active compounds in feijoa juice from four New Zealand grown cultivars by LC-MS and HS-SPME-GC-O-MS," *Food Research International*, vol. 129, p. 11, 2020.

[38] O. Prakash, R. Baskaran, and V. B. Kudachikar, "Characterization, quantification of free, esterified and bound phenolics in Kainth (Pyrus pashia Buch.-Ham. Ex D.Don) fruit pulp by UPLC-ESI-HRMS/MS and evaluation of their antioxidant activity," *Food Chemistry*, vol. 299, p. 10, 2019.

[39] S. Shakeel, M. U. Rehman, N. Tabassum, U. Amin, and M. U. R. Mir, "Effect of naringenin (a naturally occurring flavanone) against pilocarpine-induced status epilepticus and oxidative stress in mice," *Pharmacognosy Magazine*, vol. 13, no. 1, pp. S154–S160, 2017.

[40] M. K. Khan, E. H. Zill-E-Huma, and O. Dangles, "A comprehensive review on flavanones, the major citrus polyphenols," *Journal of Food Composition and Analysis*, vol. 33, no. 1, pp. 85–104, 2014.

[41] W. Xi, B. Fang, Q. Zhao, B. Jiao, and Z. Zhou, "Flavonoid composition and antioxidant activities of Chinese local pummelo (Citrus grandis Osbeck.) varieties," *Food Chemistry*, vol. 161, pp. 230–238, 2014.

[42] M. Krause and R. Galensa, "Bestimmung von naringenin und naringenin-chalkon in tomatenschalen mit RP-HPLC nach festphasenextraktion," *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, vol. 194, no. 1, pp. 29–32, 1992.

[43] M. Rauf, H. Yoon, S. Lee et al., "Evaluation of Fagopyrum esculentum Moench germplasm based on agro-morphological traits and the rutin and quercetin content of seeds under

spring cultivation," *Genetic Resources and Crop Evolution*, vol. 67, no. 6, pp. 1385–1403, 2020.

[44] F. Ali, Rahul, F. Naz, S. Jyoti, and Y. H. Siddique, "Health functionality of apigenin: a review," *International Journal of Food Properties*, vol. 20, no. 6, pp. 1197–1238, 2017.

[45] H. Guven, A. Arici, and O. Simsek, "Flavonoids in our foods: a short review," *Journal of Basic and Clinical Health Sciences*, vol. 3, no. 2, pp. 96–106, 2019.