Antioxidant Properties of Western Georgia Native Khechechuri Pear

Tamara Gabour Sad 1, Indira Djafaridze 2, Aleko Kalandia 2,* Maia Vanidze 2, Katarina Smilkov 3 and Claus Jacob 1

1 Division of Bioorganic Chemistry, School of Pharmacy, Saarland University (UdS), Campus B2 1, D-66123 Saarbruecken, Germany; tamara.sad@outlook.de (T.G.S.); c.jacob@mx.uni-saarland.de (C.J.)

2 Department of Chemistry, Faculty of Natural Sciences and Health Care, Batumi Shota Rustaveli State University (BSU), 35/32 Ninoshvili/Rustaveli str., 6010 Batumi, Georgia; indira.djafaridze@gmail.com (I.D.); vanidzemaia@gmail.com (M.V.)

3 Department of Applied Pharmacy, Division of Pharmacy, Faculty of Medical Sciences, University Goce Delcev (UGD), str. Krste Misirkov No. 10A, 2000 Shtip, North Macedonia; katarina.smilkov@ugd.edu.mk

* Correspondence: aleko.kalandia@gmail.com

Received: 30 July 2019; Accepted: 31 July 2019; Published: 9 May 2020

Abstract: An endemic pear species spreads in one region of western Georgia, Adjara, called Khechechuri. Pears are dietary source of bioactive components such as polyphenols and triterpenic acid. In addition to its gastronomic value, the aim of the article was to examine and compare phenolic compounds, flavonoids, catechins, phenolic acids, and antioxidant activities in five species of Khechechuri collected from various regions in Adjara region: Adjaristskali, Merisi, Dandalo, Shuakhevi, and Khulo. Five fruit parts, the skin, edible pulp, the whole pear (skin + pulp), juice and the pomace were analyzed and the results were compared. Our study revealed that the amount of total phenolic content found in the skin of West Georgian pear types was as much as 4650 mg/kg. Also, the pear pomace showed significant amount of total phenolic content in all species of Khechechuri. In addition, in all species of Khechechuri pears, flavonoids were found, except in the fruit juice.

Keywords: Khechechuri pear; Adjara region; phenolic compounds; catechins; flavonoids; antioxidant activity

1. Introduction

Pears are wide-spread fruit species that have been used in human diet for centuries. Being rich in various macro-, micro- nutrients and biologically active compounds, pears represent an important part of the human diet worldwide [1,2]. The Caucasus is a mountain region with extremely diversified natural conditions. A high percentage of endemics is noted in high-mountain and highland xerophytic plant formations [3,4]. The centers of diversity of the sub endemic genera are found especially in Georgia, with its Geographical isolation (with respect to longitude, latitude and altitude) as 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 endemic type of the pear family, (Pyrus communis L.), that is found only in the region of Adjara in Georgia, and is very appreciated as delicious fruit.

To date, many different studies have examined the composition of this fruit, therefore analyzing the carbohydrate, amino- and fatty-acids content, organic acids and volatile compounds content, vitamin and mineral content and bioactive compounds content (mainly phenolic content) [1,2,6–9]. The goal of the presented work was to assess the antioxidant properties of this endemic type of pear, in
terms examining and comparing the content of phenolic compounds, flavonoids, catechins, phenolic acids, and antioxidant activities. For this purpose, five species of Khechechuri pears were collected from various regions (villages) in Adjara region: Adjaristskali, Merisi, Dandalo, Shuakhevi, and Khulo.

2. Materials and Methods

2.1. Materials and Chemicals

The pears were collected from the respective villages, measured and assigned with analysis labels: Adjaristskali (A), Merisi (B), Dandalo (C) Shuakhevi (D), and Khulo (E) (Table 1). For study purposes, a total amount of 5 kg of the skin, the edible part (pulp), the whole pear (skin + pulp), juice and the pomace were homogenized and analyzed. 5 g of the mixture of each fruit part was mixed with 150 mL ethanol, and 50 mL of pear extract was used to determine the phenolic compounds, flavonoids, catechins, phenolic acid and antioxidant activity. All chemicals used in the described methods were analytical grade.

