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Quality and Quantity Properties of a Novel Natural Pear Spread in Western Georgia

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Abstract: The Caucasus is an area situated between the Black Sea and the Caspian Sea and occupied by Russia, Georgia, Azerbaijan, and Armenia. It is home to the Caucasus Mountains, including the Greater Caucasus mountain range, which has historically been considered a natural barrier between Eastern Europe and Western Asia. Georgia is a country in the Caucasus region of Eurasia. Eurasia is the largest continental landmass on Earth, comprising all of Europe and Asia. Special kind of pear spreads in one region of western Georgia (Adjara) called Khechechuri was the main research topic. Pear is a dietary source of bioactive components such as polyphenols and triterpenic acid. A total of five species of Khechechuri collected from various regions in Adjara were identified, including Adjaristskali, Merisi, Dandalo, Shuakhevi, and Khulo. Each pear fruit was examined and compared by five parts, skin, edible pulp, the whole pear (skin + pulp), Juice and the pomace. All parts were analysed for phenolic compounds, flavonoids, catechins, phenolic acids, and antioxidant activities. The total amounts of phenols, catechines, phenolic acids, and flavonoids were defined with the help of spectral methods and the antioxidant activity was established. The highest amount of total phenolic was found in the skin of Khulo and Adjaristskali which were around (4650, 4500) mg, respectively in 1 kg of the skin. pomace showed significant amount of the total phenolic in all species of khechechuri. It is worth to mention that Khechechuri parts in all different species studied contain flavonoids except the juice of the fruit species.

Keywords: Khechechuri; novel; Adjara; phenolic compounds; catechins; flavonoids; antioxidant activity; quantity; quality; UPLC

1. Introduction

The Caucasus is a mountain region with extremely diversified natural conditions (Figure 1). It is situated on the border between the temperate and subtropical zones and is concurrently influenced by the Atlantic and by the dry, continental climate of the inner regions of Eurasia [1,2]. A high percentage of endemics is noted in high-mountain and highland xerophytic plant formations [3,4] (Figure 2). The centres of diversity of the subendemic genera are in the Caucasus especially in Georgia (Table 1). Geographical isolation (with respect to longitude, latitude and altitude) is an important factor in the formation and evolution of the Caucasian flora generated by three-dimensional landscape structure of the mountains [5]. Khechechuri pear is a tropical and an endemic fruit rarely spread in the world and this is due to its growth only in the region of Adjara in Georgia. In addition to its value as a delicious fruit, it was studied to evaluate the importance of its nutritional values. The goal of the present work is to identify the quantity and quality properties of

this novel pear and to shed light on its importance as a nutrition supplement. A total of 5 g of pears + 150 mL ethanol were used to analyse the quantity and quality properties of the pear. A total of 50 mL of the extracts was utilised to determine the phenolic compounds, flavonoids, catechins, phenolic acid and antioxidant activity. A total of 100 mL of extracts was used to identify the sugar content and organic acids implementing HPLC and UPLC method.

Figure 1. Caucasus and Historical regions of Georgia.

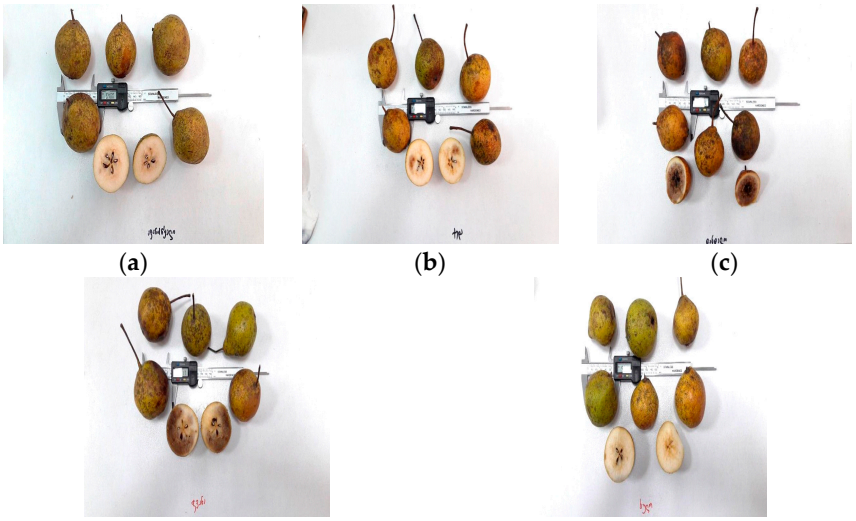


Figure 2. Khechechuri pear shapes collected from different regions and villages. They are: (**a**) Adjaristskali, a village in Qeda where the sea level rise is 150 m, (**b**) Merisi, Qeda where the sea level rise is 700 m, (**c**) Dandalo where sea level rise is 520 m, (**d**) Shuakhevi where sea level rise is 800 m, (**e**) Khechechuri–Khulo (sea level rise is 950 m).

Table 1. Khechechuri species collected from different regions and villages with their description and amounts studied.

"Pear" Khechechuri Species	Form	Colour	Mass, g	Volume, mL	Size	
					Width	Height
Adjaristskali	circle	Green with black points	136.66	130.2	67.74	64.19
Merisi, Qeda	circle	Green with black points	104.43	73.3	61.87	57.51
Dandalo, Qeda	circle	Green with black points	87.77	93.3	58.78	55.79
Shuakhevi	circle	Green with black points	150.65	67.5	68.6	63.71
Khulo	circle	Green with black points	123.08	145.0	65.80	60.75

2. Materials and Methods

The study was conducted in the Department of Chemical Analysis in Batumi Shota Rustaveli State University at west Georgia regional chromatography centre.

