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Isolation, NMR Characterization, and Comparative *In Vitro* Antioxidant Evaluation of Resveratrol from Grape Seeds (*Vitis vinifera* L.)

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ABSTRACT

Objective: The present study aimed to isolate, characterize, and evaluate the in vitro antioxidant potential of resveratrol from grape seeds (*Vitis vinifera* L.) obtained from local markets in Erzincan, Türkiye. It is hypothesized that the unique microclimate of that region enhances the bioactive profile of local grape varieties, making them a significant source of natural antioxidants.

Methods: Grape seeds (500 g) were extracted using 100% methanol via reflux for 4 hours (three replicates). The crude extract (30 g) underwent liquid-liquid extraction with ethyl acetate to yield a concentrated phenolic fraction (2 g). For isolation, Sephadex LH-20 adsorbent was used as the stationary phase and eluted with methanol. Structural characterization was conducted using ^{1}H and ^{13}C NMR spectroscopy. The antioxidant capacity of the isolated resveratrol was determined through DPPH and ABTS radical scavenging assays, comparing results with standard antioxidants (Trolox, BHA, BHT, and Ascorbic acid).

Results: Chromatographic purification yielded 10 mg of pure trans-resveratrol. The structure was firmly verified by NMR spectroscopy, with the high olefinic coupling constant (J = 16.3 Hz) suggesting the trans structure. In antioxidant experiments, Trans-resveratrol demonstrated notable DPPH radical scavenging action (IC_{50} = 17.33 ± 1.22 µg/mL). Trans-resveratrol outperformed Trolox (7.22 ± 0.24 µg/mL) and ascorbic acid (8.22 ± 0.37 µg/mL) in the ABTS test, with an IC_{50} of 6.84 ± 0.47 µg/mL.

Conclusion: The findings demonstrate that grape seeds from Erzincan markets are an excellent source of highly bioactive *trans*-resveratrol. The isolated compound exhibited exceptional antioxidant efficiency, particularly in the ABTS assay where it surpassed several commercial standards. These results suggest that local grape varieties possess significant potential as natural sources for high-purity bioactive agents in the nutraceutical and pharmaceutical sectors.

Keywords: ABTS, DPPH, Resveratrol, *Vitis vinifera*

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Introduction

Vitis vinifera L. (Grape) is among the most extensively grown fruit crops worldwide and provide a principal source of bioactive polyphenols (Xia et al., 2010). Recently, the secondary metabolites in grape seeds have attracted considerable interest for their many pharmacological benefits, such as anti-inflammatory, cardioprotective, and anticancer effects (Pezzuto, 2018). Among these secondary metabolites, resveratrol (3,5,4'-trihydroxystilbene) stands out as a strong phytoalexin that plays an important part in protecting plants against infections while delivering major health advantages to people (Sajish & Schimmel, 2015).

Resveratrol's biological value is mostly due to its advanced antioxidant effects. When the body's antioxidant defense systems are unable to sufficiently balance the generation of reactive oxygen species (ROS), oxidative stress results. This imbalance leads to cellular damage and plays a crucial role in the development of many chronic diseases, including diabetes, cardiovascular diseases, and neurodegenerative diseases such as Alzheimer's (Pryor et al., 2006). Resveratrol functions both as a direct scavenger of free radicals and as a regulator of intracellular signaling pathways and gene expression associated with antioxidant enzymes (Galiniak et al., 2019). This secondary metabolite is essential in avoiding DNA mutations and lipid peroxidation by neutralizing oxidative damage at the cellular level, both of which are significant precursors to oncogenesis and atherosclerosis (Truong et al., 2018).

The accurate identification of isolated secondary metabolite is crucial for correlating chemical structure with biological activity. Nuclear Magnetic Resonance (NMR) spectroscopy, particularly ^{1}H and ^{13}C NMR, is the definitive method for structural elucidation, facilitating the verification of molecular frameworks (Hansen, 2021). Furthermore, examining antioxidant efficacy by different mechanisms such as the DPPH and ABTS radical scavenging tests is crucial for giving a thorough understanding of the molecule's redox potential (Benzie & Strain, 1996). In this study, we report the isolation of resveratrol from grape seeds purchased from local markets in Erzincan, its structural characterization via NMR, and a detailed assessment of its *in vitro* antioxidant capacity.

Methods

Plant Material and Extraction

The grape samples (*Vitis vinifera* L.) were purchased from local markets in Erzincan, Turkey. The seeds were manually separated from the fruit pulp and dried under shade at room temperature to preserve heat-sensitive phenolic compounds. For the extraction procedure, 500 g of the ground grape seeds were subjected to reflux extraction using 100% methanol (MeOH) as the solvent. The extraction was carried out for 4 hours and repeated three times to ensure maximum yield of the bioactive constituents. After each cycle, the extracts were filtered through Whatman No. 1 filter paper. The combined filtrates were concentrated to dryness under reduced pressure using a rotary evaporator at room temperature, yielding 30 g of crude MeOH extract.