Table 1. Khechechuri pears collected from different regions (villages) with their respective description and characteristics.

| Sample       | Appearance       | Average Mass (g) | Average Volume (mL) | Size                        |
|--------------|------------------|------------------|---------------------|----------------------------|
|              |                  |                  |                     | Average Width (cm)       |
|              |                  |                  |                     | Average Height (cm)      |
| Adjaristskali| Green with black points | 136.66           | 130.2               | 67.74                      |
| (A)          |                  |                  |                     | 64.19                      |
| Merisi       | Green with black points | 104.43           | 73.3                | 61.87                      |
| (B)          |                  |                  |                     | 57.51                      |
| Dandalo      | Green with black points | 87.77            | 93.3                | 58.78                      |
| (C)          |                  |                  |                     | 55.79                      |
| Shuakhevi    | Green with black points | 150.65           | 67.5                | 68.6                       |
| (D)          |                  |                  |                     | 63.71                      |
| Khulo        | Green with black points | 123.08           | 145.0               | 65.80                      |
| (E)          |                  |                  |                     | 60.75                      |
All chemical compounds used in these analysis were of analytical grade.

2.2. Determination of Antioxidant Action

Antioxidant activity was determined by using DPPH (2,2-Diphenyl-1-picrylhydrazil) method [10,11]. In this context, 1 mL of the prepared sample was added to 3 mL of DPPH extract (0.1 mM DPPH in ethanol) and after 30 min the change in absorbance was registered at 517 nm. DPPH and 96% ethyl alcohol were used as blanks. For determination of the action of free radical inhibition (DPPH), the following formula was used:

$$In\% = A_C - A_S \times 100$$

where $A_C$ indicates absorption of DPPH/Alcohol solution and $A_S$ indicates absorption of the extract [12]. The analyses were performed in triplicates, and results were expressed as mean value.

2.3. Determination of Total Phenolic Compounds

Total phenolic compounds were assessed using Folin-Ciocalteu method [13,14]. Extraction of the samples was conducted using 80% ethanol; 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 at 25 °C, 10.0 mL of 7% Na$_2$CO$_3$ was added, the flask was then filled to the mark with water and left at room temperature for 2 h. Absorbance was measured at 750 nm. As control 1 mL of reagent was used. The calculations of the obtained values were made using the calibration curve of gallic acid. For determination of the phenols the following formula was used:

$$X = (D K V F) \times 1000/m$$

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 (g). The analyses were performed in triplicates, and results were expressed as mean value.

2.4. Determination of Catechines

Catechines content was determined using Swain and Hill method [15]. 1 mL of each sample was pipetted to 3 mL of 1% vanillin reagent (1 g vanillin in 70% sulfur acid solution). 1 mL ethanol was used as control. After 15 min, the solution became red, and then, after 15 min, the absorption at 750 nm was determined [16]. The analyses were performed in triplicates, and results were expressed as mean value.

2.5. Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined by aluminium chloride colorimetric method. The samples (0.5 mL) were mixed with 2 mL of distilled water and 150 µL of 5% NaNO$_2$ solution. After 5 min, 150 µL of 10% AlCl$_3$ was added in the mixture and, after 6 min, 2 mL 1 mol/L NaOH solution. The end volume was increased to 5 mL with distilled water. The absorbance was measured at 510 nm and the results were expressed in mg/L of catechin (or ruthin) [17]. The analyses were performed in triplicates, and results were expressed as mean value.

3. Results and Discussion

Our analyses resulted in variable, yet still comparable values of Total Phenolic Content (TPC), Total Flavonoid Content, Catechines content, Antioxidant activity and Phenolic acid content in the analyzed five different parts of Khechechuri pear (fruit, skin, pulp, juice and pomace). The results are presented in Figures 1–5, accordingly.
The skin of the pears, as a group of flavonoid compounds, were also present in the pomace and juice. The highest flavonoid content was found in the skin and pomace of the pear collected from the Khulo region. Our analyses resulted in variable, yet still comparable values of Total Phenolic Content (TPC), Catechins content, Antioxidant activity, and Phenolic acid content in the fruit parts.

