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Valorization of Selected Plant Raw Materials and Processing By-Products Commonly Found in Georgia Using Circular Economy Principles

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Abstract: The aim of the study was to investigate the chemical composition of several endemic plant species of Georgia, including *Prunus laurocerasus*, *Senecio platyphyllus*, *Rhododendron* L., *Juglans regia*, as well as residues generated during wine production from different grape varieties, using high-performance liquid chromatography coupled with various detection systems (UPLC-PDA-MS, Waters UPLC Acquity QDa). For each raw material, environmentally friendly (“green”) technologies were developed for the production of biologically

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active compound preparations using supercritical water extraction (SWE) with different cosolvents. The bioactive compound profiles of each raw material were investigated, and protocols for their identification and standardization based on active constituents were developed. The proposed technologies ensure not only the production of valuable preparations from the studied raw materials but also the almost complete valorization of production residues generated during processing. The antioxidant activities of the obtained preparations were evaluated, and correlations between antioxidant potential and the content of biologically active compounds were established.

Keywords: Plant raw materials; Production of bioactive preparations; UPLC-PDA-MS analysis

Introduction

Natural compounds have been widely used as biologically active components in the pharmaceutical and food industries. They have antimicrobial, antibacterial, antifungal, antiviral, and antitumor effects. Numerous studies have confirmed a proportional correlation between diet and disease. The issue of the use of artificial additives is especially acute. The medicinal and pharmaceutical properties of plant raw materials are mainly associated with secondary plant metabolites (Kashani et al., 2012; Ramawat & Mérillon, 2009). Naturally, plants produce these compounds because they are necessary for survival, although in many cases their physiological role remains unknown (Heinrich et al., 2012; Sarker & Nahar, 2006). The healing properties of secondary metabolites are frequently associated with waxes, fatty acids, alkaloids, terpenes, phenols, glycosides, phytosterols, and related compounds (Kashani et al., 2012; Sarker & Nahar, 2006).

The activity of phenolic compounds is of particular interest. Rosmarinic acid, carnosic acid, carnosol, rosmanol, epirosmanol, and methyl carnosate isolated from *Rosmarinus officinalis* exhibit antitumor activity (Sánchez-Camargo et al., 2014). Pharmacological studies of *Cordia verbenacea* have demonstrated anti-inflammatory activity due to α -caryophyllene and trans-caryophyllene (Parisotto et al., 2012). The biological activity of *Thymus vulgaris*, *Thymus hyemalis*, and *Thymus zygis* has been associated with thymol, carvacrol, and borneol (Santoyo et al., 2014). Likewise, *Salvia officinalis* possesses anti-inflammatory properties attributed to camphor, borneol, and 1,8-cineole (Arranz et al., 2014). Perillyl alcohol, a hydroxylated monoterpene identified in *Prunus avium*, has demonstrated activity against several progressive cancers (Serra et al., 2010). To obtain bioactive compounds, efficient extraction and recovery are required. This process generally consists of desorption and solubilization, internal diffusion through the solid matrix, and external diffusion through the boundary layer. Optimization of extraction depends on careful control of analytical conditions and sample preparation (Camel, 2001; Martínez, 2008; Barbosa-Cánovas et al., 2014).

Recent literature demonstrates increasing emphasis on green extraction technologies, including ultrasound-assisted extraction, subcritical water extraction, and supercritical fluid extraction (Herrero et al., 2006; Barbosa-Cánovas et al., 2014). Supercritical fluid extraction (SFE) offers several advantages over conventional extraction techniques, including improved transport properties, enhanced diffusivity, environmentally friendly solvents, and simplified coupling with chromatographic systems (Herrero et al., 2006). Carbon dioxide remains the most widely used supercritical solvent because it is inexpensive, non-toxic, and leaves no solvent residue after depressurization (Herrero et al., 2006; Zougagh et al., 2004; Solana et al., 2014). The addition of polar co-solvents can further improve extraction efficiency (Herrero et al., 2006; Barbosa-Cánovas et al., 2014). Bioactive compounds, also known as nutraceuticals, are naturally occurring substances with demonstrated biological activity and therapeutic potential (Lindy, 2014; Biesalski et al., 2009). Many of these metabolites function as defense compounds in plants while exhibiting pharmacological activity in humans (Colegate & Molyneux, 2011). Supercritical fluid technology has become increasingly important for obtaining compounds used in pharmaceuticals, foods, and cosmetics, as well as for developing novel treatments for diseases such as cancer, stroke, and Alzheimer's disease (Lindy, 2014; Sovilj et al., 2011).