Biochemical and chemical analyses were carried out by different physo-chemical and instrumental methods. Separation and quantitative analysis were carried out using UPLC. The samples were filtered by Cartridge Solid Phase Extraction (SPE) of Waters Sep-Pak C18 (500 mg). This was in conditions of 2 mL methanol, equilibration—2 mL water, load of 2 mL samples, wash—2 mL water + 0.1% formic acid and elute—4 × 1 mL acetonitrile + 0.1% formic acid. All the samples were filtered (0.45 μm) before the chromatography [6].

2.1. Determination of Antioxidant Action

By using DPPH (2,2-Diphenyl-1-pic-rylhydrazil) methods, antioxidant activity was determined [7,8]. In this context, 1 mL of the sample was added to 3 mL of DPPH extract (0.1 mM DPPH–0.004 g/100 mL ethyl alcohol) and after 30 min the optical density was evaluated on spectrophotometer. DPPH and 96% ethyl alcohol were used as blanks. For determination of action of free radical inhibition (DPPH) the following formula was used: In % = AC − AS/AC × 100%, where Ac indicates absorption of DPPH/Alcohol solution and As indicates absorption of the extract [9].

2.2. Determination of Total Phenolic Compounds

Total phenolic compounds were defined using Folin-Ciocalteus spectral methods [10,11]. Extraction of the samples was conducted using 80% ethyl alcohol; 0.5 or 1.0 mL of the extract was transferred to 25 mL volumetric flask, and 5.0 mL of water was added, with 1.0 mL of Folin-Ciocalteu reagent. After 8 min of delay at 25 °C it was added by 10.0 mL of 7% Na_2CO_3, the flask was then filled with water and left at room temperature for 2 h. Determination was conducted at 750 nm. For control, 1 mL of extragent was used. The calculations of obtained values were performed using the calibration curve of Gallic Acid. For determination of the phenols the following formula was used: X = (D K V F) × 1000/m, where X is the amount of phenols (mg/kg); D—optical density; K—coefficient; F—factor of dilution; V—volume of extract in mL; m—mass of the raw material used for extraction (gr).

2.3. Determination of Catechines

Catechin contents were determined by Swain and Hill spectral method [12]. A total of 1 mL of each sample was pipetted to 3 mL of 1% vanillin reagents (1 g vanillin added by 70% sulfur acid solution). Further, 1 mL ethanol was utilised as a control. (All the solutions except the samples were used as blank). After 15 min, the solution would be red, and then after 15 min, spectral adsorption was determined at 750 nm [13].

2.4. Determination of Flavonoid

The total flavonoid content (TFC) was determined by the aluminium chloride colorimetric method as previously described. khechechuri samples (0.5 mL) were mixed with 2 mL of distilled water and 150 μL of 5% $NaNO_2$ solution. After 5 min, the mixture was added by 150 μL of 10%

AlCl3 and, after 6 min, by 2 mL of 1 mol/L NaOH solution. The end volume was increased to 5 mL with distilled water. Finally, the absorbance was measured at 510 nm. Results were expressed in mg/L of catechin (or Ruthin) [14].

2.5. Determination of Individual Compounds

Individual compounds were identified by HPLC (high performance liquid chromatography) and UPLC-PDA-MS analysis methods: column BEN C18, 1.7 µm, BEN Amide1.7 µm; eluents acetonitrile 0.1% formic acid, 0.1% formic acid (gradient); flow 0.4 mL/min, column temperature 40 °C; samplers temperature 10 °C; MS-scan 100–1100 da; probe 600 °C; negative 0.8 kV, Capilarity 1.5 kV [15].

3. Results and Discussion

Quantity and quality properties existing in five different parts of Khechechuri (fruit, skin, pulp, juice and pomace) produced varied results. The results are presented in Figures 3–7.

3.1. The Total Phenolic

Figure 3 shows the total phenolic content (TPC)existing in five Species of Khechechuri collected from different areas in Adjara. The skin of the pears show a significant amount of TPC whereas crumbs (pomace) have similar content of phenolics in all species, around 3800 mg. In contrast, pulp, juice and the whole fruit contain very low percentage of total phenolic. The total phenolic compounds are: 1965–2981 mg/kg in the fruit, 4275–4644 mg/kg in the skin, 1191–2533 mg/kg in pulp, 581 to 1320 mg/kg in the juice, 3151–3717 mg/kg per unit in the crude mass (Figure 3). In this respect, the fruits obtained in the dominant Khulo (2981; 4644; 2533; 1320; 3717 mg/kg). Less than in the Ajaristskali sample (1965; 4275; 1425; 943; 3616 mg/kg).

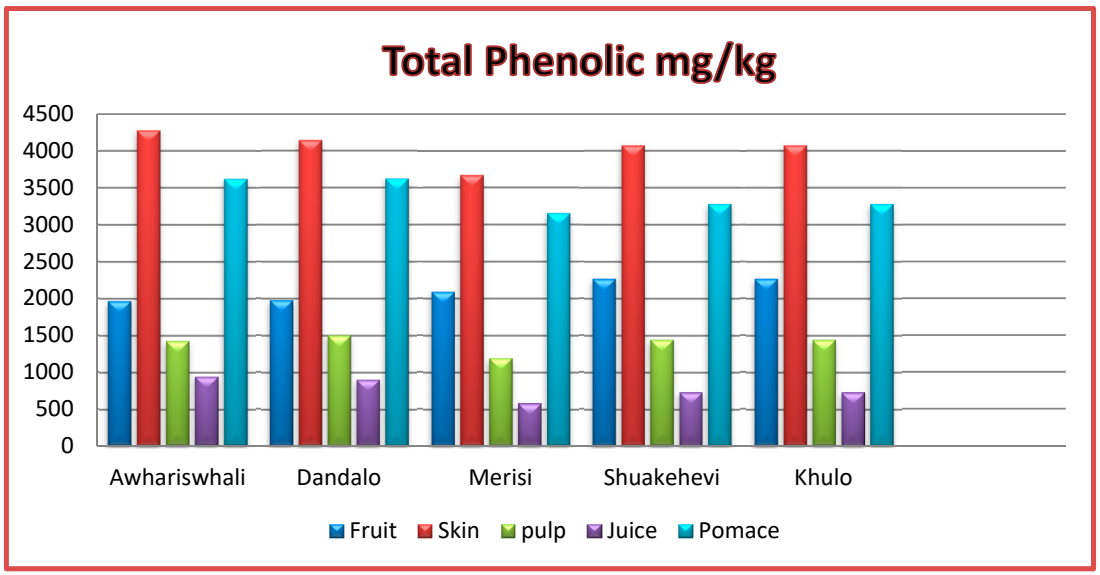


Figure 3. Total Phenolic amount per (mg) in each (kg) of the parts studied from the Khechechuri species.

