ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Comparative Evaluation of the Antioxidant Potential of Besni Grape (*Vitis vinifera L.*) Extracts Obtained Using Different Solvents

Farklı Çözücüler Kullanılarak Elde Edilen Besni Üzümü (Vitis vinifera L.) Ekstraktlarının Antioksidan Potansiyelinin Karşılaştırmalı Değerlendirilmesi

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ABSTRACT

Aim: *The in vitro antioxidant properties of water, ethanol/water, and methanol extracts obtained from commercially available Besni grape (Vitis vinifera L.) were compared.*

Materials and methods: *Dried Besni grape samples were extracted using three solvent systems with different polarities. Antioxidant activity was evaluated by the ferric thiocyanate method, total phenolic content by the Folin-Ciocalteu assay, and reducing power by the potassium ferricyanide method. Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple comparison test.*

Results: *The ethanol/water extract demonstrated the highest activity in all assays. In this group, total antioxidant activity inhibition values ranged from 77.04% to 80.79%. Reducing power absorbance values varied between 0.114±0.002 and 0.465±0.002, while total phenolic content ranged from 15.93±0.05 to 19.03±0.04 mg GAE/g lyophilizate (p <0, 05). The methanol extract also exhibited considerable antioxidant activity, whereas the aqueous extract showed comparatively lower values. Increased concentration was associated with higher antioxidant activity and reducing power in all extracts.*

Conclusion: *The antioxidant capacity of Besni grape extracts was influenced by the extraction solvent. The ethanol/water extract, in particular, showed higher phenolic content together with stronger antioxidant activity. These findings suggest that Besni grape may serve as a valuable natural antioxidant source for functional food and nutraceutical applications.*

Key words: *Besni grape, Vitis vinifera, antioxidant activity, phenolic compounds, extraction solvents*

ÖZET

Amaç: *Piyasada satılan Besni üzümünden (Vitis vinifera L.) elde edilen su, etanol/su ve metanol özütlerinin in vitro antioksidan özellikleri karşılaştırılmıştır.*

Gereç ve Yöntem: *Kurutulmuş Besni üzüm örnekleri, farklı polaritelere sahip üç çözücü sistemi kullanılarak ekstrakte edildi. Antioksidan aktivite ferrik tiyosiyanat yöntemi ile, toplam fenolik içerik Folin-Ciocalteu testi ile ve indirgeyici güç potasyum ferrisiyanür yöntemi ile değerlendirildi. İstatistiksel analiz, tek yönlü ANOVA ve ardından Duncan'ın çoklu karşılaştırma testi kullanılarak gerçekleştirildi.*

Bulgular: *Etanol/su ekstresi, tüm testlerde en yüksek aktiviteyi göstermiştir. Bu grupta, toplam antioksidan aktivite inhibisyon değerleri %77,04 ile %80,79 arasında değişmiştir İndirgeme gücü absorbans değerleri 0,114±0,002 ile 0,465±0,002 arasında değişirken, toplam fenolik içerik 15,93±0,05 ile 19,03±0,04 mg GAE/g liyofilizat arasında değişmiştir (p<0,05). Metanol ekstresi de önemli ölçüde antioksidan aktivite gösterirken, sulu ekstresi nispeten daha düşük değerler sergilemiştir. Konsantrasyonun artması, tüm ekstraktlarda daha yüksek antioksidan aktivite ve indirgeyici güç ile ilişkilendirilmiştir.*

Sonuç: *Besni üzüm ekstraktlarının antioksidan kapasitesi, ekstraksiyon çözücüsünden etkilenmiştir. Özellikle etanol/su ekstraktı, daha yüksek fenolik içerik ile birlikte daha güçlü antioksidan aktivite göstermiştir. Bu bulgular, Besni üzümünün fonksiyonel gıda ve nutrasötik uygulamalar için değerli bir doğal antioksidan kaynağı olabileceğini göstermektedir.*

Anahtar kelimeler: *Besni üzümü, Vitis vinifera, antioksidan aktivite, fenolik bileşikler, ekstraksiyon çözücüleri*

