

Antioxidant and Lipoygenase Inhibition Activities of Ultrasound-Assisted Extracts from Prokupac Grape Skins

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Abstract: The antioxidant and anti-inflammatory properties of grape skin extracts derived from the indigenous Serbian grape variety *Prokupac*, using ultrasound-assisted extraction (UAE), are showed in this contribution. Extraction was performed with 50% ethanol, absolute ethanol, and ethyl acetate, yielding values between 4.9% and 9.8%. The highest total phenolic content (TPC) was obtained for extracts in 50% ethanol without prior maceration - PSE0 (22.4 mg GAE/g GSE), followed by ethyl acetate - PSE3 (9.8 mg GAE/g GSE), while ethanol-based extracts contained markedly lower phenolic levels. Antioxidant activity, assessed by DPPH, ABTS, and FRAP assays, confirmed that PSE0 exhibited the strongest radical scavenging ability (SC50 = 0.3 µg/mL), comparable to standard antioxidants, whereas PSE3 also showed significant activity. Anti-inflammatory activity, evaluated through lipoygenase (LOX) inhibition, demonstrated that PSE0 (IC50 = 7.9 µg/mL) outperformed quercetin and approached NDGA, the reference inhibitor. These results highlight grape skins, a winemaking by-product, as a sustainable source of bioactive compounds with potential functional food and nutraceutical applications.

Keywords: Prokupac grape-skin extracts, antioxidant activity, DPPH, LOX inhibition activity.

1. Introduction

The wine industry produces significant amounts of waste, primarily composed of grape skins and seeds, which represent a valuable source of biologically active compounds [1]. Among these, grape skins are particularly rich in polyphenols and flavonoids with potent antioxidant, antimicrobial, and anticancer properties. Modern extraction techniques, such as ultrasound- and microwave-assisted methods, have been developed to improve the recovery of these compounds while reducing degradation during processing. The Šumadija region of Serbia, with its favorable climate and viticultural traditions, yields grape varieties especially abundant in anthocyanins and other phenolic constituents [2]. Extracts derived from these grapes show promising

health benefits, including protection against oxidative stress and inflammation. Consequently, grape skins stand out as a sustainable raw material for functional food and pharmaceutical applications [1,2]. The results of antioxidant and LOX-inhibitory activities of Prokupac grape skin extracts, previously published in our recent study [2], are summarized in this contribution.

2. Methodology

2.1 Samples of grape skins and ultrasound-assisted Extraction

Grape skin samples were obtained as by-products of rosé wine production from the Prokupac variety at Miletić Vineyards and Winery in the Šumadija region of Serbia. This autochthonous grape is known for its thick skins, high sugar content, and richness in phenolic compounds, which makes it particularly suitable for extraction. After harvesting, the skins were washed thoroughly with distilled water, dried at ambient temperature in a dark, ventilated room for seven days, and then stored in airtight, light-protected containers at 5 °C until further analysis. Ultrasound-assisted extraction was performed by dissolving 20 g of dried skin in 200 mL of three solvents: 50% ethanol (with and without maceration, PSE1 and PSE0), absolute ethanol (PSE2), and ethyl acetate (PSE3). The extraction was carried out as it was described earlier [2].

2.2 Determination of Total Phenolic Content, In Vitro Antioxidant Potential, and LOX inhibition activity of grape skin extracts

The total phenolic content (TPC) of extracts was determined using the Folin-Ciocalteu method, with gallic acid as the standard, and results were expressed as milligrams of gallic acid equivalents per gram of dry extract. Antioxidant potential was evaluated by two widely used assays, DPPH and ABTS radical scavenging, which are based on the reduction of stable free radicals and allow comparison with standard antioxidants such as quercetin and ascorbic acid. The Ferric Reducing Antioxidant (FRAP) Assay was used to determine the reducing power of samples in the system containing Fe^{3+} ions. The anti-inflammatory potential of extracts was tested through lipoxygenase (LOX) inhibition assay, where extracts dissolved in DMSO were incubated with soybean LOX enzyme and sodium linoleate substrate, and enzymatic activity was monitored at 234 nm. Results were expressed as IC_{50} values, indicating the concentration required to inhibit 50% of LOX activity, with nordihydroguaiaretic acid (NDGA) as the positive control. All assays were performed in triplicate.