3.2. Flavonoids

Flavonoids in polyphonic compounds in saline samples consist of about 38%–61% of the total phenol, (958–1211 mg/kg), 29%–46% in the skin, (1336–1734 mg/kg), 39%–64% in pulp, (657–992 mg/kg), 24%–29% in the juice, (158–319 mg/kg), and 33%–68% in the discharge (1080–1908 mg/kg) (Figure 4). The quantity of flavonoids is 1211 mg/kg in adjacent fruit, almost equivalent to dandelo (1131 mg/kg), Mayer (1187 mg/kg) and Khulo (1147 mg/kg). Comparatively, there is less than 958

mg/kg of Shuakhevi fruit. The content of flavonoids was most pronounced in the skin and crumbs of Awhariswhali when compared with the other species tested. It demonstrates around (1800–1750) of flavonoids in skin and crumbs, respectively. In addition, Skin and crumbs are the richest parts of flavonoids in all Species studied. Fruit and pulp contain a remarkable number of flavonoids, unlike the juice, which is very poor in flavonoids.

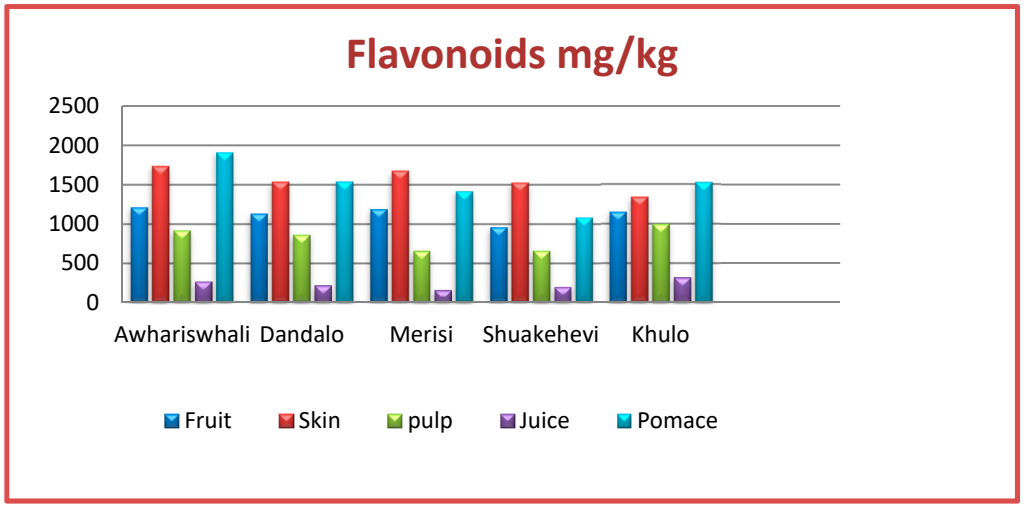


Figure 4. Flvonoids amount per (mg) in each (kg) of the parts studied from the Khechechuri species.

3.3. Charecterization of Catechines

Catechins have flavonoids. In particular, the content of catechins in pear analysis in the range of 167–688 mg/kg unit is calculated on the basis of the crude mass. 688 mg/kg is in the fruit of the hull and is comparatively less in Dandalo (167 mg/kg) and Shuakhevi (282 mg/kg). Catechins skins are differently distributed from separate parts of the pear, compared with 793 to 1452 mg/kg. The juice is less than 38.18–196 mg/kg.

Figure 5 demonstrates the significant amount of catechins present in the skin part. Other parts tested did not show significant content of catechins The skin of khechechuri sample which collected from Khulo was the richest part of catechin. It contained 1250 mg, approximately in each 1 kg of Khulo skin.