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Introduction

Aerobic metabolism is intrinsically pro-oxidant. Mitochondrial electron leak at complexes I and III, NADPH oxidase activity in phagocytes and vascular wall cells, and peroxisomal fatty-acid β-oxidation together produce reactive oxygen and nitrogen species (ROS/RNS) as obligate metabolic byproducts. Under basal conditions, these intermediates are restrained by enzymatic scavengers, including superoxide dismutase, catalase, and the glutathione peroxidase system, together with low–molecular-weight reductants, so that redox chemistry remains a controlled signaling resource rather than indiscriminate damage (1). Once this equilibrium collapses, oxidative modification of membrane lipids, structural and enzymatic proteins, and genomic DNA proceeds at rates exceeding cellular repair (2,3). Chronic redox imbalance underpins atherogenic plaque progression, β-cell exhaustion in type 2 diabetes mellitus, dopaminergic and cholinergic neuronal attrition, and the mutagenic burden of early tumorigenesis (2,4). Plant-derived phenolics intervene in this chemistry through well-defined structural features: ortho-dihydroxy substitution on the B-ring, conjugation across the C-ring, and a resonance-stabilized phenoxyl radical that follows hydrogen atom donation. Flavonols, flavan-3-ols, stilbenes, and anthocyanins each exploit some combination of these features to terminate lipid peroxidation chains and quench peroxyl radicals before they reach biological targets (5,6).

Besni grape (*Vitis vinifera L.)* is grown on the semi-arid uplands of Adiyaman in southeastern Türkiye and carries Protected Geographical Indication status. The cultivar is white-fleshed, seeded, and processed primarily as sun-dried raisins. Its persistence under high solar irradiance, scant precipitation, and wide diurnal thermal amplitude is biologically instructive: each of these stressors upregulates phenylalanine ammonia-lyase and downstream phenylpropanoid enzymes, redirecting fixed carbon toward stilbenoid and flavonoid biosynthesis as a UV- and ROS-defensive output. Despite this favorable biology, the phytochemical literature on Besni grape remains thin compared with the well-mapped red European cultivars. Resveratrol, catechin, epicatechin, quercetin glycosides, gallate esters, and oligomeric proanthocyanidins have been documented across *V. vinifera* tissues (7,8); the relative weighting of these classes is markedly cultivar-dependent. Pigmented varieties are anthocyanin-dominated, whereas white cultivars rely on flavonols, flavan-3-ols, and hydroxycinnamic acid derivatives for the bulk of their redox-active fraction (9,10). Recovery of phenolics from a plant matrix is governed by the physicochemistry of the extraction medium more than by the source biology alone. Solvent polarity, dielectric constant, and hydrogen-bond donor capacity together dictate which structural classes partition out of the tissue. Hydroalcoholic mixtures combine the cell-wall hydrolytic action of water with the solubilization of moderately polar aglycones by alcohol and, consequently, recover a broader spectrum than either pure water or pure alcohol can achieve alone (11). In grape and grape-byproduct matrices, ethanol-water systems repeatedly outperform single-solvent alternatives in total phenolic yield and in downstream antioxidant readouts (12,13). For Besni grape, no systematic polarity-resolved comparison has been published, and the question of how solvent geometry shapes the redox profile of this geographically indicated cultivar remains open.

Antioxidant capacity is mechanistically heterogeneous, and no single assay captures the full range of relevant chemistries. We therefore selected three complementary readouts. The ferric thiocyanate (FTC) assay tracks hydroperoxide accumulation during linoleic acid autoxidation and reflects protection against chain-propagating lipid peroxyl radicals. The Folin-Ciocalteu reaction, although cross-reactive with non-phenolic reductants, remains the most reproducible total reducing-phenolic descriptor available in food chemistry and was calibrated here against gallic acid (14). The potassium ferricyanide reduction assay quantifies single-electron-transfer (SET) activity through Fe(III) → Fe(II) conversion (15). The three probes therefore interrogate mixed hydrogen-atom-transfer, peroxide-suppressive, and pure SET behavior, respectively. The present work applies this triadic framework to water, ethanol/water, and methanol extracts of commercially sourced Besni grape to identify which solvent geometry maximizes the recovery of physiologically meaningful antioxidant activity.

Material and Methods

Analytical-grade reagents, comprising methanol, ethanol, the Folin-Ciocalteu phenol reagent, linoleic acid, ammonium thiocyanate, potassium ferricyanide, ferric chloride, and trichloroacetic acid, were supplied by Sigma-Aldrich (St. Louis, MO, USA) and used without further purification.

