Phytochemical screening of RED grape pomace extracts enhanced with *Polygonum cuspidatum* flower extract for potential cosmetic formulations

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Abstract. *This study aims to investigate the phytochemical profile of pomace extract of the NOVA hybrid grape variety sourced from a vineyard located in the central region of Transylvania, Romania. Additionally, P. cuspidatum flower extract was added to grape pomace extract to increase its bioactive compounds content, to be used in various products such as dermatocosmetics, nutraceuticals, and cosmetics. These investigations suggested that the mixture of grape pomace and P. cuspidatum flower extracts has promising potential, in terms of bioactive compounds, for application to various skincare formulations.*

Keywords: grape pomace; *Polygonum cuspidatum*; extract; polyphenols; antioxidant activity; statistical analysis.

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1. Introduction

Grape pomace, a by-product of the winemaking process, is a valuable source of active principles, transforming grapes into natural medicines. The explanation is straightforward: the skin, pulp, and seeds of white and red grapes are rich in polyphenols, which provide significant antioxidant benefits to human health. This has changed our understanding of the concepts of fortified foods [1,2] and natural cosmetic beauty elixirs [3-8] in the 21st century. Grape pomace can be an invaluable source of beauty, a true elixir for dry and wrinkled skin that has lost its radiance.

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The cosmetic industry has a full advantage in terms of the therapeutic qualities of grape extracts, i.e., anti-aging compounds [9-11].

In today's world, many people are striving for a healthier lifestyle by reconnecting with nature. This has led to an increased use of products derived from natural compounds and a noticeable shift in consumer behavior. Integrative medicine provides a holistic approach to addressing health issues by using high-quality natural products [12-19] tailored to meet each individual's specific needs.

Taking into consideration these findings, in last ten years several studies of the authors' team have explored the phytochemical composition and biological activities of various plant species [8-11, 20-24], as well as grape [6,10, 25] or grape pomace [8,9,26,27] extracts, illustrating the increasing interest in phytochemical characterization as further application in the cosmetic or food fields.

Polygonum is a genus in the *Polygonaceae* family, consisting of over 300 perennial species found in northern temperate regions, as well as tropical and subtropical areas. These ubiquitous herbs are well known for their therapeutic properties in traditional medicine. In the theory of Traditional Chinese Medicine (TCM), the plant *P. cuspidatum* is described as having heat-clearing, detoxifying, dampness-draining, and blood-activating properties. It has long been prescribed for conditions such as jaundice, sores, arthralgia, amenorrhea, and pulmonary illnesses. Phytochemical analyses have revealed a variety of bioactive compounds, primarily stilbenes, anthraquinones, flavonoids, and polyphenols. Among these, resveratrol (i.e., (E)-3,5,4′-trihydroxystilbene), polydatin (i.e., 3,4,5-trihydroxystilbene-3-beta-monoglucoside) - the glycoside form of resveratrol -, quercetin (i.e., 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one), and emodin (i.e., 1,3,8-trihydroxy-6-methylanthracene-9,10-dione), all in both forms *cis* and *trans*, are acknowledged as the main active compounds in terms of pharmacological activity [28].

Hybrid grape varieties have been developed since the 19th century in response to diseases caused by *Phylloxera vastatrix*. These hybrids have demonstrated greater resistance to diseases and pests, adaptability to various climatic conditions, high productivity, and ease of cultivation and maintenance. Some older hybrids may have a less robust flavor, but second-generation hybrids can produce higher-quality wines that feature complex aromas. A hybrid red grape variety that meets the aforementioned properties is the NOVA variety, successfully adapted to all wine-growing areas in Romania. This red variety has demonstrated good resistance to temperatures and various cryptogamic diseases. Specific to this variety is the authentic old taste of provincial grapes.