The MeOH extract was dissolved in distilled water and subjected to liquid-liquid extraction with ethyl acetate (EtOAc). The ethyl acetate phase was separated (three times) and subsequently evaporated to dryness at room temperature to prevent the thermal degradation of stilbenes. This process resulted in a final yield of 2 g of ethyl acetate extract, which was kept in dark vials at -20°C for further chromatographic separation and characterization.

Extraction and Isolation

The EtOAc extract (2 g) was dissolved in a minimum amount of analytical grade methanol and loaded onto a chromatography column (100*2.5cm) packed with Sephadex LH-20 (100 g) as the stationary phase. The separation was performed using 100% methanol as the mobile phase under gravity flow. A total of 35 fractions (10 mL) were collected. Using silica gel 60 F_{254} plates, Thin Layer Chromatography (TLC) was used to analyze the elution profile and purity of the fractions. The plates were controlled with a mobile system of ethyl acetate/acetic acid/formic acid/water (100:11:11:26, v/v) and visualized under UV light at 254 and 366 nm. Fractions (21-35) (10 mg) were obtained.

DPPH· Free Radical Scavenging Ability

The method developed by Şimşek et al. was adjusted to evaluate the free radical scavenging activity (Şimşek et al., 2026). A new 0.26 mM DPPH· solution was made before analysis. After making extract stock solutions (1 mg/mL), the proper aliquots (20–400 µL) were put into test tubes. To provide a range of test concentrations, the volume was increased to 3 mL using methanol. Each reaction mixture was then supplemented with 1 milliliter of the DPPH· solution. To guarantee homogeneity, the samples were vortexed, and to stop the radical from photo-degrading, they were incubated for 30 minutes at room temperature in the dark. After incubation, absorbance was recorded at 517 nm against a methanol blank using a UV–Vis spectrophotometer. Experiments were conducted in six replicates, and results were expressed as mean ± SD. Radical scavenging activity (%) and IC_{50} values (µg/mL) were calculated.

ABTS·$^{+}$ Radical Scavenging Ability

The extract's ABTS·$^{+}$ radical scavenging activity was evaluated by altering the Re et al. Technique (Re et al., 1999). The ABTS radical cation was produced by allowing ABTS (2 mM) to react with potassium persulfate (2.45 mM) in phosphate buffer (0.1 mM, pH 7.4). The reaction mixture was prepared at a volume ratio of 1:2 and maintained at room temperature in the absence of light for 12 h to permit full generation and stabilization of the ABTS radical species.

Test sample stock solutions were made with a concentration of 1 mg/mL. To achieve varying test concentrations, 20–400 µL quantities of each extract were combined with 3 mL of phosphate buffer. 1 mL of pre-

formed ABTS·⁺ solution was then added. For sixty minutes, the reaction combinations were incubated at room temperature in a dark setting.

Absorbance was measured at 734 nm against a suitable blank using a UV–Vis spectrophotometer. Radical scavenging activity (%) and IC_{50} values (µg/mL), representing the concentration required to decrease ABTS·⁺ absorbance by 50%, were determined from concentration–response curves. Antioxidant activity was compared with standard antioxidants BHT, BHA, and Trolox.

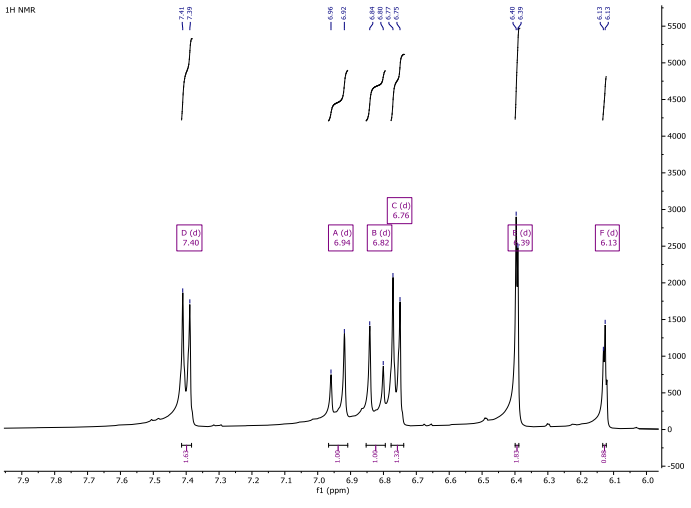
Results

The isolated secondary metabolite (Resveratrol) was obtained as an off- yellowish amorphous powder (10 mg). The chemical structure was rigorously confirmed as trans-resveratrol using 1D NMR (^{1}H and ^{13}C) spectroscopy.

