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# EXPLORING THE ANTIOXIDANT ACTIVITY OF BLEEDING SAP FROM VARIOUS GRAPE VARIETIES

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**Abstract:** This investigation systematically evaluated the total phenolic content (TPC) and antioxidant properties of bleeding sap obtained from six grape cultivars—Evita, Cabernet Sauvignon, Prokupac, Smederevka, Muscat Hamburg, and Othello. The study employed quantitative assays to measure TPC, alongside three complementary methods (DPPH, ABTS, and CUPRAC) to determine the antioxidant capacity of the samples. The bleeding sap exhibited a wide range of phenolic concentrations, with TPC values spanning from 5.03 to 359.38 μg GAE/mL, suggesting significant variability among the cultivars. Antioxidant activity, as assessed by the DPPH assay, ranged between 83.68 and 96.29 μg TE/mL, while the ABTS and CUPRAC assays revealed activities from 13.07 to 37.48 μg TE/mL and 60.78 to 121.94 μg TE/mL, respectively. These findings underscore the potential of grape bleeding sap as a natural source of antioxidants and provide a scientific foundation for future studies aimed at exploring its application in food and pharmaceutical industries.

**Keywords**: bleeding sap, total phenolc, antioxidant activity

## Introduction

With a long history of cultivation, consumption, and trade, viticulture in Serbia is an important branch of agriculture with significant economic value. Most grape cultivation and production is carried out by local wineries operating as small family businesses. The beneficial effects of consuming grapes and wine on human health are well documented. Additionally, there is considerable interest in other edible parts of the *Vitis vinifera* plant, as they are regarded as having high nutritional value. Potential bioactivities and medicinal properties have been attributed to all plant parts, but especially to pomace,

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shoots, stems, and leaves, which are used in the formulation of dietary antioxidant supplements (Handoussa et al., 2013).

When the vines are pruned in the spring, the cut branches exude sap, known as grape bleeding sap (Le et al., 2017). Grape bleeding sap contains many nutrients, including calcium, potassium, glutamic acid, as well as other components such as kinins and polyphenols. It is commonly used in traditional medicine as a tonic and more generally to promote health and prevent aging (Tohit et al., 2009), which may be related to its radical scavenging properties.

The main objective of this study was to determine the polyphenolic content and antioxidant capacity of bleeding sap of grape of different grape varieties and to compare the antioxidant capacity of these samples applying most widely used spectrophotometric methods: DPPH, ABTS, CUPRAC and to estimate correlation of antioxidant capacities with total phenolics.

## Materials and methods

### Materials

GBS was collected from vineyards and grape fields in Zemun in April 2024. The source plants were identified as *Vitis vinifera* L.

### Chemicals and Reagents

Gallic acid, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich. Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) were obtained from Fluka. Copper (II) chloride, potassium persulfate, and sodium carbonate were acquired from Merck. All solvents and reagents used in this study were of HPLC or analytical grade.

### Determination of Total Phenolics Content

The spectrophotometric determination of total phenolic content (TPC) was performed using the Folin-Ciocalteu method adapted for wine analysis (Matthaus, 2002), with gallic acid as the standard. This method is based on the reduction of a phosphotungstenphophomolybdate complex by phenolics to yield blue reaction products.

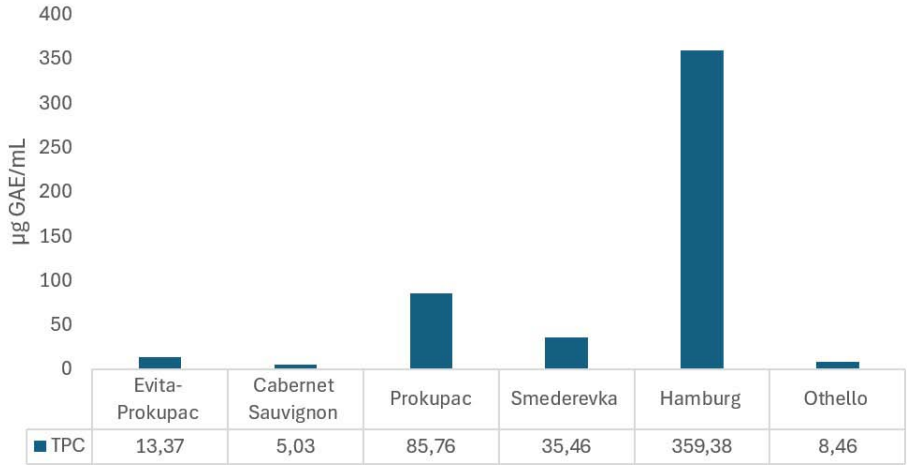
**Antioxidant Activity Assays**

The antioxidant capacities of grape bleeding sap were evaluated using three assays: DPPH radical scavenging [Brand-Williams et al., 1995], ABTS radical cation scavenging [Re et al., 1999], and the cupric ion reducing antioxidant capacity (CUPRAC) assay [Apak et al., 2006]. The antioxidant activities determined from all assays were expressed as μg of Trolox equivalent (TE) per mL of fresh grape bleeding sap.