To our knowledge, this hybrid variety has not been characterized from a phytochemical point of view to date, nor has it been included in the International

Organization of Vine and Wine (OIV) database. This was an asset of this research, the fact that for the first time, the NOVA variety is included in scientific research with the aim of carrying out a phytochemical screening regarding the active compounds responsible for the aroma and the authentic old taste. This new red grape hybrid, harvested from Batos vineyards, Transylvania region, Romania, was investigated in terms of the phytochemical profile of pomace extract. Specific to this region are slopes with different shapes, northern exposure, and inclinations between 15-25°, which are cultivated with vines. A brief description of a NOVA grape variety was: *(i)* the grape has large red berries; *(ii)* bunches rich in berries; *(iii)* oval grape; *(iv)* red thin skin; *(v)* authentic old taste and aroma of provincial grapes; *(vi)* genetic resistance; *(vii)* ripening takes place at the end of September. This interspecific hybrid has good resistance to temperature variations, to several cryptogamic diseases (i.e., gray rot, scab, and powdery mildew), and to harmful insects like phylloxera. Furthermore, to increase the complexity of this research, it was chosen to study the NOVA red grape pomace extract, as well as the addition of Japanese knotweed flower extract, to offer consumers unexplored natural product options rich in active compounds beneficial to human health. The chemical composition of knotweed flower extracts in terms of polyphenol content was already determined in previous research by Radulescu et al [8, 11]. This study stands out due to two main aspects. The first objective was to characterize the red grape pomace from the NOVA variety and the flowers of *P. cuspidatum* extracts in terms of phytochemical profile. The second objective was to investigate the phytochemical profile of a mixture of NOVA grape pomace and the flower of knotweed extracts through statistical analysis.

2. Materials and methods

2.1. Materials and reagents

The red grape pomace was obtained by fermentation in the autumn of the year 2024 and kept semi-dried until the extraction. *P. cuspidatum* was collected from Batos village using sterile equipment. The plant samples were carefully separated, with the flowers set apart from the roots, and then subsequently dried at a controlled temperature of 20°C to preserve their quality. The pomace and *P. cuspidatum* extracts were coded according to the data presented in Table 1.

All reagents (Merck KGaA, Darmstadt, Germany) were of HPLC grade (e.g., hydrochloric acid, HCl 37% 1.19 kg/L; ethyl alcohol, C_2H_5OH p.a., min. 99% (v/v); methyl alcohol, CH_3OH 60%; vanillin $C_8H_8O_3$; (+)-catechin $C_{15}H_{14}O_6$; quercetin dihydrate, $C_{15}H_{14}O_9$, 3,3',4',5,7-pentahydroxyflavone, p.a., min. 95%; anhydrous gallic acid, $C_7H_6O_5$, 3,4,5-acid trihydroxybenzoic, p.a. ACS, min. 98%; Folin-Ciocalteu reagent; Tris base 2-amino-2-(hydroxymethyl)propane-1,3-diol,

p.a., $H_2NC(CH_2OH)_3$, ACS.ISO.Reag.Ph.Eur; sodium acetate trihydrate, $CH_3COONa \cdot 3H_2O$, p.a., min. 98.5%, sodium carbonate Na_2CO_3, p.a., min.96%, and aluminum chloride hexahydrate, $AlCl_3 \cdot 6H_2O$, p.a., min. 96%; sodium hydroxide, NaOH, p.a., min. 99.02% and sodium nitrite, $NaNO_2$, p.a., 99.5%). For dilution and sample preparation, deionized water with a conductivity below 0.5 µS/cm at 25°C was used.

Table 1. Hydroalcoholic extract samples with assigned code.

Sample code	*Extract*
N	NOVA grape pomace hydroalcoholic extract
N/PcF	NOVA grape pomace and *P. cuspidatum* flower hydroalcoholic extracts, in a 3:1 ratio (v:v)
PcF	*P. cuspidatum* flower hydroalcoholic extract

2.2. Sample preparation

The NOVA grape pomace and Japanese knotweed flower hydroalcoholic extracts were obtained in an original pilot plant in two stages: *(i)* countercurrent extraction under pressure, and *(ii)* extract concentration process, owned by SC NIRVANA SRL Batos, Mureș County, Romania. The extraction procedure was conducted using 70% ethanol, with a plant-to-solvent ratio of 1:2 (m/v) for the pomace and 1:1 (m/v) for the Japanese knotweed flower. The extraction time was set to 25 cycles of 14 minutes each, as illustrated in Figure 1.