Materials and Methods

Prior to chromatographic analysis, all extracts were concentrated under reduced pressure using a vacuum evaporator, followed by centrifugation and filtration through *Waters Acrodisc LC PVDF syringe filters* (13 mm, 0.45 μ m). Qualitative identification and quantitative determination of the target compounds were carried out using a *Waters ACQUITY UPLC* system equipped with a photodiode array detector (PDA) and a single quadrupole mass spectrometer (QDa) operating with an electrospray ionization (ESI) source. Chromatographic separation was performed on an *ACQUITY BEH C18* column (2.1 $\times$ 100 mm, 1.7 μ m). The mobile phase consisted of 0.1%

formic acid in water (solvent A) and acetonitrile (solvent B). Elution was achieved using the following gradient program: 0–2.0 min, 5% B; 2.0–8.0 min, 20% B; 8.0–10.0 min, 35% B; 10.0–11.0 min, 95% B; 12.0 min, 5% B; and maintained at 5% B until 15.0 min. The flow rate was set at 0.3 mL min⁻¹, the injection volume was 2 µL, and the column temperature was maintained at 40°C. Mass spectrometric detection was performed in the negative electrospray ionization mode (ESI⁻). The operating conditions were as follows: capillary voltage, 0.8 kV; cone voltage, 15 V; source temperature, 120°C; and desolvation temperature, 500°C. Data acquisition was conducted in selected ion recording (SIR) mode over an *m/z* range of 100–1000.

Different extraction techniques and solvent systems were applied to evaluate the content of biologically active compounds. Frozen green walnut husks (*Juglans regia* L.) were extracted using 30%, 40%, and 50% aqueous ethanol by ultrasonic bath-assisted extraction, ultrasonic probe-assisted extraction, and hot reflux extraction. For *Rhododendron ponticum* L. and *Rhododendron luteum* Sweet, 20% aqueous ethanol was used as the extraction solvent, while ultrasonic bath and ultrasonic probe extraction were employed. Each sample was extracted in triplicate. The resulting extracts were analyzed for total phenolic content, total flavonoid content, total catechin content, and antioxidant activity. Quantitative measurements were performed using spectrophotometric methods.

Determination of Total Phenolic Content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric assay. Briefly, 1 mL of the ethanolic extract was mixed with 5 mL of distilled water and 1 mL of Folin–Ciocalteu reagent. After incubation for 5 min, 10 mL of sodium carbonate (Na₂CO₃) solution was added. The reaction mixture was diluted to a final volume of 25 mL with distilled water and incubated for 2 h in the dark. A blank solution containing all reagents except the sample extract was prepared under identical conditions. Following incubation, the absorbance was measured at 750 nm using a spectrophotometer. The total phenolic content was quantified using a gallic acid calibration curve and calculated according to the following equation:

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

Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method. Briefly, 1 mL of the ethanolic extract was transferred into a 10 mL volumetric flask containing 5 mL of distilled water and 0.3 mL of 5% sodium nitrite (NaNO₂). After 5 min, 0.3 mL of 10% aluminum chloride (AlCl₃) solution was added. Following an additional 6 min of incubation, 2 mL of 1 N sodium hydroxide (NaOH) was introduced into the reaction mixture. The absorbance was measured at 510 nm. A blank prepared with the corresponding ethanol solution instead of the sample extract was analyzed under identical conditions. The total flavonoid content was quantified using a rutin calibration curve.

