TOTAL PHENOLIC CONTENT CORRELATED WITH ANTIOXIDANT ACTIVITY OF SOME GRAPE POMACE BIOMASS HYDROALCOHOLIC EXTRACTS, WHITE AND RED VARIETIES

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The aim of this paper was to evaluate the total phenolic content and comparative antioxidant activity by three methods, photo chemiluminescence FRAP, DPPH of some grape pomace polyphenolic extracts from white and red varieties of Vitif vinifera (L)., obtained by maceration for 12 days, at room temperature in 40%, 70% aqueous ethanol solution or ethanol. The grape pomace was obtained after the secondary winemaking process of five grape varieties Vitis vinifera (L.) for white wines, Sauvignon Blanc, Muscat Ottonel, and red wines, Feteasca Neagra, Cabernet Sauvignon and Mamaia, created at Research Station for Viticulture and Oenology of Murfatlar, Constanta County, Romania. The results emphasize that an increased phenolic content and antioxidant capacity were obtained for the white varieties, Muscat Ottonel and for the red ones, Cabernet Sauvignon. Regarding the extraction efficiency, for all analyzed grape pomace 70% hydroalcoholic extracts present an increased activity. Based on our preliminary results regarding the radical scavenger activity of grape marc hydroalcoholic extracts, we can consider these by product as a promising resource for dermato-cosmetics with anti-aging effect.

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1. Introduction

World wine industry transforms 10% - 25% of grapes into residues, mainly consisted of lees, seeds and stems. These are a rich source of polyphenols and therefore, they can be used to produce new products [1]. Grape pomace results as the main by product in wine industry, obtained either by pressing white grapes to obtain the wine or by pressing the fermented pomace from red grapes. Red wines ferment in contact with grape skins to extract polyphenols and anthocyanins helps achieving and stabilizing the color. Due to its high-water content of 55 - 75%, the chemical and microbiological stability is low, such as its use is limited. The complex valorization of grape pomace as an important source of bioactive phytocompounds with applications in the pharmaceutical, cosmetic and food industries represents an efficient, profitable, and ecological alternative [2, 3]. Grape pomace is rich in polyphenols, which exhibit antioxidant properties, including resveratrol, procyanidins/condensed tannins, anthocyanins, flavanols, and catechins [4]. For instance, it was reported a total phenolic contents (TPC) of grape pomace ranged from 0.12 to 7.48 g gallic acid equivalents/100 g dry matter (DM), while the antioxidant activity was in the range of 20-75 mM Trolox equivalents [5]. Phenolic compounds are mostly distributed in grape seeds (60%–70%), skin (28%–35%), and pulp (< 10%) [6]. Flavonoids are the most abundant and studied compounds, for their biological properties, including antioxidant, anti-inflammatory, anticarcinogenic, antimicrobial, antiviral, cardioprotective, neuroprotective and hepatoprotective activities [7]. Grape pomace extracts can be applied in food, pharmaceutical, cosmetic, and other industries in the form of liquid extracts, or powder [8]. Grape pomace extracts were used to prevent lipid oxidation in fish-based products [9], meat products [10], and as an antimicrobial agent against *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter coli*, *Escherichia coli O157:H7* and *Salmonella infantis* strains [11]. In the food industry, grape pomace extracts can be used as a functional supplement to enrich beverages and as an ingredient of an osmotic solution to obtain dehydrated fruits with enhanced phenolic content [12 - 15].

The extraction of bioactive compounds depends on several factors, such as the extraction parameters, raw materials and the solvent [16, 17]. Conventional extraction requires the use of organic solvents, temperature and stirring. Examples of this type of technique include Soxhlet extraction, maceration and hydrodistillation. Modern techniques, or non-conventional extraction, are green or clean processes because require low energy consumption or not need organic solvent [18 - 20]. Because of concern about the use of synthetic preservatives (e.g. parabens), colorants, aromas, and stabilizers that can cause allergies, skin irritations and health

risks from long-term exposure, cosmetic formulations based on natural compounds are desired. Polyphenols from grape pomace due to their radical scavenging activity could be an alternative, new cost-effective source for pharmaceutical and cosmetics industries to manufacture products with tissue regeneration capacity and anti-aging properties [21, 22].

