Grape Skin Extract–Enriched Hydrogels for Wound Healing: An Antioxidant-Based Approach

Aleksandar Kocovic[1,2,*], Jovana Bradic[1,2], Anica Petrovic[1,2], Marko Simic[1], Vladimir Jakovljevic[2,3,4], Edina Avdovic[5]

[1] Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovica, 69, 34000 Kragujevac, Serbia; e-mail: jovanabradickg@gmail.com, salekkg91@gmail.com, petkovicanica0@gmail.com, simic.marko.kg@gmail.com
[2] Center of Excellence for Redox Balance Research in Cardiovascular and Metabolic Disorders, Zmaj Jovina 30, 34000 Kragujevac, Serbia; jovanabradickg@gmail.com, salekkg91@gmail.com, petkovicanica0@gmail.com, simic.marko.kg@gmail.com, drvladakgbg@yahoo.com
[3] Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovica, 69, 34000 Kragujevac, Serbia; e-mail: drvladakgbg@yahoo.com
[4] Department of Human Pathology, Sechenov First Moscow State Medical University, 8 Trubetskaya Street St., 119991 Moscow, Russia; e-mail: drvladakgbg@yahoo.com
[5] Institute for Information Technologies, University of Kragujevac, Jovana Cvijica bb, 34000 Kragujevac, Serbia; e-mail: edina.avdovic@pmf.kg.ac.rs

* *Corresponding author*

DOI: 10.46793/ICCBIKG25.673K

Abstract: Gelatin–alginate hydrogels incorporating grape skin extract (GSE) from the Šumadija region were prepared by blending alginate and gelatin (3:1, 3:2, 1:1), casting, freezing, and $CaCl_2$ cross-linking. Organoleptic properties, total phenolic content (TPC, Folin–Ciocalteu), and antioxidant activity (DPPH and ABTS microplate assays with Trolox) were evaluated. GSE-loaded hydrogels formed uniform, solid gels. Compared with blank matrices, GSE increased TPC by approximately 46–79% and produced modest but consistent reductions in SC_{50} values: 0.3–1.8% in ABTS and 0.5–1.6% in DPPH (Trolox 6.909 and 5.034 μg/mL, respectively). Across GSE formulations, higher gelatin fractions correlated with higher TPC and lower ABTS SC_{50}, suggesting protein–polyphenol interactions that enhance aqueous availability, whereas DPPH responses were minimally affected. These findings indicate that GSE incorporation preserves antioxidant functionality and yields small in-assay gains, with composition exerting a stronger effect in ABTS than in DPPH. The platform is a sustainable route to bioactive wound dressings and merits further optimization and biological validation
Keywords: grape skin, hydrogel, antioxidant, polyphenols

1. Introduction

Hydrogels are three-dimensional, cross-linked polymer networks that imbibe large amounts of water and maintain a moist, protective interface, which makes them attractive wound dressings [1,2]. In addition to hydration and exudate control, hydrogel matrices can carry bioactive agents and enable local, sustained delivery at the wound

site [3,4]. Synthetic hydrogels, however, can raise concerns about biocompatibility, residual monomers or initiators, and limited biodegradability, which has accelerated interest in natural polymers. Gelatin provides cell-interactive motifs and enzymatic degradability, while alginate forms mild, ionically cross-linked gels with strong fluid uptake; together they yield tunable matrices that couple tissue affinity with mechanical stability for wound care [3].

Oxidative stress is a hallmark of impaired healing. Excess reactive oxygen species (ROS) disrupt signaling, damage extracellular matrix components, and prolong inflammation, which ultimately delays repair. Antioxidant-based strategies aim to temper this imbalance and support orderly progression through the phases of healing [5]. Grape skin, a readily available by-product of winemaking, is rich in polyphenols (for example, anthocyanins, flavonols, proanthocyanidins, and stilbenes) that can scavenge ROS and modulate inflammatory pathways relevant to cutaneous repair [6]. Valorizing grape skins from the Šumadija region (Serbia) also advances sustainability by converting local agro-industrial waste into a high-value ingredient for biomedical materials.

The objective of this study was to investigate antioxidant activity of gelatin–alginate hydrogel enriched with GSE derived from wine sort present in Šumadija.

2. Methodlogy

2.1 Preparation of GSE-loaded hydrogels (HG+GSE)

Grape skin extract (GSE) was produced by ultrasound-assisted extraction with ethyl acetate and stored at 4 °C. Hydrogel formulations were obtained by adjusting the alginate-to-gelatin ratio as follows: Alg/Gel-1 (3:1), Alg/Gel-2 (3:2), and Alg/Gel-3 (1:1). A solution of GSE (8 mg/mL) was blended with alginate and gelatin, homogenized, cast into molds, frozen, and then cross-linked with $CaCl_2$. Control hydrogels without GSE (HG) were prepared in the same manner, replacing the extract with water.

2.2 Organoleptic properties of HG+GSE

Organoleptic features of HG+GSE—appearance, color, and homogeneity—were evaluated by visual inspection and tactile assessment.