Determination of Total Catechin Content

The total catechin content was determined using the vanillin assay. For the analysis, 1 mL of the sample extract was mixed with 3 mL of freshly prepared vanillin reagent, obtained by dissolving 1 g of vanillin in 100 mL of concentrated hydrochloric acid. The blank solution consisted of the vanillin reagent and the corresponding extraction solvent. After 3 min of incubation, the absorbance was recorded at 500 nm.

Determination of Antioxidant Activity

The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, one of the most widely applied spectrophotometric methods for assessing the antioxidant capacity of natural products. For the assay, 1 mL of the ethanolic extract was mixed with 3 mL of DPPH ethanolic solution and incubated in the dark for 30 min at room temperature. The absorbance was subsequently measured at 515 nm. A control solution containing DPPH and the corresponding extraction solvent was prepared under identical conditions. The free radical scavenging activity was expressed as the percentage inhibition of DPPH radicals.

Results and Discussion

The analysis of *walnut green husk* extracts demonstrated that the highest levels of biologically active compounds were obtained by conventional extraction using 50% ethanol as the extraction solvent. Under these conditions, the total phenolic content reached 18.47 and 20.21 mg/g, while the total flavonoid content was 12.78 mg/g. The highest catechin content was also observed in the same extract, reaching 0.66 mg/g. Ultrasonic probe-assisted extraction produced comparable results; however, the obtained values were slightly lower than those achieved by the conventional extraction method.

The antioxidant activity assay also confirmed the effectiveness of conventional extraction with 50% ethanol. Extracts obtained from both walnut forms exhibited the highest antioxidant activity, with 50% inhibition of DPPH radicals achieved by 12.92 and 14.28 mg of sample, respectively. A comparative evaluation of the two walnut forms revealed no substantial differences in the content of the investigated bioactive compounds or their antioxidant capacity. In addition, a positive correlation was observed between total phenolic content and antioxidant activity, suggesting that phenolic compounds are the major contributors to the antioxidant potential of walnut green husk extracts. The extracts of *Rhododendron ponticum* L. and *Rhododendron luteum* Sweet were evaluated for their total phenolic, flavonoid, and catechin contents, as well as their antioxidant activity using the DPPH radical scavenging assay.

The results demonstrated that the highest concentrations of bioactive compounds were consistently obtained in the first extract (Extract I), whereas their contents gradually decreased in the subsequent extraction cycles. A similar trend was observed for antioxidant activity, as progressively larger amounts of extract were required to achieve 50% DPPH radical inhibition, indicating a reduction in antioxidant capacity. Among the *R. ponticum* samples, the ultrasonic probe-assisted extract from the Machakhela population exhibited the highest total phenolic content (120.98 mg/g). This extract also showed the highest catechin content (19.68 mg/g), while only 2.67 mg of sample was required to achieve 50% DPPH inhibition, demonstrating its strong antioxidant potential.

Comparison of the extraction techniques revealed that ultrasonic probe-assisted extraction was more effective than ultrasonic bath extraction for the Machakhela samples. The total phenolic content reached 120.98 mg/g in the probe-assisted extract, compared with 100.58 mg/g in the ultrasonic bath extract. Accordingly, the probe-assisted extracts also exhibited superior antioxidant activity. In contrast, *R. luteum* extracts contained lower amounts of total phenolics, flavonoids, and catechins than *R. ponticum*. Nevertheless, the first extract showed the highest antioxidant activity, requiring only 0.54 mg of sample to achieve 50% DPPH radical inhibition. This finding suggests that antioxidant activity depends not only on the overall concentration of phenolic compounds but also on their qualitative composition. Furthermore, a positive correlation was observed between the contents of phenolic compounds, flavonoids, and catechins and the antioxidant activity of the extracts. Extracts enriched with these bioactive constituents exhibited greater free radical scavenging capacity, highlighting the important contribution of phenolic compounds to the antioxidant potential of *Rhododendron* extracts.