2. Experimental

2.1. Materials

Five *Vitis vinifera* (L.) grape marc varieties were used in this study, three red varieties (Feteasca Neagra, Cabernet Sauvignon, Mamaia) and two white varieties (Muscat Ottonel and Sauvignon Blanc), collected from the experimental field of the Murfatlar Research Station for Viticulture and Oenology, Constanta County, Romania. All grapes were harvested at technological maturity, in 2018 year. The winemaking technology applied to the grapes as raw material was the classic one, consisting in crushing and destemming. In the case of red wine varieties, the maceration took place in static stainless-steel vessels for 5 days, followed by pressing (using a classic manual press), the resulted pomace being recovered and conditioned by drying at room temperature. Every 24 hours, the grape pomace was mechanically aerated in order to facilitate the evaporation of water and thus preventing the development of bacteria and fungi.

2.2. Obtaining of hydroalcoholic extracts

The extracts from grape pomace varieties were obtained from 1 g of dry vegetable material placed into a volumetric flask (100 mL) and 40% or 70% aqueous ethanolic solution was added up to the mark. Extraction was carried out for 12 days, at room temperature, in dark conditions, and the flasks were periodically shaken. The extracts were filtered off and they have clear aspect, with specific odor and color depending on the grape pomace variety. Extract samples named stock solutions were storage at +4 °C to prevent degradation of compounds with antioxidant potential. Aliquots of 10 μL from extracts were used for different measurements, [23 - 26].

2.3. Determination of total phenolic content

The total phenolic content (TPC) of extracts was determined using Folin-Ciocalteu reagent and gallic acid as standard compound. The extract (0.1 mL) was mixed with 46 mL distilled water and 1 mL Folin-Ciocalteu reagent; the solution was thoroughly shaken. After 3 min, 3 mL aqueous solution of 2% Na_2CO_3 was added and the samples were incubated 2 hours, at room temperature. The absorbance of each solution was measured at 765 nm using UV-Vis spectrophotometer Jasco V-630 (Deutschland GmbH) and TPC values were determined based on the calibration curve of gallic acid, y=11.47+281.15*x (R^2=0.9999) [27 - 31].

2.4 Determination of antioxidant activity using the photochemiluminescence method

ACL (Antioxidative Capacity in Lipid Soluble Substances) procedure developed by Analytik Jena AG using Photochem apparatus for the total antioxidant capacity determination of hydroalcoholic extracts, was applied. All measurements were made in triplicate and the values are given as average ones and expressed as Trolox® equivalents (nmol Trolox/μL). Before the measurement, each sample was rapidly homogenized using a Vortex mixer (Velp Scientifica, Italy) and 10 μL aliquot from the supernatant were withdrawn. Each determination lasted 120 sec., [21 - 26]. In the case of antioxidant activity determination through photo-chemiluminescence method (Analytik Jena AG, Germany), further dilutions of stock solutions were performed when was necessary, to reach calibration range of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) used as standard compound, $1/y=0.65+3.36*1/x$ ($R^2=0.9848$).

2.5. Determination of antioxidant activity using the Ferric Reducing Antioxidant Power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay has been performed according to Benzie & Strain, and Benzie & Szeto [32, 33]. FRAP reagent was prepared by mixing 2.5 mL 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 2.5 mL of 300 mM acetate buffer (*p*H = 3.6) and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$. The FRAP reagent was added to 1 mL of each extract and the solution was homogenised. Blank reagent was prepared by adding 1 mL water instead of the extract. The absorbance solution of the samples and the reagent blank have been measured after 4 min, at λ=593 nm. The antioxidant activity of the samples was calculated based on a calibration curve for gallic acid, $y = 297.03x$, in the range of 0.8–16.6 mM ($R^2 = 0.9953$).

2.6. Determination of Antioxidant Activity using the DPPH radical scavenging assay

The radical scavenging activity of vegetal samples has determined by DPPH (1,1 diphenyl-2-picryl hydrazyl) method according to Brand-Williams et al. [34] with minor changes. Every extract sample (0.1 mL) was added to 2.9 mL of DPPH solution. After the solution stands in dark conditions for 60 min., its absorbance was measured at λ=517 nm. A calibration curve for gallic acid was plotted against the percentage of DPPH radical scavenging activity, $y=2.4024x+0.8899$, in 0.16 - 3.32 mM range ($R^2=0.9913$). The results have been expressed as mg gallic acid / L extract.