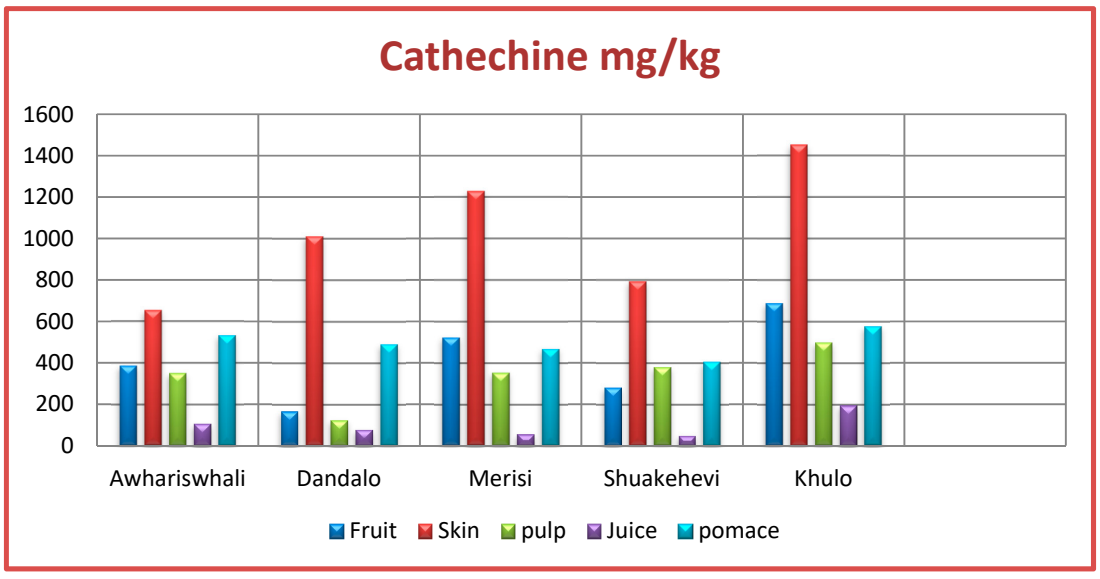


Figure 5. Catechins amount per (mg) in each (kg) of the parts studied from the Khechechuri species.

3.4. Antioxidant Activity

Antioxidant activity was determined by Khechechuri in different regions of the Adjara (sea level rise is 150 m Adjaristskali, 520 m—Dandalo, 700 m—Merisi, 800 m—Shuakhevi, and 950 m—Khulo).The antioxidant activity, which is expressed in 100 mg sample of 50% inhibition of 0.1 molar solution, has been established to determine the secretion of fruit and part of the pear (skin, pulp, juice, pomace). In particular, where the content of phenolic compounds is higher, the antioxidant activity is more likely increased (more than 100 mg sample indicator) (Figure 6).

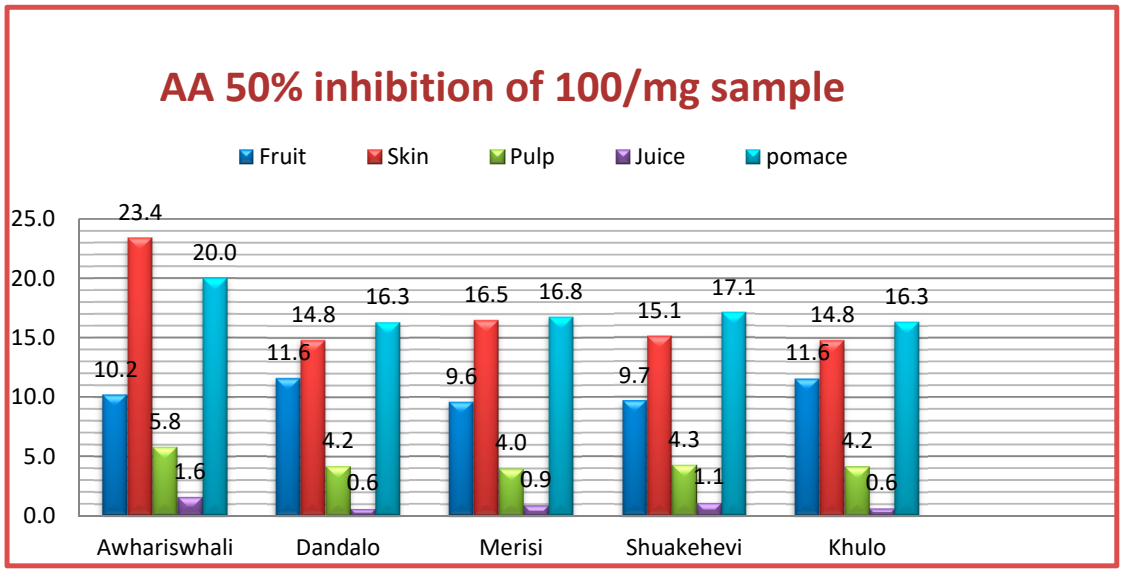


Figure 6. Antioxidant activity of Khechechuri in 5 regions of Adjara.

3.5. Phenolic Acid

Skin and pomace exhibit a significant amount of phenolic acids in the samples studied, whereas the pomace was the richest part in Dandalo and Awhariswhali.

The phenolic compounds are also confirmed from the phenolic compounds that contain phenolic acid, which is represented in the fruit by 330–465 mg/kg. The sample size is 338 mg/kg, and dandavalo (377 mg/kg) and Shuakhevi (370 mg/kg) are more than 688 mg/kg in the samples of the acharatsklisa (331 mg/kg) and Mayer (330 mg/kg).

Compared to other phenolic compounds, the relatively high content of phenolic acid is found in the skin (623–781 mg/kg) and in the pomace (403–703 mg/kg). From the fruit to the juice will go to 11–21% (37–78 mg/kg) of the total content of phenolic acid (Figure 7).