For the quantitative assessment of biologically active compounds, various extraction methods and solvents were applied. In the case of frozen green walnut husk (*Juglans regia*), extraction was performed using 30%, 40%, and 50% ethanol through three different approaches: ultrasonic bath-assisted extraction, ultrasonic probe-assisted extraction, and conventional hot extraction using a reflux condenser. For *Rhododendron ponticum* L. and *Rhododendron luteum* Sweet samples, 20% ethanol was used as the extraction solvent, and extraction was carried out using an ultrasonic bath and an ultrasonic probe. All samples underwent three consecutive extraction cycles. The obtained extracts were analyzed for the content of total phenolic compounds, flavonoids, and catechins, and their antioxidant activity was evaluated. Quantitative determination of these parameters was performed using spectroscopic methods.

The analysis of walnut green husk extracts demonstrated that the highest content of biologically active compounds was achieved using conventional extraction with 50% ethanol. In extracts obtained by this method, the total phenolic content reached 18.47 and 20.21 mg/g, while the total flavonoid content was 12.78 mg/g. The maximum catechin concentration was also observed under the same extraction conditions and amounted to 0.66 mg/g. High values were also obtained using ultrasonic probe-assisted extraction; however, these results were slightly lower compared with conventional extraction. The evaluation of antioxidant activity further confirmed the superiority of conventional extraction with 50% ethanol. Both studied forms of walnut extracts exhibited high free radical scavenging capacity, while the amount of extract required to achieve 50% inhibition of DPPH radicals was 12.92 and 14.28 mg, respectively.

Within the framework of the study, two different morphological forms of walnut were compared. According to the obtained results, no significant differences were observed between them in terms of biologically active compound content and antioxidant activity. Furthermore, a positive correlation was established between total

phenolic content and antioxidant activity, indicating the important role of phenolic compounds in determining the antioxidant properties of walnut extracts. The study also evaluated the content of total phenolic compounds, flavonoids, and catechins in extracts of *Rhododendron ponticum* L. and *Rhododendron luteum* Sweet, as well as their antioxidant activity using the DPPH assay.

The results demonstrated that in both species, the highest levels of biologically active compounds were detected in the first extract (Extract I), while their concentrations gradually decreased with each subsequent extraction cycle. A similar trend was observed in the assessment of antioxidant activity. As the content of biologically active compounds decreased, the amount of sample required to achieve 50% inhibition of DPPH radicals increased, indicating a reduction in antioxidant efficiency. Among the *Rhododendron ponticum* samples, the highest total phenolic content was detected in the ultrasonic probe-assisted extract obtained from the Machakhela population, reaching 120.98 mg/g. The same extract contained 19.68 mg/g catechins, and 50% inhibition of DPPH radicals was achieved using only 2.67 mg of extract, indicating its high antioxidant activity.

Comparison of Machakhela samples showed that ultrasonic probe-assisted extraction was more efficient than ultrasonic bath extraction. Specifically, the total phenolic content in the probe-assisted extract was 120.98 mg/g, whereas in the ultrasonic bath extract it was 100.58 mg/g. Accordingly, probe-assisted extracts also demonstrated higher antioxidant activity. The content of total phenolics, flavonoids, and catechins in *Rhododendron luteum* extracts was lower compared with *R. ponticum*. Nevertheless, the first extract exhibited the highest antioxidant activity, as only 0.54 mg of sample was required to achieve 50% DPPH inhibition. This result indicates that antioxidant activity is determined not only by the total amount of phenolic compounds but also by their qualitative composition. Analysis of the results also revealed positive correlations between the contents of total phenolic compounds, flavonoids, and catechins and antioxidant activity. Extracts rich in biologically active compounds demonstrated stronger free radical scavenging capacity, confirming the significant role of phenolic compounds in the formation of the antioxidant potential of *Rhododendron* extracts.

The distribution of bioactive compounds among different fractions of *Prunus laurocerasus* demonstrates the significant potential of processing residues as a valuable source of phytochemicals. The total phenolic content, flavonoids, catechins, leucoanthocyanins, and anthocyanins were evaluated in pulp, juice, and pomace fractions. Although the juice contained considerable amounts of soluble phenolic compounds, the pomace exhibited a remarkable accumulation of several biologically important metabolites, highlighting its potential for valorization. The most pronounced difference was observed for *anthocyanins*, which are among the most valuable secondary metabolites due to their antioxidant, anti-inflammatory, and cytoprotective properties. The pomace contained 11.39 mg/g dry weight of total anthocyanins, compared with only 1.35 mg/g in juice, demonstrating an approximately 8-fold enrichment of anthocyanins in the processing residue [Figure 1.].