3. Results and discussion

3.1. Total phenolic content determination

The obtained hydroalcoholic extracts from grape pomace were analyzed spectrophotometrically in order to quantify the total phenolic compounds, the results being presented in Fig. 1. By analyzing the experimental data, it can be observed that in the case of unfermented grape pomace (varieties for white wines, Sauvignon Blanc and Muscat Ottonel), the amount of total phenolic compounds showed values that ranged from 320.4 to 485.7 mg/L, while for the extracts obtained from fermented pomace (varieties for red wines), the values were in the range of 330.1-531.2 mg/L, values relatively close to those obtained in the case of white varieties. The highest values were noticed for Cabernet Sauvignon (516.9 - 531.2 mg/L), followed by Feteasca Neagra (381.1 - 428.3 mg/L) and Mamaia (330.5 - 348.2 mg/L), while for white pomace varieties, Muscat Ottonel (475.6 - 485.7 mg/L), followed by Sauvignon Blanc (320.4- 359.2 mg/L). Noteworthy is the high TPC values of Cabernet Sauvignon extracts, 34.94 % and 12.22 % higher than in the case of 70 % ethanolic extract and 40% ethanolic extract, respectively, from Feteasca Neagra grape pomace. Among white grape pomace, from Muscat Ottonel variety were obtained the richiest in phenolic compound extracts, though the TPC values are lower than for the Cabernet Sauvignon extracts. Regarding the influence of the concentration of ethanolic aqueous solution (70% and 40%) on the extraction process, it can be concluded that 70% content of ethanol fovored the recovery of phenolic compounds. Ethanolic extracts yielded much higher phenolic content than methanolic extracts values found in literature data, for red varieties Feteasca Neagra and Cabernet Sauvignon (40 - 80 mg/L GAE) [35]. The assessment of total phenolic content is one of the most commonly used method to quantify the phenolic content of a plant extract. The high sensitivity, reproducibility and convenience of this assay have made it popular in routine screening of natural extracts. The antioxidant activity of extracts is also very important feature, which should correlate with TPC value.

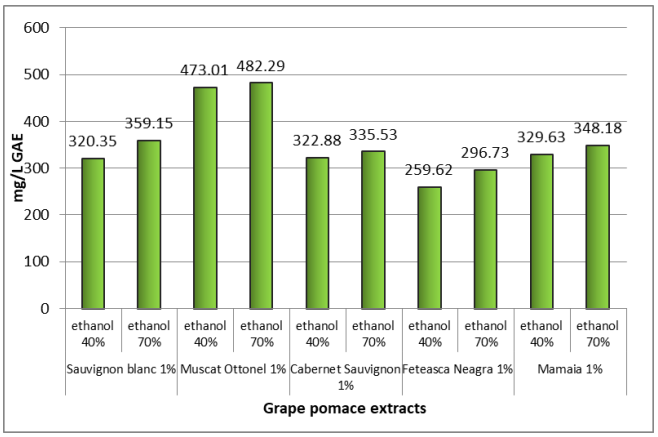

Fig. 1. Total phenolic content of prepared hydroalcoholic extracts (mg/L GAE)

3.2. Antioxidant activity by photochemiluminescence method

Our results emphasize an increased total antioxidant capacity (TEAC) (Fig. 2) for the extracts obtained from red grape pomace varieties, Cabernet Sauvignon (1.742 - 1.795 nmol eq. Trolox/μL) and Feteasca Neagra (1.703 - 1.758 nmol eq. Trolox/μL) having very close values, followed by the white varieties, Muscat Ottonel (1.638 - 1.676 nmol eq. Trolox/μL) and Sauvignon Blanc (1.623 - 1.645 nmol eq. Trolox/μL). As regard the solvent used for extraction, for all analyzed grape marc 70% ethanolic extracts presented higher radical scavenger activity than 40% ethanolic extracts. Based on the antioxidant activity results determined through photo-chemiluminescence method, showed that grape marc is a promising source of biocomponents for anti-aging dermato-cosmetics. It was observed that the samples macerated in 70% ethanolic aqueous solution led to extracts with higher total radical scavenging capacity than maceration in 40% ethanolic aqueous solution for all grape pomace varities.