2.3. Total condensed tannins

The total condensed tannins (TCT) were determined according to the method proposed by Broadhurst and Jones [29]. This method was described in previous research [8,11,21,22]. Briefly, the stages of sample preparation for TCT analysis were: *(i)* in a 0.5 mL extract sample, 3 mL of 2.4% vanillin solution prepared in 60% methanol was added, then the mixture was homogenized by vortex; *(ii)* 1.5 mL of 30% hydrochloric acid was added to the mixture, followed by homogenization; *(iii)* the mixture was first maintained for 15 minutes at 20 ± 2°C, then second at 13 minutes at 25°C. The absorbance of the sample was accurately measured at 500 nm using the Evolution 260 Bio UV-Visible spectrophotometer (Thermo Fisher Scientific Inc., Madison, WI, USA). This measurement carefully considered the absorbance of the blank and any interference from the correction sample. A calibration curve based on (+)-catechin was used to evaluate the total condensed tannins content (TCT). The results are presented in catechin equivalents (CE), quantified as mg CE/mL, ensuring a clear understanding of the sample's quality and potency.

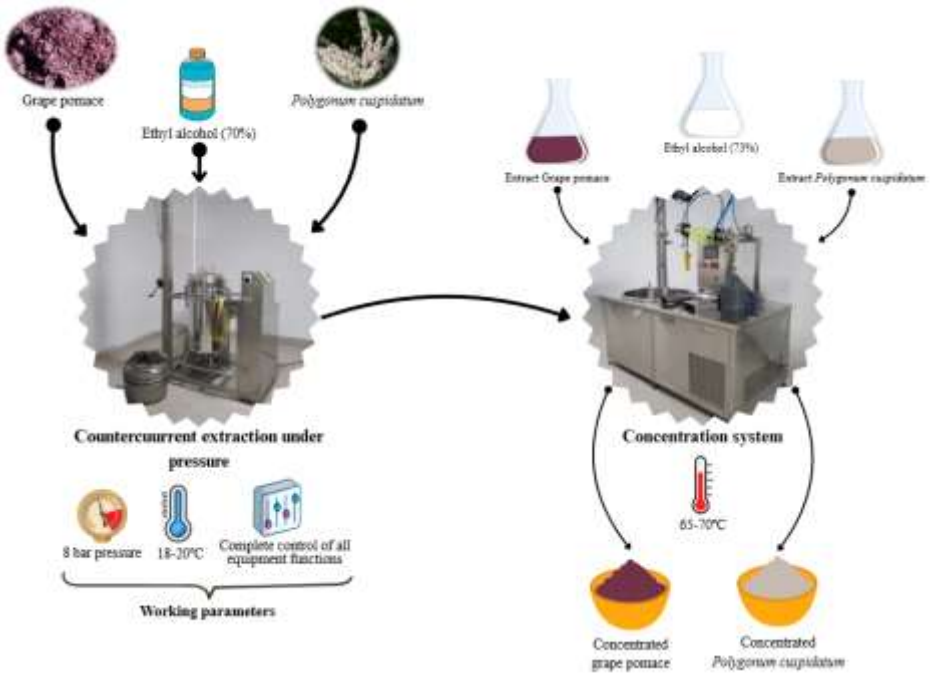

Fig. 1. Preparation stages of grape pomace and *P. cuspidatum* flower hydroalcoholic extracts.

2.4. Total anthocyanins content

The total anthocyanins content (TAC) of extracts was assessed by the method described by Giusti and Wrolstad [30] and applied by Radulescu et al. in previous research [8, 11]. The procedure consists of several steps: *(i)* establishing the dilution factor (DF) for each sample using a 0.025 M KCl buffer solution (pH = 1.0),; *(ii)* registering the spectrum of the sample from 260 to 800 nm, using distilled water as a blank; *(iii)* preparing two dilutions of each extract: one with 0.025 M KCl buffer solution (pH = 1.0) and the other with 0.4 M CH_3COONa buffer solution (pH = 4.5); *(iv)* measuring the absorbance of the two sample dilutions at 700 nm ($A_{700\ nm}$) and at the wavelength corresponding to maximum absorption ($A_{\lambda max}$); (v) calculating the absorbance of the diluted sample using Equation (1):

$$A = (A_{520\ nm} - A_{700\ nm})_{pH=1.0} - (A_{520\ nm} - A_{700\ nm})_{pH=4.5} \quad (1)$$