Preparation of Vitis vinifera L. Extracts

Sun-dried Besni grape (*Vitis vinifera* L.) was procured in sealed retail packaging carrying its Protected Geographical Indication labeling; provenance was verified against regional morphological descriptors. Pedicels and extraneous plant debris were removed manually. The cleaned material was milled to uniform particle size in a laboratory blender, passed through a stainless-steel sieve, and stored in amber glass vials at room temperature, shielded from light and atmospheric moisture, until extraction.

Three solvent systems were chosen to span a defined polarity gradient and bias extraction toward overlapping but non-identical phenolic subsets: distilled water, anhydrous methanol, and a 1:1 (v/v) ethanol-water mixture. Each extraction was loaded with 100 g of milled material at a fixed 1:10 (w/v) solid-to-solvent ratio.

Aqueous extraction was carried out in an open glass beaker on a heated magnetic stirrer (90–95°C) under constant agitation. The crude infusion was clarified through Whatman filter paper, and the filtrate was dried in a low-temperature convection oven. The resulting residue was sealed and held at +4°C until assay.

Hydroalcoholic extraction employed a Soxhlet apparatus charged with 100 g of milled material and 1000 mL of a 1:1 ethanol-water mixture. Cycling proceeded at 50°C for 48 h, with full solvent replacement at the 24-h mark to maintain a favorable concentration gradient. The pooled extractant was reduced to dryness under vacuum on a rotary evaporator and stored under refrigeration.

Methanol extraction followed the same Soxhlet geometry: 100 g of grape powder, 750 mL methanol, 50°C, 48 h, with solvent renewal at 24 h. The collected solvent was filtered, methanol was removed under reduced pressure, and the concentrate was dried at 47°C before transfer to sterile amber vials for storage.

Total Antioxidant Activity Assay

Suppression of lipid autoxidation was assessed by the ferric thiocyanate (FTC) protocol, in which the rate of hydroperoxide accumulation in an emulsified linoleic acid system serves as the readout for antioxidant protection. Reaction mixtures contained extract at the working concentration, 0.2 M phosphate buffer (pH 7.0), and a linoleic acid emulsion; incubation proceeded at 37°C in the dark to exclude photochemical artifacts. At defined intervals, aliquots were withdrawn and developed sequentially with ammonium thiocyanate and $FeCl_2$ dissolved in dilute HCl, generating the red Fe(III)-thiocyanate chromophore in proportion to the peroxide formed. Absorbance was read at 500 nm; lower values corresponded to greater suppression of peroxidation. Ascorbic acid and Trolox served as positive controls, and every assay was run six replicates.

Determination of Total Phenolic Content

Total reducing-phenolic content was quantified by the Folin-Ciocalteu chromogenic reaction. Diluted extract was combined with the phosphotungstic-phosphomolybdic reagent and allowed to react for 5 min at 30°C. The pH of the medium was then raised by addition of 7.5% (w/v) sodium carbonate, driving formation of the blue molybdotungstate complex. After a 90-min incubation at 30°C in darkness, absorbance was recorded at 765 nm against the reagent blank on a UV-Vis spectrophotometer. Quantification was performed against a gallic acid calibration series prepared across the working concentration range, and results were reported as milligrams of gallic acid equivalents (GAE).

Reducing power assay

Single-electron-transfer activity was assayed essentially as described by Ferreira and coworkers (15) with procedural adjustments to suit the matrix examined here. Extract was combined with 0.02 M phosphate buffer (pH 6.6) and 1% (w/v) potassium ferricyanide, and the mixture was warmed at 50°C for 30 min to drive the $Fe(CN)_6^{3-} \rightarrow Fe(CN)_6^{4-}$ reduction. The reaction was stopped with 10% (w/v) trichloroacetic acid, the precipitate was pelleted at 3000 rpm for 10 min, and an aliquot of the clarified supernatant was treated with 0.1% (w/v) ferric chloride to develop the Prussian-blue chromophore. Absorbance at 700 nm scaled directly with the electron-donating capacity of the extract.

Statistical Analysis

Each experimental condition was run as six independent technical replicates, and results are presented as mean ± SE. Distributional normality was verified prior to parametric testing. Between-group differences were resolved by one-way ANOVA, with Duncan's multiple range test applied post hoc. Computations were carried out in IBM Statistical Package for Social Sciences (SPSS) program version 25.0 (IBM Corp., Armonk, NY, USA), and the significance threshold was set at $p < 0.05$.