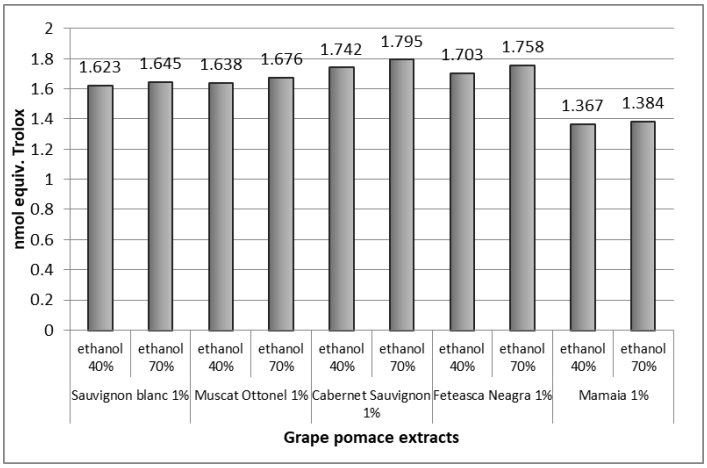

Fig. 2. Total antioxidant capacity (TEAC), nmol eq. Trolox®/μL.

3.3. Antioxidant activity by FRAP assay

The antioxidant activity determined by FRAP assay are presented in Fig.3. One can notice that the highest radical scavenger capacity values were obtained for the extracts from red Feteasca Neagra grape pomace (0.576 mg GAE /L and 1.233 mg GAE/L for 40% ethanolic extract and 70% ethanolic extract, respectively), followed by Cabernet Sauvignon (0.421 mg GAE/L extract and 0.046 mg GAE/L for 40% ethanolic extract and 70% ethanolic extract, respectively). For most of the grape marc varieties, 70% ethanolic extracts showed higher FRAP values than 40% ethanolic ones, except the extracts from Cabernet Sauvignon grape pomace (0.421 mg GAE/L for the 40% ethanolic extract), which was higher than for 70% ethanolic extract (0.046 mg GAE/L) (Fig. 3).

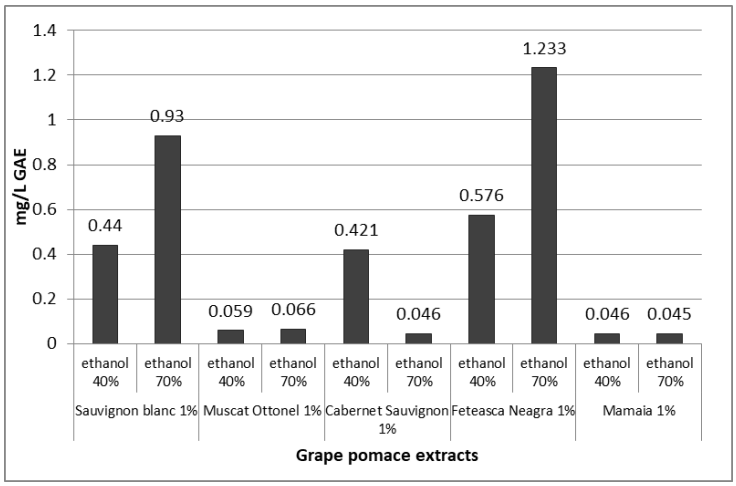

Fig. 3. Radical scavenger activity obtained by FRAP assay, expressed in mg GAE /L extract (GAE – gallic acid equivalents)

3.4. Antioxidant activity by DPPH assay

In figure 4 are presented the values obtained for radical scavenger activity for prepared extracts determined by DPPH assay. As can be seen, the highest antioxidant capacity value was determined for 70% ethanolic extract from Feteasca Neagra (17.81 mg GAE/L), followed by 70% ethanolic extract from Sauvignon Blanc (15.75 mg GAE/L) and 70% ethanolic extract from Muscat Ottonel (14.88 mg GAE/L). For all grape pomace varieties, higher radical scavenging activity values were obtained for 70% ethanolic extracts than for 40% ethanolic ones (Fig. 4).