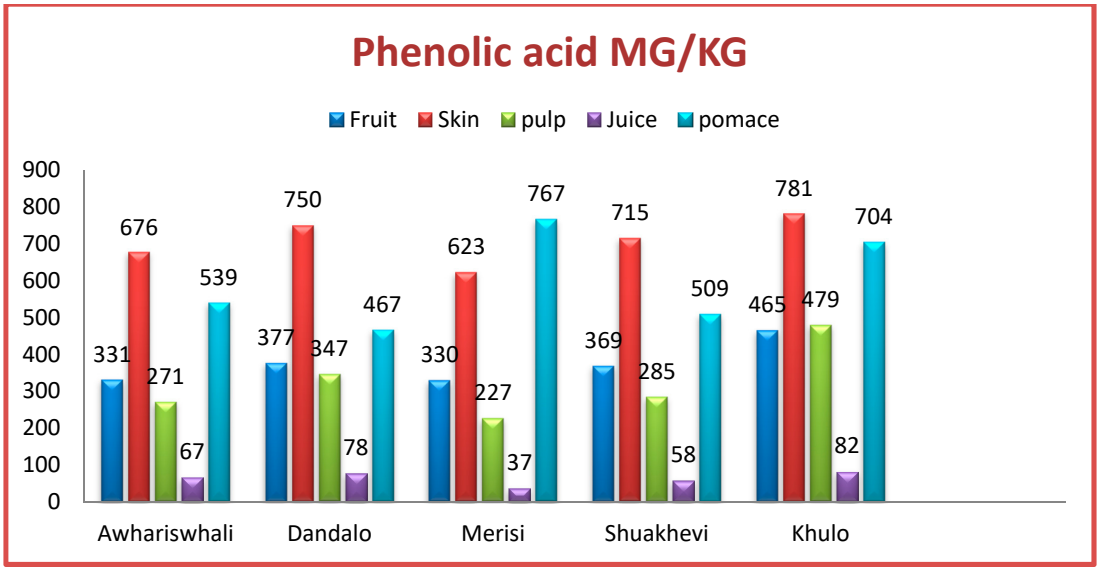


Figure 7. Phenolic acid amount per (mg) in each (kg) of the parts studied from the Khechechuri species.

The promising results obtained from this study of the biological activity of flavones and catechins, antioxidant and phenolics encouraged us to test the chemical activities.

The following physicochemical methods have been used for the research: The quantitative content of phenolic compounds with HPLC-UV, RI, UPLC-PDA, MS chromatography method. Substances were identified using standard compounds and the free data base https://metlin.scripps.edu of substance masses, as well as comparing data from peer-reviewed literary publications Khechechuri was found to contain a rich assortment of phenolic compounds, with at least 5 distinct peaks visible on a UPLC chromatogram, (Figure 8).

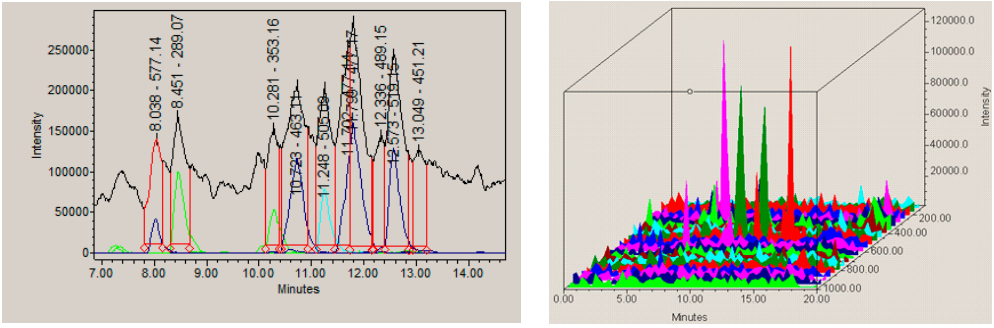
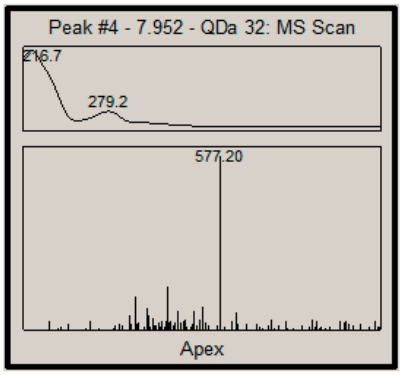
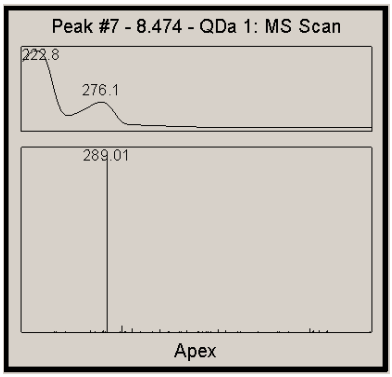


Figure 8. UPLC of phenolic compounds MS-scan *m*/*z* H^- (100–700 Da) isolated from Pear extract—B-type procyanidin dimer, (±)-Catechin, Chlorogenic Acid, Quercetin-3-O-galactoside, Quercetin-3-O-glucoside, Isorhamnetin-3-O-galactoside, Isorhamnetin-3-O-glucoside and Isorhamnetin-acylated-galactoside.

Compound **2** was identified as (+)-catechin (Figure 9) based on its retention time (8.474), UV spectrum and $[M–H]^-$ion at m/w 289.11, which match those of the reference compound.



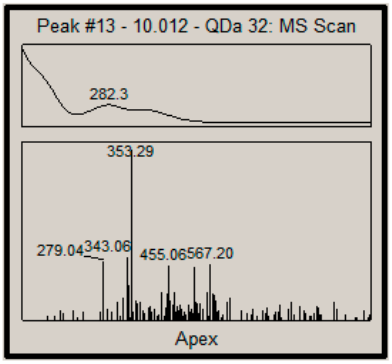
Compound **1**: B-type procyanidin dimer
Rt (min) 7.952;
[M–H] (*m*/*z*) 577.20



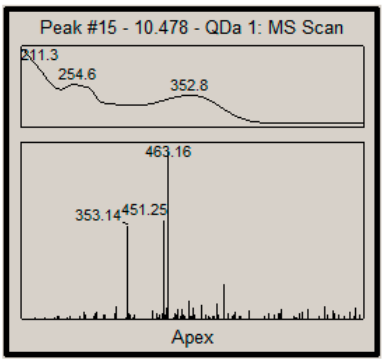
Compound **2**
(+)-catechin

Figure 9. UPLC of phenolic compounds, B-type procyanidin dimer, (±)-Catechin.