The TAC was determined as monomeric anthocyanin pigment (MAP) concentration (mg/L), and calculated by taking into consideration Equation (2):

$$MAP = \frac{A \cdot MW \cdot DF \cdot 1000}{\varepsilon \cdot l} \quad (2)$$

where A - diluted sample absorbance; MW - molecular mass, g/mol; DF - dilution factor; ε - molar absorptivity, L/(mol·cm); l - optical path length (1 cm). The method highlighted that, if the molar absorptivity for the primary pigment is not available, or if the composition of the sample is unknown, the pigment content should be calculated as cyanidin-3-glucoside, for which MW = 449.2 g/mol and ε = 26900 L/(mol·cm) [8,24].

2.5. Total flavonoids content

The aluminum chloride assay method was used for the determination of total flavonoids content (TFC). The method was described by Radulescu et al in previous research [8,11], and in brief, include several steps: *(i)* over 1 mL of extract sample, 4 mL of distilled water, and 0.3 mL of $NaNO_2$ in a concentration of 5% were added, then followed by stirring; *(ii)* after a 5-minute rest, over the mixture was added 0.3 mL of 10% $AlCl_3$, then followed by stirring and resting for another 5 minutes; *(iii)* finally, 2 mL of 1 M NaOH solution was added to the mixture, followed by stirring, then brought to 10 mL volume with distilled water. The blank sample was prepared in a similar manner, using 1 mL of distilled water in place of the extract sample. The absorbance of the sample was measured at 510 nm using an Evolution 260 Bio UV-Visible spectrophotometer (Thermo Fisher Scientific Inc., Madison, WI, USA). The TFC was then determined in triplicate, based on the calibration curve of quercetin (Q), and the results were expressed in quercetin equivalents (QE) as mg QE/mL.

2.6. Total polyphenols content

The total polyphenols content (TPC) was measured based on a standardized procedure [31], slightly improved according to Radulescu et al research [8;18]. The proposed method involved several steps: *(i)* into a brown-walled test tube over 0.5 mL of diluted extract sample, 2.5 mL of 10% Folin-Ciocalteu reagent were added, then the mixture was stirred for 8 minutes; *(ii)* over the initial mixture were added 2 mL of 8% Na_2CO_3, followed by stirring; *(iii)* after a 60-minute rest at room temperature, the absorbance was recorded at 765 nm. The TPC was determined based on gallic acid (GA) calibration curve, and the results were expressed in gallic acid equivalents (GAE) as mg GAE/mL extract.

2.7. DPPH radical scavenging capacity

The DPPH radical scavenging assay of extracts was achieved according to the method related by Shimamura et al. [32] in terms of inhibition concentration at 50% (IC_{50}) [33]. The method had several steps: *(i)* DPPH solution preparation described by Radulescu et al [8,18]; *(ii)* over 0.2 mL of the extract sample, 0.8 mL of Tris-

HCl buffer, and 1 mL of DPPH solution were added, then the mixture was stirred briefly and allowed to rest in the dark for 30 minutes. For each extract sample, five different concentrations were prepared using either simple or successive dilution methods, along with a control sample that consisted of 0.2 mL of ethanol, 0.8 mL of Tris-HCl buffer solution, and 1 mL of DPPH solution. *(iii)* for the sample (A_{sample}) and control sample ($A_{control}$), the absorbance was measured at 517 nm, using a blank (i.e., 1.2 mL of ethanol and 0.8 mL of Tris-HCl buffer). The Inhibition Ratio (IR% %) was calculated using Equation (3):

$$IR\ (\%) = \left[\left(A_{control} - A_{sample}\right)/A_{control}\right] \cdot 100 \quad (3)$$

The reference value required to reduce the initial DPPH absorbance by 50% was obtained by using a calibration curve of gallic acid (GA) (IC_{50}). DPPH radical scavenging capacity was indicated as IC_{50} in μg GAE/mL extract [8,11].