Results

Total Antioxidant Activity, Reducing Power, and Total Phenolic Content of Besni Grape Water Extract (BGWE)

The total antioxidant activity, reducing power, and total phenolic compound levels of the Besni grape water extract (BGWE) are presented in Table 1. Total antioxidant activity absorbance values ranged from 0.724±0.001 to 0.595±0.002, while inhibition percentages varied between 65.65% and 71.77%. Reducing power absorbance values were measured between 0.073±0.001 and 0.245±0.002. Total phenolic content values ranged from 14.76±0.03 to 16.66±0.04 mg GAE/g lyophilizate.

Total Antioxidant Activity, Reducing Power, and Total Phenolic Content of Besni Grape Ethanol-Water Extract (BGEWE)

The findings obtained from the Besni grape ethanol-water extract (BGEWE) are shown in Table 2. Total antioxidant activity absorbance values ranged between 0.484±0.005 and 0.405±0.001, whereas inhibition percentages were found between 77.04% and 80.79%. Reducing power absorbance values varied from 0.114±0.002 to 0.465±0.002. Total phenolic content values ranged from 15.93±0.05 to 19.03±0.04 mg GAE/g lyophilizate.

Total Antioxidant Activity, Reducing Power, and Total Phenolic Content of Besni Grape Methanol Extract (BGME)

The results obtained from the Besni grape methanol extract (BGME) are summarized in Table 3. Total antioxidant activity absorbance values ranged from 0.565±0.002 to 0.444±0.002, while inhibition percentages varied between 73.20% and 78.94%. Reducing power absorbance values were determined between 0.102±0.003 and 0.424±0.001. Total phenolic content values ranged from 15.44±0.06 to 17.66±0.04 mg GAE/g lyophilizate.

Discussion

The antioxidant properties of Besni grape extracts varied depending on the solvent used during extraction. Among the tested groups, the ethanol–water extract produced the highest total phenolic content together with the strongest antioxidant and reducing activity values. Methanol extracts also showed considerable activity, whereas the aqueous extract yielded comparatively lower values in all three assays.

The phenolic composition of grape cultivars is strongly influenced by genetic background, climatic conditions,

Table 1. *Comparison of the total antioxidant activity (TAA), reducing power (RP) and phenolic compound content (TPC) of Besni grape water extract (BGWE)*

Examples	Dose (mg/mL)	Total antioxidant activity		Reducing power	Amount of phenolic compound
		Avg. Abs. (60. hours, 500 nm)	% Inhibition	Avg. Abs. (700 nm)	(mg GAE/g lyophilized)
BGWE	1	0.724±0.001 d	65.65	0.073±0.001 g	14.76±0.03 i
	5	0.663±0.001 c	68.55	0.126±0.001 h	15.62±0.02 j
	10	0.595±0.002 b	71.77	0.245±0.002 ı	16.66±0.04 k
AA	1	0.956±0.001 e	54.65	—	—
Trolox	1	0.455±0.002 a	78.42	—	—
Control (water)		2.108±0.002 f	—	—	—

Results are given as the mean (± standard error) of six parallel measurements.
Values with the same letter are not statistically different according to Duncan's test ($p < 0.05$).

Table 2. *Comparison of the total antioxidant activity (TAA), reducing power (RP) and phenolic compound content (TPC) of Besni grape ethanol-water extract (BGEWE)*

Examples	Dose (mg/ml)	Total antioxidant activity		Reducing power	Amount of phenolic compound
		Avg. Abs. (60. hours , 500 nm)	% Inhibition	Avg. Abs. (700 nm)	(mg GAE/g lyophilized)
BGEWE	1	0.484±0.005 c	77.04	0.114±0.002 f	15.93±0.05 ı
	5	0.455±0.001 b	78.42	0.296±0.002 g	17.21±0.04 i
	10	0.405±0.001 a	80.79	0.465±0.002 h	19.03±0.04 j
AA	1	0.956±0.001 d	54.65	—	—
Trolox	1	0.455±0.001 b	78.42	—	—
Control (water)		2.108±0.002 e	—	—	—

Results are given as the mean (± standard error) of six parallel measurements.
Values with the same letter are not statistically different according to Duncan's test ($p < 0.05$).