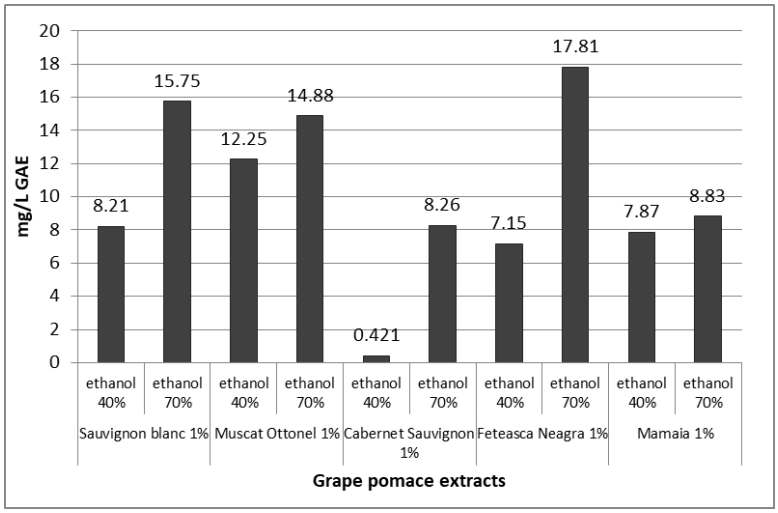

Fig. 4. Radical scavenging activity determined DPPH assay, mg GAE /L extract

One can observe that in the case of Feteasca Neagra extracts the highest antioxidant activity values determined by two of three methods, while in the case of

white grape pomace varieties, from Sauvignon blanc were prepared polyphenolic extracts with the strongest antioxidant capacity as photo-chemiluminescence and FRAP methods showed.

All three methods used for antioxidant activity assessment act based on a similar mechanism, which explains the correlation observed between the results of assays. Ideally, the evaluation of the antioxidant activity should be made by several assays, encompassing the behaviour of extract components towards several reactive oxygen species. The Pearson correlation coefficient applied to the set of data reflecting the total phenolic content of five types of wine submitted to our study and the antioxidant activity measured by three different methods: (TEAC) Determination of antioxidant activity using the photo-chemiluminescence method, Ferric Reducing Antioxidant Power (FRAP) assay, and DPPH (1,1 diphenyl-2-picryl hydrazyl) method. According to the correlation coefficient applied to the set of data measured on the extract samples of red and white wine with 40% ethanol content, the results shows a high positive correlation (r=0.694641) between the FRAP and TEAC measurement of antioxidant activity, meanwhile the TPC is in a high negative correlation with the antioxidant activity of the samples measured by FRAP method. The smallest linear correlation is shown between TCP and TEAC method either in 40% ethanol samples (Table 1), as well as for 70% ethanol content samples (Table 2).

Table 1.

Pearson Correlation Coefficient applied to the extracts from grape pomace varieties with 40% ethanol content, established between the TPC and antioxidant activity of the samples measured by three different laboratory methods.

	TPC	*TEAC*	*FRAP*	*DPPH*
TPC	1			
TEAC	-0.08147	1		
FRAP	-0.74149	0.694641	1	
DPPH	0.567386	-0.36236	-0.50473	1

Table 2.

Pearson Correlation Coefficient applied to the extracts from grape pomace varieties with 70% ethanol content, established between the TPC and antioxidant activity of the samples measured by three different laboratory methods.

	TPC	*TEAC*	*FRAP*	*DPPH*
TPC	1			
TEAC	-0.09049	1		
FRAP	-0.51369	0.327296	1	
DPPH	0.071554	0.351395	0.806141	1

According to the correlation coefficient applied to the set of data measured on the extract samples of red and white wine with 70% ethanol content, the results

shows a high enough positive correlation (r=0.806141) between the FRAP and DPPH measurement of antioxidant activity.

4. Conclusions

This study underlines that the grape pomace as by product resulted from winemaking process is an important source of phenolic compounds that depends on the variety and solvent. Among studied grape pomace varieties, the best results in term of total antioxidant capacity were obtained for Cabernet Sauvignon from red grape pomace varieties and Muscat Ottonel from white one determined through photo-chemilunimescence method, and in the case of Feteasca Neagra and Sauvignon Blanc when FRAP and DPPH assays were used.

Regarding the prepared polyphenolic extracts from grape pomace, cold maceration in 70% aqueous ethanol for 12 days, in dark conditions, at room temperature, led to the preparation of extracts with higher radical scavenger capacity than maceration in 40% aqueous ethanolic solution in the case of all grape pomace varieties.

According to the correlation results, the antioxidant activity of the extract of grape pomace varieties is proportional with the total phenolic content of the investigated samples in an average ratio of 0.625.

These preliminary results of antioxidant activity of grape marc hydroalcoholic extracts show that grape pomace varieties represent a promising source of phenolic compounds with good radical scavenger capacity, which can be valorised in pharmaceutical preparations or cosmetics industry.

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