2.8. Statistical analysis

Numerous methods and tools for data analysis have been created for the statistical field. Data analysis, combined with detection and/or inference, is a crucial step in any statistical study. For this purpose, a number of statistical software programs are available, including IBM SPSS [34]. A scatterplot matrix, Pearson correlations, and cluster analysis were among the statistical methods used with IBM SPSS Statistics v. 26 (Armonk, NY: IBM Corp, USA). The obtained data (i.e., TCT, TAC, TFC, TPC, and IC_{50}) characterizing the extracts of pomace and Japanese knotweed (*Polygonum cuspidatum*) were subjected to further statistical evaluation.

3. Results and discussion

Flavonoids, anthocyanins, quercetin, tannins, organic acids, microelements, oligo-elements, lipids, and water-soluble vitamins from different natural products (cosmetics, dermatocosmetics, pharmaceuticals, fortifying foods) can enhance and support human health. In this regard, the goal of this study was to provide a comprehensive overview of the polyphenols and antioxidant activity found in flower extracts of *P. cuspidatum*, found in Romania, which are relatively under-researched in terms of their phytochemical composition and medical purposes. Hydroalcoholic extracts of grape pomace, *Polygonum cuspidatum* flower, and their mixture showed significant variations related to the content of condensed tannins, anthocyanins, flavonoids, and polyphenols (Table 2 and Figures 2-5), reflecting the antioxidant potential (Table 2 and Figure 6) and distinct phytochemical composition of each plant source.

Table 2. Phytochemical screening results of hydroalcoholic extracts.

Sample	*TCT [mg CE/mL]*	*TAC MAP* [μg/mL]*	*TFC [mg QE/mL]*	*TPC [mg GAE/mL]*	*IC_{50} [μg GAE/mL]*
N	0.884±0.029	13.387±0.337	1.937±0.067	0.797±0.023	228.60±0.52
N/PcF	3.472±0.076	11.912±0.293	6.769 ± 0.134	2.557±0.042	115.41±0.21
PcF	15.682±0.026	0.167±0.005	30.679±0.393	10.920±0.268	28.04±1.12

**Concentration of monomeric anthocyanin pigment (MAP)*
Note: Results are expressed as mean ± standard deviation (triplicate)

Notable differences are observed between the two types of extracts (grape pomace and *P. cuspidatum* flower) in the case of total anthocyanin content (13.387 ± 0.337 and 0.167 ± 0.005 MAP, μg/mL, respectively), where, being derived from grape skin, the pomace extract contains significant amounts of anthocyanins.

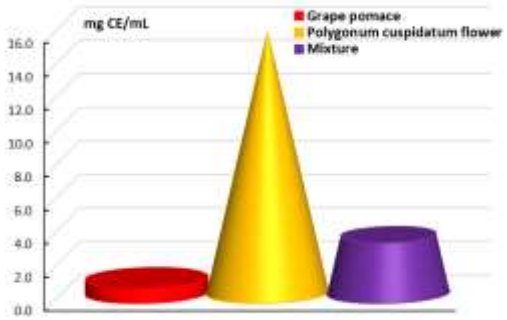

Fig. 2. The content of condensed tannins for extracts of pomace, *Polygonum cuspidatum* flower, and pomace-flower mixture.

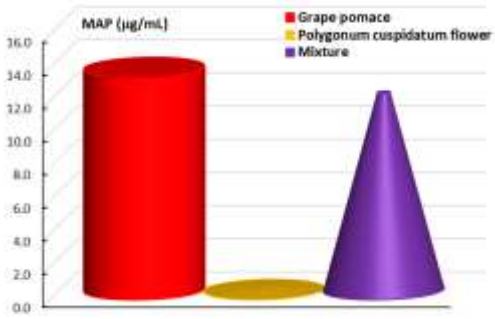

Fig. 3. Total anthocyanin content for extracts of pomace, *Polygonum cuspidatum* flower, and pomace-flower mixture.