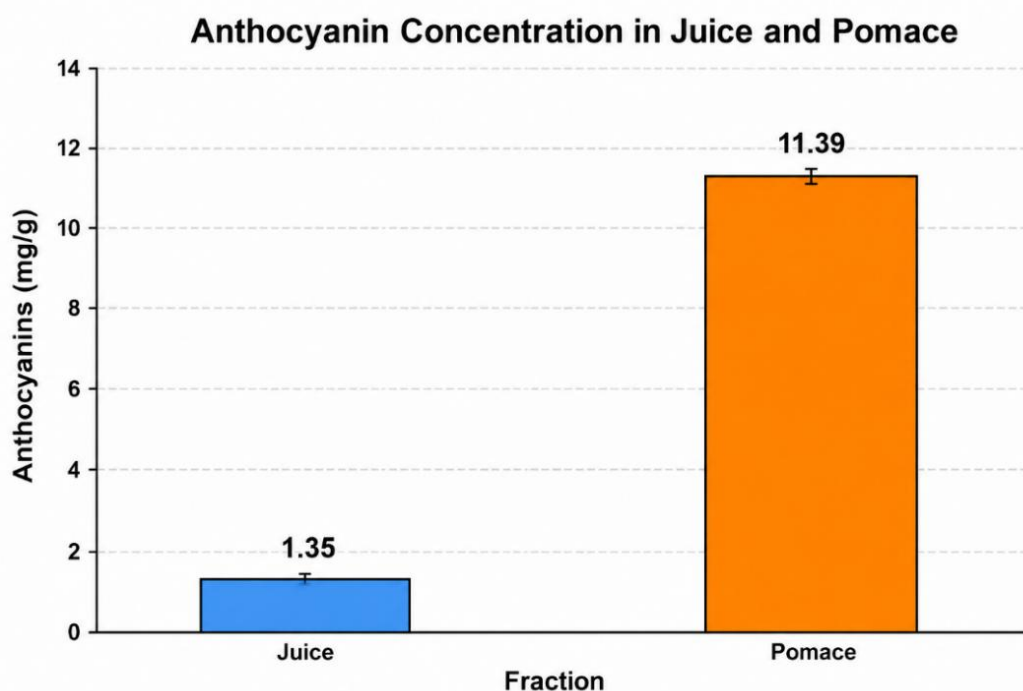


Figure 1. Comparison of anthocyanin concentration in juice and pomace of *P. laurocerasus*

Moreover, mass balance analysis revealed that approximately 90% of total anthocyanins generated during processing remained concentrated in the pomace, while only 10% was recovered in the juice fraction. This confirms that *P. laurocerasus* pomace represents the primary reservoir of anthocyanin compounds. A similar tendency was observed for *leucoanthocyanins*, with pomace containing the highest concentration (15.693 mg/g) and retaining approximately 66% of the total *leucoanthocyanin* content after juice extraction. Catechins were also preferentially accumulated in the pomace, where their content exceeded that of the juice fraction. These findings indicate that mechanical processing does not eliminate the biological value of *P. laurocerasus* residues; instead, it results in the concentration of specific bioactive constituents within the solid fraction.

Antioxidant activity, determined by the DPPH radical scavenging assay (expressed as the amount of sample required to achieve 50% inhibition; lower values indicate stronger antioxidant activity), was highest in the pomace fraction (2.796 mg), followed by the pulp (3.315 mg), whereas the juice exhibited the lowest antioxidant activity (10.666 mg). This pattern is consistent with the higher concentrations of anthocyanins and proanthocyanidins retained in the pomace, both of which are recognized as potent free radical scavengers [Figure 2].