Compound **3** was identified as chlorogenic acid (Figure 10) based on its $[M–H]^-$ ion at *m*/*z* 352.08 and with authentic standards. Rt (min) 10,012



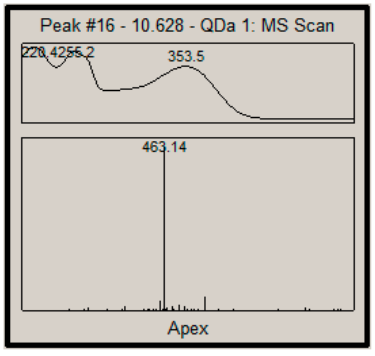
Compound **3**: Chlorogenic Acid
(3-O-Caffeoylquinic acid)
Rt (min) 10.012; [M–H] (*m*/*z*) 353.29



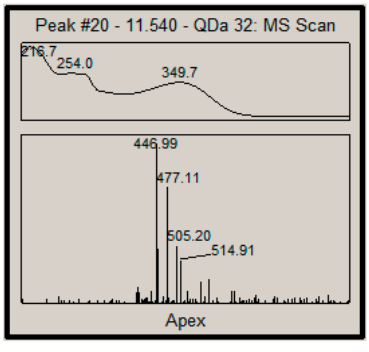
Compound **4**: Quercetin-3-O-galactoside
(Hyperoside)
Rt (min) 10.478; [M–H] (*m*/*z*) 463.16

Figure 10. UPLC of phenolic compounds, Chlorogenic Acid, Quercetin-3-O-galactoside.

Compound **5** and Compound **6** were identified based on its $[M–H]^-$ ion at (*m*/*z*) 463.14, 477.11 as Quercetin-3-O-glucoside and Isorhamnetin-3-O-galactoside, respectively (Figure 11).



Compound **5**: Quercetin-3-O-glucoside
Rt (min) 10.628; [M–H] (*m*/*z*) 463.14

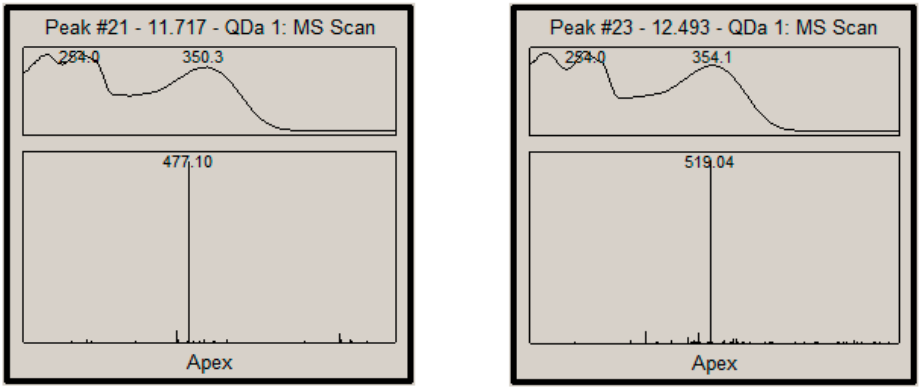


Compound **6**: Isorhamnetin-3-O-galactoside
Rt (min) 11.540; [M–H] (*m*/*z*) 477.11

Figure 11. UPLC of phenolic compounds, Quercetin-3-O-glucoside, Isorhamnetin-3-O-galactoside.

Compound **7** $[M–H]^-$ *m*/*z* 477.09 Retention time—11.717 min, maximum absorption—UV-341.7 nm. According to the base of METLIN compound masses, the substance 4 corresponds to Isorhamnetin-3-O-galactoside as shown in (Figure 12).

Compound **8** $[M–H]^-$ *m*/*z* 519.11, the result of fragmentation is *m*/*z*. Retention time—12.493 min, maximum absorption—UV-312.5 nm. According to the base of METLIN compound masses, the compound **3** corresponds to Isorhamnetin–acetylglucoside) (Figure 12).



Compound **7**: Isorhamnetin-3-O-glucoside
Rt (min) 11.717; [M–H] (*m*/*z*) 477.10

Compound **8**: Isorhamnetin-acylated-galactoside
Rt (min) 12.493; [M–H] (*m*/*z*) 519.04

Figure 12. UPLC of phenolic compounds, Isorhamnetin-3-O-glucoside and Isorhamnetin-acylated-galactoside.

The research object was the fruit of the Khechechuri—the most popular winter variety in western Georgia, which was compared with other pear varieties, developed in western Georgia, namely, the summer variety—the Saselo, the autumn one—the Tapla and the winter ones—the Tavrejuli, the Katsistava and the Khechechuri.

The summer variety (Saselo)—common phenols—1300—1501 mg/kg, the autumn variety (Tapla)—2470–2762 mg/kg, winter varieties—Katsistava—2260—2846 mg/kg, Tavrejula—2460—2826 mg/kg. The number of phenolic compounds accumulated in autumn and winter varieties is greater than in summer samples.

By comparing the results of quantitative analysis of biologically active compounds presented in Figures 13–16, we can conclude that in the fetus of varieties Saselo, Katsistava, Tapla, Tavrejuli, the amount of flavonoids, catechins and phenol carboxylic acids with respect to common phenols is presented in the following ratio—1: 0.5: 0.23: 0.2, and in the peel it is 1: 0.5: 0.3: 0.2 (average indicator).