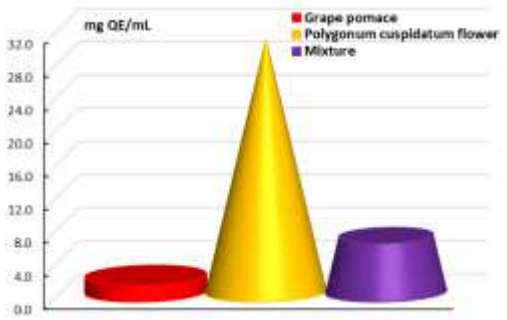

Fig. 4. Total flavonoids content for extracts of pomace, *Polygonum cuspidatum* flower, and pomace-flower mixture.

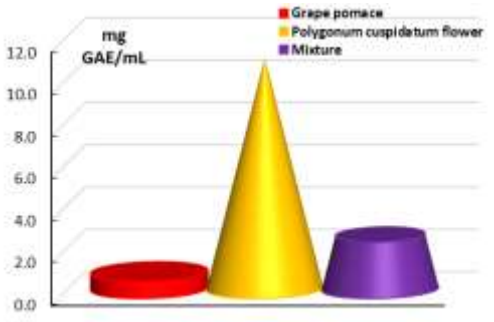

Fig. 5. Total polyphenols content for extracts of pomace, *Polygonum cuspidatum* flower, and pomace-flower mixture.

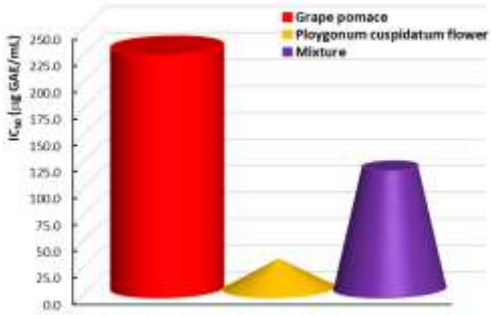

Fig. 6. Antioxidant activity for extracts of pomace, *Polygonum cuspidatum* flower, and pomace-flower mixture.

On the other hand, the *P. cuspidatum* extract, compared to the pomace extract, is superior in terms of condensed tannins content (15.682 ± 0.026 versus 0.884 ± 0.029 mg CE/mL), total flavonoid content (30.679 ± 0.393 versus 1.937 ± 0.067 mg QE/mL), and total polyphenol content (10.920 ± 0.268 versus 0.7974 ± 0.023 mg GAE/mL).

The combination of two extracts (grape pomace and *P. cuspidatum* flower) could lead to an increase in the values of the analyzed phytochemical parameters, through cumulative effects. However, the results indicate intermediate values for the extract resulting from mixing, but significantly higher compared to those of the grape pomace extract. This aspect could be attributed to: the dilution effect (the mixture of the two extracts with different concentrations leads to an averaging of the values); partial synergy or lack of synergy (there is the possibility that the compounds in the two extracts do not interact synergistically, but only add and if there is no mutual potentiation, the antioxidant activity will not exceed the individual values); complementary phytochemical composition and possible antagonistic interactions (in some cases, the compounds may interfere with each other, tannins may precipitate flavonoids or some polyphenols may inhibit the activity of others, these interactions may reduce the overall efficiency, keeping the values below the theoretical maximum level). Intermediate values of the mixture, in terms of condensed tannins, anthocyanins, flavonoids, polyphenols, and antioxidant activity, indicate an additive combination without strong synergy or major antagonism.

Scatterplot matrix analysis (Figure 7) allows the simultaneous evaluation of bivariate relationships between multiple variables, providing a compact graphical representation of all possible pairs. In the present study, scatterplot matrix analysis was employed to investigate the relationships between phytochemical parameters and the antioxidant activity of the analyzed samples.

The scatterplot matrix shown in Figure 7 highlights the bivariate relationships between the analyzed phytochemical parameters — total condensed tannins content (TCT), total anthocyanins content (TAC), total flavonoids content (TFC), and total polyphenols content (TPC) — as well as the antioxidant activity (IC_{50}). The investigated samples include grape pomace extract (N), Japanese knotweed flower extract (*Polygonum cuspidatum*, PcF), and a mixture of them (N/PcF). The distribution of values highlights a clear differentiation between the three types of extracts, reflecting significant variations in the polyphenolic profile and the associated antioxidant capacity. Overall, the values for the chemical parameters follow the trend N < N/PcF < PcF, suggesting a progressive increase in the concentrations of phenolic metabolites with the intake of P. cuspidatum extract.