## Results and discussion

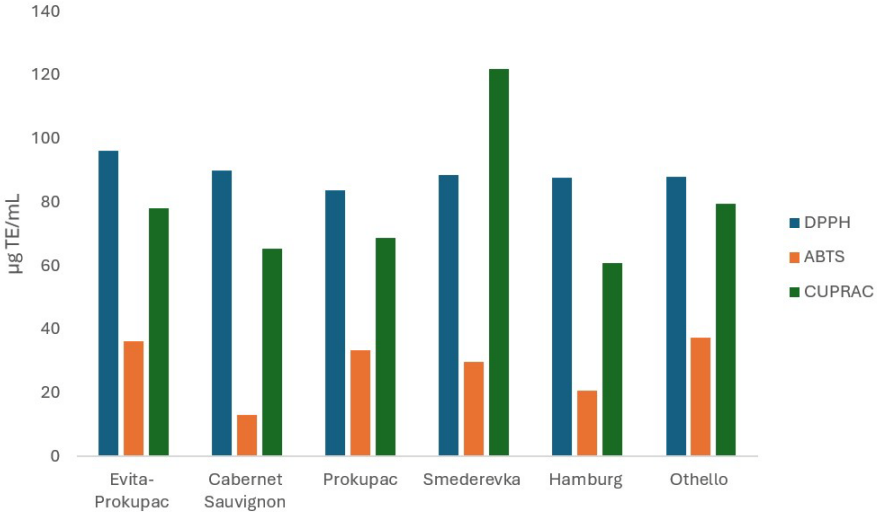
The total phenolic content (TPC) of grape bleeding sap from different varieties is presented in Figure 1. TPC values of the bleeding sap from the investigated varieties ranged from 5.03 μg GAE/mL (Cabernet Sauvignon) to 359.38 μg GAE/mL (Muscat Hamburg). Le et al. (2017) reported that the phenolic content of Xinjiang (China) grapevine bleeding sap ranges from 2.6 to 6.6 μg/mL. The significant variability in the total phenolic content of grape bleeding sap from different countries is likely attributable to factors such as grape variety, vineyard location, climate, and soil type.

Figure 1. Total phenolic contents of bleeding sap of grape samples



For individual grape varieties, the TPC of the bleeding sap decreased in the order: Muscat Hamburg > Prokupac > Smederevka > Evita > Othello > Cabernet Sauvignon. In contrast, the total phenolic content of white wines is significantly lower than that of red wines (Tekos et al., 2021). Li et al. (2009) reported TPC values of 2268 mg GAE/L for Cabernet Sauvignon red wine and 1086 mg GAE/L for Muscat Hamburg rosé wine. The free radical-scavenging activity of the bleeding sap from different grape varieties was determined using the DPPH and ABTS assays, as shown in Table 1 and Fig. 2. The $ABTS^{\bullet+}$ and DPPH radicals are among the most widely used and stable chromogenic reagents for measuring antioxidant activity in biological materials. High antioxidant activity indicates that the samples act as hydrogen donors, thereby terminating the oxidation process by converting free radicals into stable forms.

Figure 2. Antioxidant activity of bleeding sap of grape samples




For DPPH, the values ranged from 83.68 to 96.29 µg TE/mL. In individual grape varieties, the DPPH values of the bleeding sap decreased in the following order: Evita > Cabernet Sauvignon > Smederevka > Othello > Muscat Hamburg > Prokupac.

For ABTS, the values ranged from 13.07 to 37.07 µg TE/mL. In individual grape varieties, the ABTS values of the bleeding sap decreased in the following

order: Othello > Evita > Prokupac > Smederevka > Muscat Hamburg > Cabernet Sauvignon.

In the present study, we used the CUPRAC assay, which is based on the reduction of Cu(II) to Cu(I) by antioxidants. All analyzed samples of grape bleeding sap demonstrated significant antioxidant capacity in the CUPRAC test (Table 1, Fig. 2). The mean CUPRAC value of the bleeding sap was 79.10 μg TE/mL. In individual grape varieties, the CUPRAC values of the bleeding sap decreased in the following order: Smederevka > Othello > Evita > Prokupac > Cabernet Sauvignon > Muscat Hamburg.

Additionally, contrary to the results for wine, there was no correlation between the polyphenol content of grape bleeding sap and its antioxidant activity. There are clear differences in the total phenolic content and the antioxidant effect of grape bleeding sap from different origins.

The benefits of grapes, wine, and grape bleeding sap are often attributed to their polyphenol content and antioxidant activity. However, this study differentiates between the polyphenol content of grape bleeding sap and its antioxidant activity. Clearly, antioxidant activity is not limited to polyphenols, as has also been found with other grape-derived products. We confirm the need to specify the origin and composition of the plant extract when describing its effects.

## Conclusion

Our results indicate that grape bleeding sap has high total phenolic content and strong antioxidant properties. However, further in vivo studies and clinical evaluations are required to validate these findings and explore potential applications in various industries.

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