Table 3. *Comparison of the total antioxidant activity (TAA), reducing power (RP) and phenolic compound content (TPC) of Besni Grape methanol extract (BGME)*

Examples	Dose (mg/ml)	Total antioxidant activity		Reducing power	Amount of phenolic compound
		Avg. Abs. (60. hours, 500 nm)	% Inhibition	Avg. Abs. (700 nm)	(mg GAE/g lyophilized)
BGME	1	0.565±0.002 d	73.20	0.102±0.003 g	15.44±0.06 i
	5	0.504±0.002 c	76.09	0.263±0.001 h	16.46±0.03 j
	10	0.444±0.002 a	78.94	0.424±0.001 ı	17.66±0.04 k
AA	1	0.956±0.002 e	54.65	—	—
Trolox	1	0.455±0.002 b	78.42	—	—
Control (water)		2.108±0.002 f	—	—	—

Results are given as the mean (± standard error) of six parallel measurements.
Values with the same letter are not statistically different according to Duncan's test ($p < 0.05$).

cultivation region, drying procedures, and extraction techniques. Phenolic compounds in Vitis vinifera species mainly consist of flavonoids, phenolic acids, proanthocyanidins, and anthocyanins (13,16). Their antioxidant activity is largely associated with radical scavenging ability, suppression of lipid peroxidation, and electron-donating capacity (5).

The higher antioxidant activity observed in the hydroalcoholic extract is consistent with previous studies reporting that solvent polarity plays a major role in the extraction of phenolic compounds. Burin et al. demonstrated that ethanol-containing solvent systems produced higher total phenolic content and antioxidant capacity in different grape varieties (17). Similarly, Rockenbach et al. reported that hydroalcoholic extraction improved the recovery of phenolic compounds from grape-derived materials (18). The elevated phenolic content and reducing power values obtained from the ethanol-water extract in the present study support these observations.

The antioxidant activity detected in the methanol extract was also noteworthy. Methanol is recognized as an effective solvent for low-molecular-weight phenolic compounds (11). However, some phenolic subclasses are extracted more efficiently in solvents with intermediate polarity, which may explain the stronger activity observed in the hydroalcoholic extract. Similar relationships between phenolic concentration and antioxidant capacity have been reported previously in grape-derived materials (19). A similar relationship was observed in the present study, where increased phenolic content was accompanied by higher antioxidant activity and reducing power values.

The antioxidant effects of phenolic compounds are closely related to their hydrogen atom transfer and electron transfer capacities. For this reason, reducing power assays are widely used to evaluate antioxidant potential (20). In the current study, the ethanol-water extract showed the highest reducing power values, suggesting a stronger electron-donating capacity compared with the other extracts. Jayaprakasha et al. similarly reported a positive association between reducing power and phenolic concentration in grape seed extracts (21).

The relatively lower antioxidant activity observed in the aqueous extract may be associated with the limited solubility of certain phenolic fractions in water. Nevertheless, antioxidant activity increased progressively with concentration in the water extract as well, indicating that Besni grape also contains water-soluble antioxidant compounds. Hydrophilic phenolic acids and certain flavanol derivatives may contribute to this activity (22).

Phenolic compounds found in grapes are known not only for their antioxidant properties but also for their anti-inflammatory, cardioprotective, and neuroprotective effects (7). Averilla et al. reported that grape-derived polyphenols may exert protective effects against oxidative stress-related disorders (23). Recent studies have also shown that grape polyphenols can reduce oxidative cellular damage, suppress lipid peroxidation, and support intracellular antioxidant defense mechanisms (24,25)

Although Besni grape is one of the geographically indicated local grape cultivars of Türkiye, available information regarding its biochemical characteristics remains limited. Therefore, the present findings provide additional data concerning the antioxidant potential of this cultivar. The relatively high phenolic content and antioxidant activity obtained particularly from the ethanol-water extract suggest that Besni grape may represent a valuable natural source of antioxidant compounds for functional food and nutraceutical applications.

Some limitations should be considered when interpreting these findings. Although total phenolic content was evaluated, individual phenolic compounds were not characterized using advanced analytical techniques such as HPLC or LC-MS/MS. In addition, antioxidant activity was assessed only through in vitro biochemical assays.

Conclusion

The antioxidant properties of Besni grape extracts varied according to the extraction solvent. The ethanol-water extract showed the highest phenolic content, along with the strongest antioxidant and reducing activities. These findings indicate that Besni grape is a promising natural source of antioxidant compounds and may have potential use in functional food and nutraceutical applications. Further studies focusing on individual phenolic compounds and biological activity may provide a more detailed understanding of its bioactive potential.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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