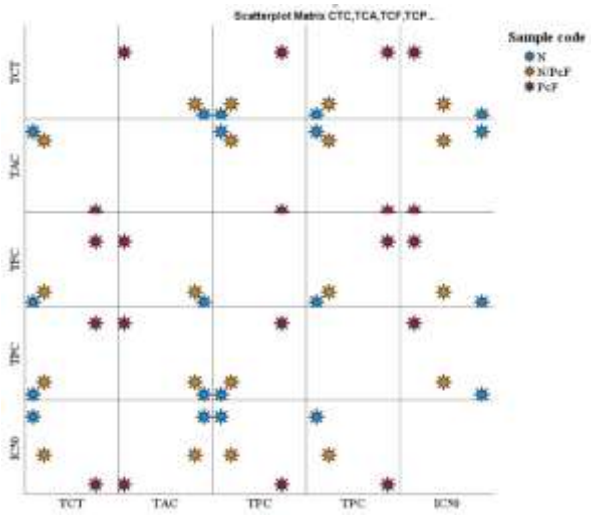

Fig. 7. Scatterplot matrix of CTC, TCA, TCF, TCP, and IC_{50} in grape pomace extracts.

This behavior is consistent with the literature [35,36], which indicates the presence of considerable amounts of resveratrol, emodin, and other bioactive polyphenols in this species, compounds that contribute substantially to the potentiation of antioxidant activity. The notable exception to this trend is observed for the IC_{50} parameter, where the order of values is reversed (N > N/PcF > PcF). Since IC_{50} is inversely proportional to the antioxidant activity (lower values correspond to a higher free radical scavenging capacity), this inversion confirms that the PcF extract exhibits the most pronounced antioxidant activity, followed by the N/PcF mixture, while the pomace extract (N) exhibits the lowest efficiency. The positive linear correlations identified between the phytochemical parameters (e.g., between TFC and TPC or between TPC and TCT) suggest a consistent co-variation between the different classes of compounds, indicating a synergistic behavior in the contribution to the total antioxidant activity. In conclusion, the graphical analysis highlights the

chemical complementarity between the pomace and *P. cuspidatum* extracts and underscores the potentiating effect of the N/PcF mixture. However, the PcF extract remains the most effective in terms of antioxidant activity, which can be attributed to the high density of polyphenolic compounds characterized by a high antioxidant activity. These results support the use of *P. cuspidatum* extract as a potential bioactive agent, either individually or in combination with extracts derived from viticulture by-products, for the development of synergistic antioxidant formulations.

The dendrogram presented in Figure 8 was achieved using the average linkage hierarchical clustering method, aiming to assess the degree of similarity between the analyzed variables (TCT, TAC, TFC, TPC, and IC_{50}). An illustration of the structural connections between the analyzed parameters is shown by the dendrogram's axes, which represent the rescaled distance at which the variables or groups of variables join.

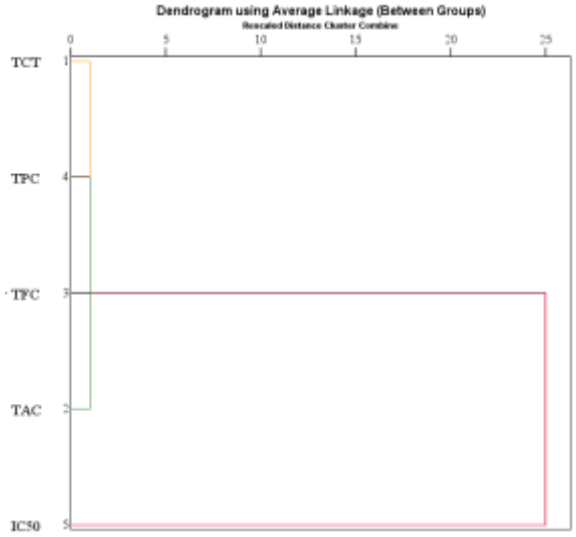

Fig. 8. Dendogram of variables of interest for the three analyzed samples (N/PcF, N, and PcF).