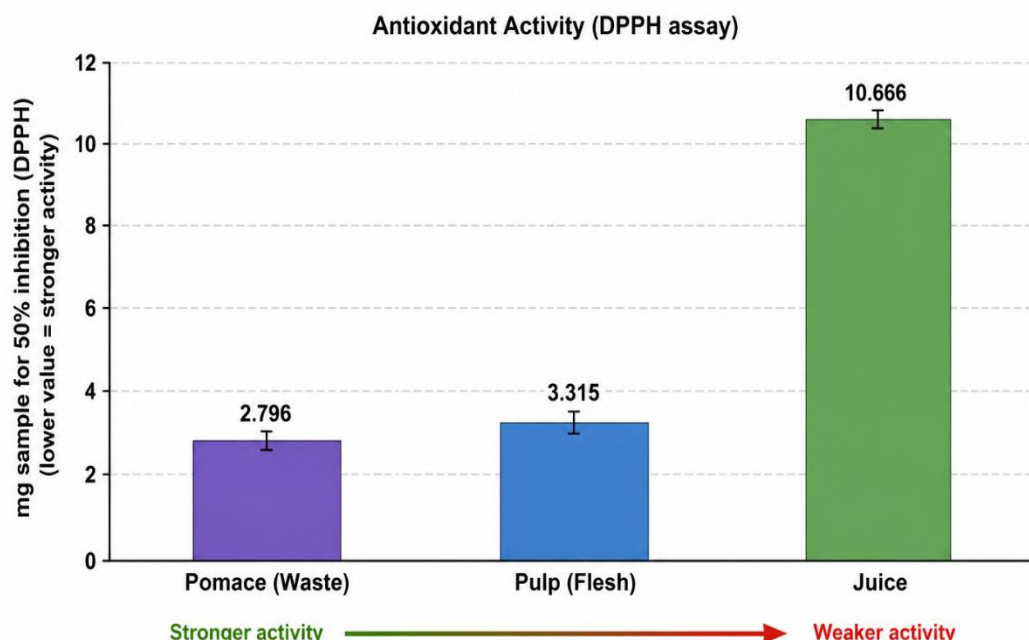


Figure 2. Antioxidant activity of *Prunus laurocerasus* fractions determined by the DPPH assay.

Considering that *Prunus laurocerasus* pomace is traditionally regarded as an agricultural by-product, the demonstrated enrichment of phenolic compounds, particularly anthocyanins, provides strong evidence for its transformation into a high-value raw material.

Conclusion

Our study demonstrated that the extraction efficiency of biologically active compounds is strongly influenced by both the extraction technique and the physicochemical characteristics of the plant material. A particularly important outcome of this study is the substantial phytochemical value retained in agro-industrial processing residues. The analysis of *Prunus laurocerasus* fractions demonstrated that pomace is not merely a waste stream but a highly enriched source of valuable secondary metabolites. Approximately 90% of the total anthocyanins remained concentrated in the pomace after juice production, while elevated levels of leucoanthocyanins and catechins were also retained in the solid residue. These findings clearly show that processing by-products can preserve, and in some cases concentrate, compounds of high functional and commercial value rather than representing a loss of useful biomass.

Collectively, these results support the valorization of agro-industrial by-products such as pomace, fruit peels, skins, green husks, seeds, and other residual plant materials as sustainable sources of high-value phytochemicals. Instead of being discarded as waste, these materials can be redirected into the production of natural antioxidants, functional food ingredients, nutraceuticals, cosmetic formulations, and pharmaceutical preparations. Such an approach is fully aligned with circular economy principles, as it promotes resource efficiency, waste minimization,

and the creation of added value from materials that are already generated within existing agricultural and food-processing chains in Georgia.

Overall, this work demonstrates that the optimization of extraction technologies, combined with the comprehensive utilization of plant raw materials and processing residues, represents a promising strategy for maximizing the recovery of bioactive compounds and advancing the sustainable exploitation of Georgia's botanical resources. Future studies should focus on process scale-up, bioavailability, biological efficacy, and industrial implementation of these valorization strategies in order to support their translation into commercially viable and environmentally responsible products.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest

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