Figure 1. Total phenolic content (mg/kg) in the fruit parts.

Figure 2. Total Flavonoid Content (mg/kg) of the fruit parts.

Figure 3. Catechins content (mg/kg) of the fruit parts.
Figure 4. Antioxidant activity of the fruit parts.

3.5. Phenolic Acid Content

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 examined from the compounds that contain phenolic acid, which is represented in the fruit by 330–465 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 11–21% (37–78 mg/kg) of the total content of phenolic acid (Figure 5).

Figure 5. Phenolic acid content (mg/kg) of the fruit parts.

3.1. Total Phenolic Content

Figure 1 shows the total phenolic content (TPC) of the Khechechuri pears, collected from different areas in Adjara region. As it was expected, in all of the collected samples, the highest amount of total phenolic compounds can be found in the skin of the pears, from 4275–4644 mg/kg. Indeed, pomace samples showed the second–highest content of phenolic compounds in all the samples tested, i.e., 3151–3717 mg/kg. On the other hand, in the pulp, the juice, and the whole fruit, lower percentage of total phenolic compounds was found, 1191–2533 mg/kg, 581 to 1320 mg/kg and 1965–2981 mg/kg, respectively.

3.2. Total Flavonoid Content

Our results showed that flavonoids make up to 38–61% of the total phenolic compounds, (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 pomace (1080–1908 mg/kg) (Figure 2). The content of flavonoids was most pronounced in the skin and pomace in the pears collected from the Adjaristskali region, when compared with the other samples tested. These results presented that the highest flavonoid content is found in the skin and pomace in all pear samples that have been tested. Unlike these parts, the juice shows remarkably lower content of flavonoids (Figure 2).
3.3. Catechins Content

The catechins, as a group of flavonoid compounds, were also assessed in these species. The content of catechins was calculated on the basis of the crude mass. As shown in Figure 3, the highest content of catechins was found in the skin of all collected samples, and the skin of the pear collected from Khulo region presented the highest content of catechins, 1250 mg/kg. Again, the pomace showed second-highest content of catechins in all samples, with also highest amount in the pear collected from Khulo region, 576 mg/kg. Catechins were least represented in the pear juice, ranging from 38–196 mg/kg in all the tested samples.

3.4. Antioxidant Activity

The antioxidant activity 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 4).

3.5. Phenolic Acid Content

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 examined from the compounds that contain phenolic acid, which is represented in the fruit by 330–465 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 5).

4. Conclusions

This study is a contribution to the knowledge about the composition of bioactive compounds in the endemic pear Khechechuri. Our results showed that skin and pomace have higher concentration of the bioactive substances examined, unlike the pulp and juice, which is probably due to their higher content of water that dilutes their concentration and therefore 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 [18]. 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. Therefore, this makes our observation valuable both in this direction. In addition, we concluded that with an increase in sea level elevation, the concentration of phenolic compounds also increases.

Some of the results from the biological assays showed encouraging data that can certainly be used in further studies on these kind of pears. 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, K.S. did scientific revision and corrections of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Universitaet des Saarlandes, Erasmus code: SAARBRU01”. Check carefully that the details given are accurate and use the standard spelling of funding agency names at https://search.crossref.org/funding, any errors may affect your future funding.