The variables in the first cluster, TCT, TPC, TAC, and TFC, are connected at comparatively low distances, suggesting a high degree of similarity between them. According to this group, there may be positive correlations between these phytochemical parameters and the corresponding structural characteristics of the examined samples, as well as intercorrelation between these parameters. Specifically, the proximity of TPC and TCT suggests a close link between these two parameters, which may result from similar structural or metabolic pathways. The IC_{50} variable represents the second cluster in isolation and is significantly further distant from the other variables. This unique placement indicates that the

IC_{50} variable, which characterizes inhibitory action, has a different nature. Therefore, IC_{50} acts as a parameter that depends on the variations of others, but is not sufficiently similar to be included in the same group. This hierarchical structure supports the observations from the scatterplot matrix, where the variables TCT, TAC, and TPC showed common trends and a potential ability to discriminate between sample types. The distinct separation of IC_{50} suggests that the chemical composition of the samples influences the antioxidant activity.

Pearson correlation analysis (Figure 9) revealed considerable correlations between the quantifiable phytochemical parameters (TCT, TAC, TFC, and TPC) and the antioxidant activity (IC_{50}). The correlation coefficients indicate a strong positive correlation between total anthocyanin content (TAC) and IC_{50} (r = 0.801), while the other phytochemical parameters (TFC, TPC) include strong negative correlations (r = –0.837 and r = –0.841, respectively).

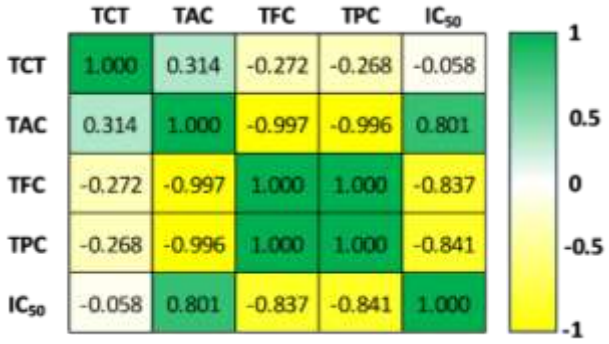

Fig. 9. Pearson's correlation coefficient matrix of multiple variables.

These negative relationships indicate that as total flavonoid and polyphenol content increase, the IC_{50} value decreases, reflecting a more intense antioxidant activity (since a lower IC_{50} value indicates a more efficient inhibition of free radicals). Consequently, it can be stated that total flavonoids and polyphenols contribute significantly to the antioxidant activity of the analyzed extracts. In contrast, the positive correlation between TAC and IC_{50} suggests that anthocyanins, although present at higher concentrations, may exert less effective antioxidant effects, possibly due to their structural instability at medium reaction pH or interactions with other compounds that may reduce their antioxidant efficiency. At the same time, the very high correlations between phytochemical parameters (r > 0.990 between TCP and TAC, respectively, TPC and TFC) confirm the coevolution and interdependence of these compounds, an aspect also highlighted by hierarchical clustering analysis (Figure 8).

The trend observed for the analyzed samples (N < N/PcF < PcF for phenolic contents, respectively N > N/PcF > PcF for IC_{50}) is in full agreement with the

correlation relationships obtained. Thus, the Japanese knotweed extract (PcF), the richest in phenolic compounds, also presented the lowest IC_{50} value, confirming a superior antioxidant capacity compared to the pomace extract (N) and their mixture (N/PcF). The Pearson correlation analysis indicates that the antioxidant activity of the studied extracts was primarily influenced by the flavonoid and polyphenol contents. In contrast, anthocyanin content appears to contribute to the overall antioxidant effect to a lesser or indirect extent.

Conclusions

Natural active ingredients act as an effective adjuvant, exhibiting important biorevitalization, protection, and trophic support activities, and also demonstrate significant pharmacological activity. This study highlights the phytochemical profile of NOVA red grape pomace and *P. cuspidatum* flower extracts, as well as their mixture in terms of key components.

While red grape pomace extract of the NOVA hybrid variety is rich in antioxidant substances, the new extract formulation, which included new naturally active compounds of *P. cuspidatum* flower extract, suggested future research directions for mixture extracts to develop new attractive natural formulations. Nevertheless, additional studies are required to clarify and support their potential therapeutic applications.

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