3. Results

The ultrasound-assisted extraction (UAE) of bioactive phenolic compounds from the indigenous Prokupac grape variety demonstrated that solvent polarity plays a crucial role in extraction efficiency and composition. Extraction yields varied between 4.9% and

9.8%, with the highest values obtained using 50% ethanol (PSE0 and PSE1), while ethyl acetate (PSE3) gave the lowest yield. Importantly, yield alone did not reflect chemical composition. When total phenolic content (TPC) was measured, PSE0 exhibited the highest concentration, reaching 22.4 mg GAE/g GSE, more than double that of PSE3 (9.8 mg GAE/g GSE) and several times greater than ethanol-based extracts (3.6 and 2.2 mg GAE/g GSE). These results show that hydroalcoholic mixtures improve bulk recovery, but careful solvent selection is essential for maximizing bioactive compound preservation.

The antioxidant activity of the extracts was evaluated using DPPH, ABTS, and FRAP assays, which together provide a broad assessment of radical scavenging and reducing capacity (Table 1). PSE0 proved to be the most effective antioxidant, with remarkably low SC₅₀ values of 0.3 µg/mL in both DPPH and ABTS assays, demonstrating a potency comparable to quercetin and NDGA, the positive control. PSE3 also displayed strong antioxidant activity (SC₅₀ values of 8.3 µg/mL for DPPH and 8.8 µg/mL for ABTS), correlating with its moderate phenolic content and the selectivity of ethyl acetate for polyphenols with high radical-scavenging ability. In contrast, PSE1 and PSE2 exhibited much weaker activity, with SC₅₀ values of 41.9 µg/mL and 99.4 µg/mL, respectively. The FRAP assay confirmed these findings, where PSE0 had the highest reducing capacity (A₇₀₀ = 0.2624), followed by PSE3 (0.1636), while ethanol-based extracts showed substantially lower values.

Table 1. Yield, antioxidant, anti-inflammatory activity, and total phenolic content of investigated grape skin extracts and standards.

Investigated extract and standards	DPPH SC ₅₀ (µg/mL)	ABTS SC ₅₀ (µg/mL)	TPC (mg GAE/g GSE)	FRAP (A _{700nm})	LOX IC ₅₀ (µg/mL)
PSE1	41.9 ± 0.8	32.5 ± 0.6	3.6 ± 0.1	0.0744 ± 0.0035	354.9 ± 0.3
PSE2	99.4 ± 0.4	60.7 ± 0.3	2.2 ± 0.1	0.0579 ± 0.0049	>500
PSE3	8.3 ± 0.2	8.8 ± 0.4	9.8 ± 0.5	0.1636 ± 0.0052	64.7 ± 0.1
PSE0	0.3 ± 0.1	0.3 ± 0.1	22.4 ± 0.1	0.2624 ± 0.0039	7.9 ± 0.1
Trolox	ND	1.3 ± 0.1	ND	ND	ND
NDGA	0.5 ± 0.1	ND	ND	ND	5.2 ± 0.1
Quercetin	0.6 ± 0.1	ND	ND	ND	13.1 ± 0.5
Ascorbic acid	18.0 ± 0.1	31.1 ± 0.1	ND	0.1249 ± 0.0022	ND

Statistical analysis by one-way ANOVA indicated no significant differences among the antioxidant results from DPPH, ABTS, and FRAP (p-values between 0.80 and 0.66), confirming consistency across methods. All assays ranked antioxidant potency in the order: PSE0 > PSE3 > PSE1 > PSE2. Taken together, the results confirm PSE0 and PSE3 as extracts containing the most biologically relevant antioxidants.

The extracts were further investigated for anti-inflammatory activity by assessing lipoxygenase (LOX) inhibition, a key pathway in the biosynthesis of pro-inflammatory leukotrienes. The LOX inhibition assay revealed pronounced differences among the

extracts. PSE0 showed the most potent effect, with an IC₅₀ of 7.9 µg/mL, surpassing quercetin (13.1 µg/mL) and only slightly weaker than NDGA (5.2 µg/mL), the reference inhibitor. PSE3 also demonstrated meaningful inhibition (64.7 µg/mL), while PSE1 and PSE2 exhibited weak or negligible activity, with IC₅₀ values of 354.9 µg/mL and >500 µg/mL, respectively. These results demonstrate that PSE0, with its high phenolic load, and PSE3, with solvent-specific selectivity, both exhibit dual antioxidant and anti-inflammatory activity.

4. Conclusions

In conclusion, Prokupac grape skin extracts, particularly PSE0 and PSE3, demonstrated significant antioxidant and anti-inflammatory activities, directly linked to their phenolic composition. The results highlight the dual benefit of valorizing viticultural waste while producing bioactive-rich extracts with potential applications in preventing oxidative stress and inflammation-related diseases. This study provides strong evidence that the Šumadija region's native grape variety represents a valuable and underutilized resource in functional food and pharmaceutical research.

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