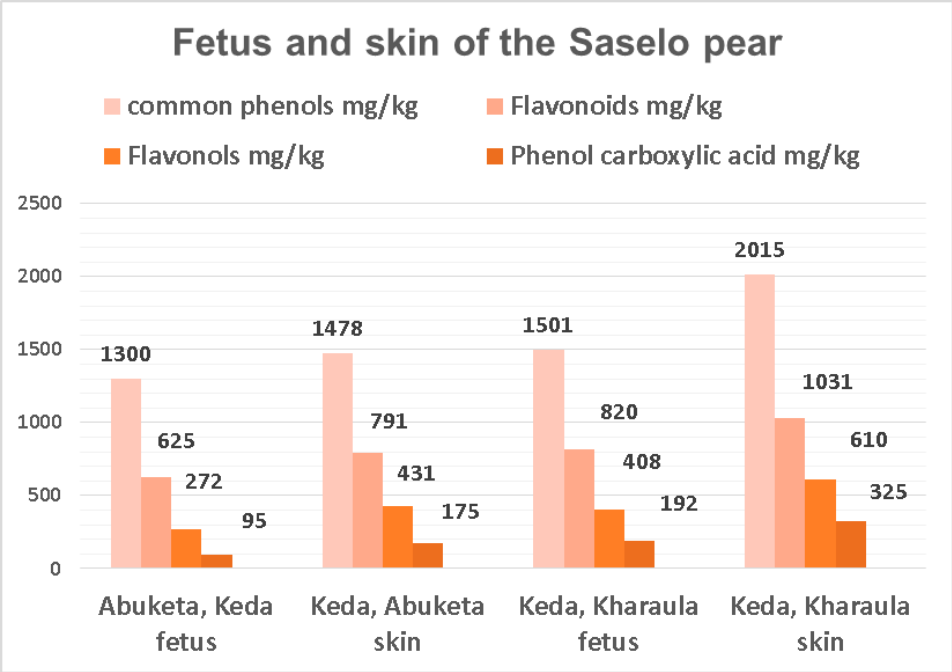


Figure 13. Common Phenols, phenol carboxylic acid, flavonoids and flavanols amount per (mg) in each (kg) of the parts studied from the Saselo species.

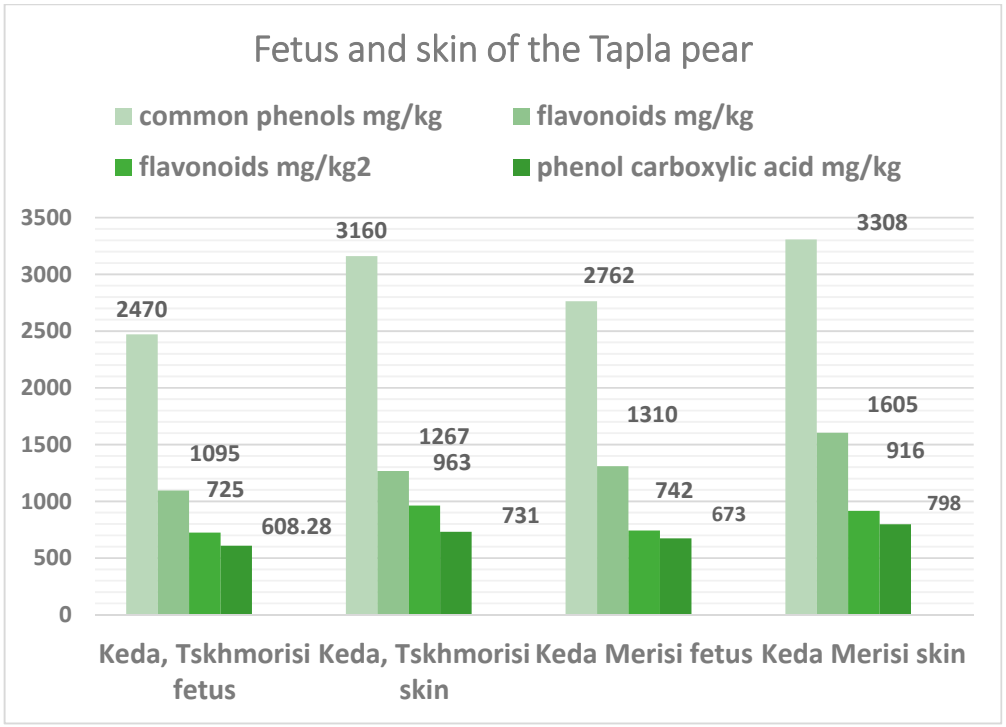


Figure 14. Common Phenols, phenol carboxylic acid, flavonoids and flavanols amount per (mg) in each (kg) of the parts studied from the Tapla species.

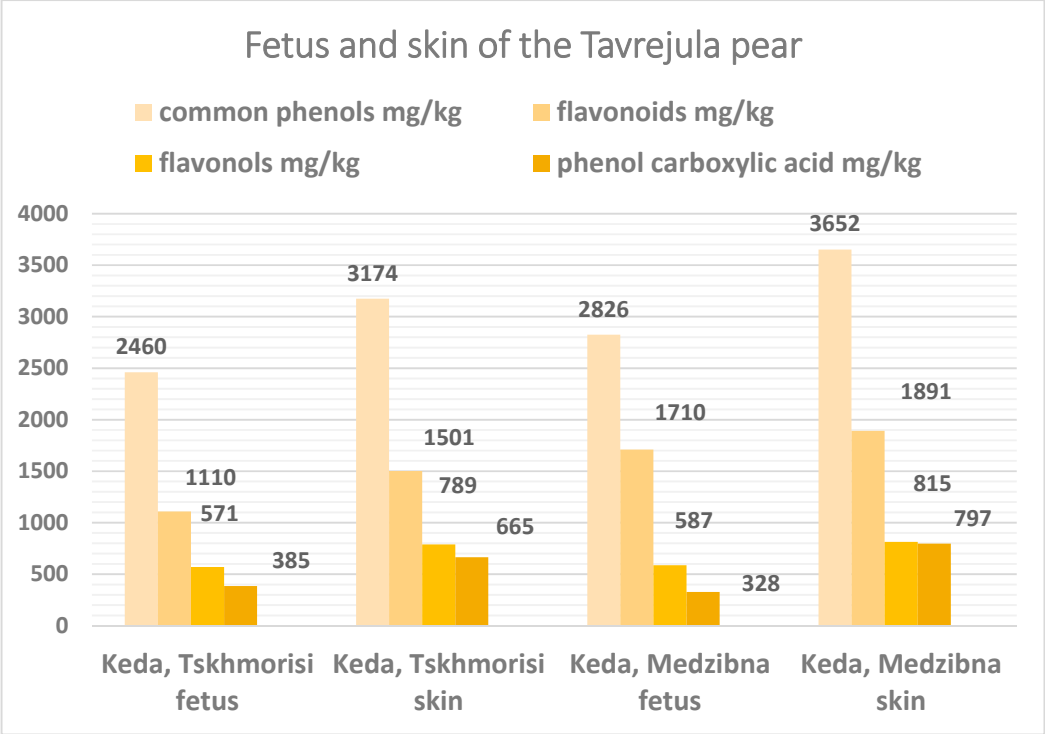


Figure 15. Common Phenols, phenol carboxylic acid, flavonoids and flavanols amount per (mg) in each (kg) of the parts studied from the Tavrejula species.