2.3 Total Phenolic Content (TPC)

TPC was quantified by a microplate Folin–Ciocalteu assay [7]. Gallic acid standards were prepared to generate a calibration curve, and samples (e.g., hydrogel supernatants) were diluted as needed. In a 96-well plate, 20 μL of standard or sample was mixed with 80 μL distilled water and 20 μL diluted Folin–Ciocalteu reagent; after a brief incubation, 80 μL of 7.5% (w/v) Na_2CO_3 was added. After 30 min at room temperature, absorbance was read at 765 nm using a microplate reader. TPC was calculated from the calibration curve and expressed as μg gallic acid equivalents per gram of xerogel (μg GAE/g xerogel).

2.4 DPPH Radical Scavenging Assay

DPPH radical scavenging was assessed by microplate method [7]. Samples and Trolox standards were combined with methanolic DPPH (0.004% w/v) to a final volume of 200 μL per well. Following 30 min incubation at room temperature in the dark, absorbance was measured at 515 nm with a microplate reader. Radical scavenging (%)

was calculated relative to a negative control (DPPH + solvent), and SC_{50} values were derived from concentration–response curves.

2.5 ABTS Radical Cation Decolorization Assay

$ABTS^{\bullet+}$ was generated by reacting ABTS stock with potassium persulfate for 16 h in the dark, as described previously [7]. The working solution was diluted to an initial absorbance of 0.700 ± 0.020 at 734 nm. In a 96-well format, 20 μL of sample or Trolox standard was mixed with 180 μL $ABTS^{\bullet+}$, and absorbance at 734 nm was recorded within 1 min using a microplate reader. All measurements were performed in triplicate.

3. Results and Discussion

The alginate–gelatin hydrogel base (HG) was solid and transparent, while HG+GSE was colored due to the grape skin extract and exhibited a coherent, uniform gel network.

Loading grape skin extract (GSE) into gelatin–alginate hydrogels produced small but consistent gains in in-vitro antioxidant readouts and a marked rise in released phenolics (Table 1). Versus the corresponding blanks (1, 2, 3), GSE-loaded gels (1E, 2E, 3E) showed lower SC_{50} in ABTS by ~0.3–1.8% (e.g., 298.700 → 291.603 μg/mL for 3→3E) and modest reductions in DPPH by ~0.5–1.6% (e.g., 348.919 → 343.234 μg/mL for 3→3E), while the reference Trolox remained far more potent (DPPH 5.034 μg/mL; ABTS 6.909 μg/mL). Total phenolic content rose substantially with GSE (≈46–79%; 1→1E, 2→2E, 3→3E), and across GSE-loaded formulations ABTS SC_{50} decreased from 1E to 3E, whereas DPPH SC_{50} increased slightly.

Table 1. Antioxidant activity and TPC of investigated hydrogel loaded with grape skin extract and standards.

HG/HG-GSE/Standard	DPPH SC_{50} (μg/mL)	ABTS SC_{50} (μg/mL)	TPC (μg GAE/g E)
1	332.165 ± 33.493	298.700 ± 29.930	4.411 ± 0.013
1E	330.494 ± 32.838	297.879 ± 22.627	6.440 ± 0.025
2	341.730 ± 32.555	297.993 ± 28.042	4.460 ± 0.015
2E	338.487 ± 33.114	293.044 ± 28.027	6.963 ± 0.012
3	348.919 ± 33.730	296.890 ± 28.366	4.238 ± 0.019
3E	343.234 ± 32.994	291.603 ± 27.133	7.576 ± 0.019
Trolox	5.034 ± 0.327	6.909 ± 0.694	/

Hydrogels without extract (1, 2, 3); grape skin extract–enriched formulations (1E, 2E, 3E); SC_{50} half-maximal scavenging concentration; TPC, total phenolic content; GAE, gallic acid equivalents; E, extract fraction of the xerogel. Data are presented as mean ± SD.

This divergence is plausible given assay media differences (aqueous $ABTS^{\bullet+}$ vs methanolic $DPPH^{\bullet}$) and matrix effects on diffusion and microenvironment. The progressive TPC rise from 1E to 3E suggests higher gelatin fractions enhance phenolic availability, likely via protein–polyphenol interactions and subsequent release. Overall, GSE incorporation preserves antioxidant functionality and composition tuning has a larger impact on ABTS than on DPPH under these conditions [3].

4. Conclusions

Gelatin–alginate hydrogels loaded with grape skin extract (GSE) formed uniform, solid gels. GSE increased released phenolics by ~46–79% and produced small but consistent antioxidant gains versus blanks, more pronounced in ABTS than DPPH. Within GSE formulations, higher gelatin content correlated with higher TPC and lower ABTS SC_{50}, consistent with protein–polyphenol interactions. Although potency remained far below Trolox, these data support a sustainable, antioxidant-based dressing; further optimization (polymer ratio/cross-linking), release profiling, and cytocompatibility testing are warranted.

Acknowledgment

This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 11067, Unleashing Nature's Potential: Using Grape Skin Extract and Sustainable Materials for Advanced Chronic Wound Therapy—GraSP_MAT and #GRANT No 14848, Unleashing nature's potential: Development of Additive-Free Prokupac Grape Skin Topping as Functional Food—GraSP, and also by the Serbian Ministry of Science, Technological Development, and Innovations Agreements No. 451-03-137/2025-03/200111, 451-03- 136/2025-03/200111 (assigned to Faculty of Medical Sciences), and Agreements No. 451-03-136/2025- 03/200378, 451-03-137/2025-03/200252, and 451-03-136/2025-03/200122 (assigned to Institute for Information Technologies).

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