Acknowledgments: The authors would like to acknowledge the effort of Ahmad Yaman Abdin who established the cooperation between the two institutes, UdS and BSU, through the framework of the ERASMUS+ program
and the visit awarded to the doctoral student Tamara Gabour Sad from the sending University; University of Saarland, to the receiving University, University of Shota Rustaveli in Batumi, Georgia to do this scientific project. The authors express special thanks to Ken Rory, Inga Kartsivadze, Tatia Gorgoshadze and many other colleagues of the “Academiacs International” network (www.academiacs.eu) in Saarland University for helpful discussions and inspiration.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Li, X.; Gao, W.-Y.; Huang, L.-J.; Zhang, J.-Y.; Guo, X.-H. Antioxidant and Antiinflammation Capacities of Some Pear Cultivars. *J. Food Sci.* 2011, 76, C985–C990. [CrossRef] [PubMed]
2. Salta, J.; Martins, A.; Santos, R.G.; Neng, N.R.; Nogueira, J.M.F.; Justino, J.; Rauter, A.P. Phenolic composition and antioxidant activity of Rocha pear and other pear cultivars—A comparative study. *J. Funct. Foods* 2010, 2, 153–157. [CrossRef]
3. Zimina, R. The main features of the Caucasian natural landscapes and their conservation, USSR. *Arct. Alp. Res.* 1978, 10, 479–488. [CrossRef]
4. Catford, J.C. Mountain of tongues: The languages of the Caucasus. *Ann. Rev. Anthropol.* 1977, 6, 283–314. [CrossRef]
5. Gagnidze, R.; Gviniashvili, T.; Shetekauri, S.; Margalitadze, N. Endemic genera of the Caucasian flora. *Feddes Repertorium Zeitsschrift Botanische Taxonomie Geobotanik* 2002, 113, 616–630. [CrossRef]
6. Galvis-Sánchez, A.C.; Gil-Izquierdo, A.; Gil, M.I. Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *J. Sci. Food Agric.* 2003, 83, 995–1003. [CrossRef]
7. Ferreira, D.; Guyot, S.; Marnet, N.; Delgadillo, I.; Renard, C.M.; Coimbra, M. Composition of phenolic compounds in a Portuguese pear (*Pyrus communis* L. var. S. Bartolomeu) and changes after sun-drying. *J. Agric. Food Chem.* 2002, 50, 4537–4544. [CrossRef] [PubMed]
8. Wang, J.; Xu, J.Z.; Chen, H.J.; Zhang, H.J.; Li, S.L. The determination of volatile matters and amino acids content in the fruit of Ya pear. *Food Technol.* 2002, 9, 71–73.
9. Chen, J.; Wang, Z.; Wu, J.; Wang, Q.; Hu, X. Chemical compositional characterization of eight pear cultivars grown in China. *Food Chem.* 2007, 104, 268–275. [CrossRef]
10. Okawa, M.; Kinjo, J.; Nohara, T.; Ono, M. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. *Biol. Pharm. Bull.* 2001, 24, 1202–1205. [CrossRef] [PubMed]
11. Menstor, L.L.; Menezes, F.S.; Leitão, G.G.; Reis, A.S.; Santos, T.C.D.; Coube, C.S.; Leitão, S.G. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.* 2001, 15, 127–130. [CrossRef] [PubMed]
12. Kedare, S.B.; Singh, R. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* 2011, 48, 412–422. [CrossRef] [PubMed]
13. Stratil, P.; Klejdus, B.; Kubiáň, V. Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods. *J. Agric. Food Chem.* 2006, 54, 607–616. [CrossRef] [PubMed]
14. Petkovšek, M.M.; Stampar, F.; Veberic, R. Increased phenolic content in apple leaves infected with the apple scab pathogen. *J. Plant. Pathol.* 2008, 90, 49–55.
15. González-Rodríguez, J.; Pérez-Juan, P.; de Castro, M.L. Flow injection determination of total catechins and procyanidins in white and red wines. *Innov. Food Sci. Emerg. Technol.* 2002, 3, 289–293. [CrossRef]
16. Kharadze, M.; Djaparidze, I.; Shalashvili, A.; Vanidze, M.; Kalandia, A. Phenolic compounds and antioxidant properties of some white varieties of grape wines spread in Western Georgia. *Bull. Georg. Natl. Acad. Sci.* 2018, 12, 103–109.
17. Pekal, A.; Pyrzynska, K. Evaluation of aluminium complexion reaction for flavonoid content assay. *Food Anal. Methods* 2014, 7, 1776–1782. [CrossRef]
18. Available online: https://www.sciencedirect.com/topics/physics-and-astronomy/phenolic-compound (accessed on 18 March 2020).