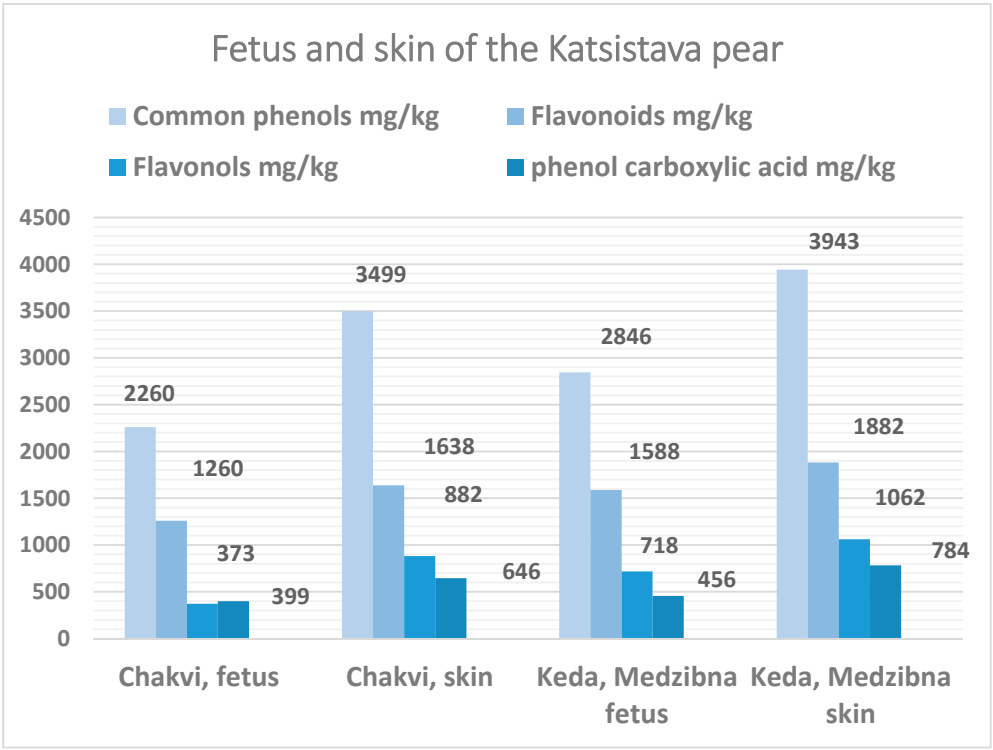


Figure 16. Common Phenols, phenol carboxylic acid, flavonoids and flavanols amount per (mg) in each (kg) of the parts studied from the Katsistava species.

Saselo samples were taken in 2 villages of the Keda district, at different heights from sea level, in particular, in Abuketa—344 m and Kharaula—780 m. The content of phenolic compounds was

studied both in the fetus and in the peel. The amount of total phenols in the fruit of Saselo is 1300 mg/kg, and in the peel—1478 mg/kg. Accordingly, the number of flavonoids is 625 and 791, catechins—272 and 431, phenol carboxylic acids—95 and 175 mg/kg.

4. Conclusions

This study is a contribution to knowledge about the phenolic compounds, antioxidant activity, catechins and flavonoids of special kind of pear "Khechechuri". By comparing the results obtained, we can conclude that the content of the phenol in the digestive tract on the various locks is different. In the mountainous Adjara, there is an increase in height by 150 m, in 1965 mg/kg, 1976 mg/kg, diameter of 1976 mg/kg, 700 m in 2089 mg/kg, 800 m shua 2265, 950 m in Khulo 2981 mg/kg, which is likely to be caused by strenuous climatic conditions for planting more fermentation and thus boosting biologically active substances. Skin and pomace were the most effective parts biologically due to their high concentration of the active substances, unlike the pomace and juice, which contain water that dilute the concentration and reduces effectiveness. It has been well documented that fruit by-products, such as peels, seeds and leaves, contain high levels of various health-enhancing substances, e.g., phenolic compounds (https://www.sciencedirect.com/topics/physics-and-astronomy/phenolic-compound). With an increase in the height of sea level, the number of phenolic compounds also increases, in particular, if the number of phenols in the fruit of Saselo in Abuketa is 1300 mg/kg, then in the Kharula fruits it is 1501 mg/kg. A similar increase is observed in relation to other phenolic compounds.

The utilization of by-products has become an important aspect in waste management in contributing to more sustainable production in the food and pharmaceutical industries.

Some of the results from the biological assays showed encouraging data that can certainly be used in further studies on these kind of pears in the future. Additional experiments are obviously required in the future in order to investigate the precise mechanism or mechanisms responsible for the pronounced biological activity. It might be interesting to study other technological variables of the production process, as well as different varieties of pears.

Author Contributions: T.G.S. conceived the study. T.G.S. and I.D. performed the Khechechuri formation study, conducted the biological and chemical studies and analyzed the data while A.K. and M.V. contributed to drafting the manuscript which was written by T.G.S., Y.A. corrected the language, A.K. and C.J. coordinated the studies at their respective institutes and drafted the manuscript.

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