The ^{1}H NMR (400 MHz, DMSO-d_6) spectrum exhibited characteristic signals of a stilbene framework. The aromatic protons of the resorcinol ring appeared as a doublet at δ 6.39 (J = 2.1 Hz, 2H, H-2,6) and a doublet at δ 6.13 (J = 2.2 Hz, 1H, H-4). The AA'BB' spin system of the para-substituted phenol ring was observed at δ 7.40 (J = 8.7 Hz, 2H, H-2',6') and δ 6.76 (J = 8.6 Hz, 2H, H-3',5'). The trans configuration of the olefinic double bond was confirmed by the large coupling constant (J = 16.3 Hz) of the signals at δ 6.94 and 6.82, corresponding to the vinylic protons (Figure 1).

Figure 1

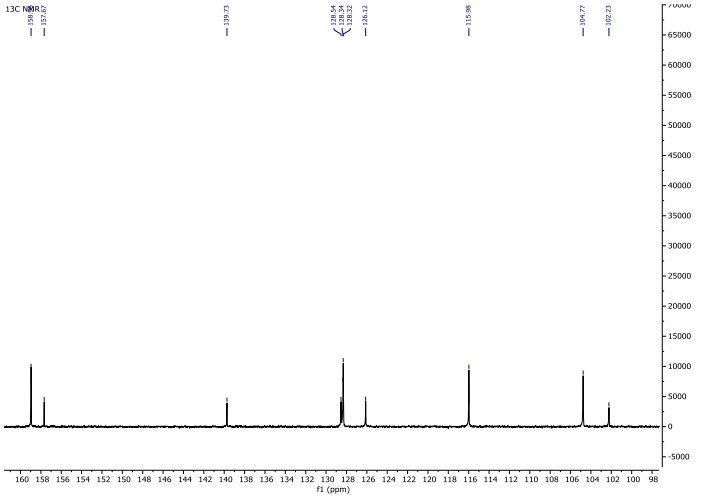
The ^{1}H-NMR (DMSO-d6, 400 MHz) spectrum of resveratrol.



The ^{13}C NMR spectrum further supported the proposed structure, displaying ten distinct carbon signals (including equivalent carbons). Key chemical shifts were recorded at δ 158.96 (C-3,5), 157.67 (C-4'), 139.73 (C-1), 128.54 and 128.32 (C-2,6 and C-α,β), 126.12 (C-1'), 115.98 (C-3',5'), 104.77 (C-2,6), and 102.23 (C-4). These data are in excellent agreement with the literature values reported for trans-resveratrol (Figure 2).

Figure 2

The ^{13}C-NMR (DMSO-d6, 100 MHz) spectrum of resveratrol



The antioxidant capacity of the isolated resveratrol was assessed using DPPH and ABTS radical scavenging assays and compared with standard antioxidants, including Trolox, BHA, BHT, and ascorbic acid.

In the DPPH assay, the isolated resveratrol demonstrated notable radical scavenging activity, with an IC_{50} value of 17.33 ± 1.22 µg/mL. Although this activity was lower than that of BHA (8.01 ± 0.42 µg/mL) and Trolox (7.98 ± 0.68 µg/mL), it still indicated a considerable ability to neutralize DPPH radicals relative to many naturally occurring phenolic compounds (Table 1).

In contrast, the ABTS assay revealed stronger antioxidant performance. The isolated resveratrol exhibited an IC_{50} value of 6.84 ± 0.47 µg/mL, reflecting greater potency than Trolox (7.22 ± 0.24 µg/mL), ascorbic acid (8.22 ± 0.37 µg/mL), and BHT (9.19 ± 0.94 µg/mL), and a comparable activity to the synthetic antioxidant BHA (6.54 ± 0.21 µg/mL). The enhanced activity observed in the ABTS system suggests that the resveratrol isolated from grape seeds may be particularly effective in electron-transfer-based radical scavenging processes (Table 1).

Discussion

The isolated compound obtained from grape seeds was identified as trans-resveratrol based on detailed NMR spectroscopic analysis. In the ^{1}H NMR spectrum, the olefinic protons displayed a large coupling constant (J = 16.3 Hz), which is characteristic of a trans-configuration. In contrast, the corresponding cis-isomer typically exhibits significantly smaller coupling constants (about J = 5 Hz) (Aydin et al., 2014).

Table 1

DPPH and ABTS antioxidant activities of resveratrol

Sample and standards	DPPH IC_{50} (µg mL^{-1})	ABTS IC_{50} (µg mL^{-1})
Resveratrol	17.33±1.22	6.84±0.47
Trolox	7.98±0.68	7.22±0.24
BHA	8.01±0.42	6.54± 0.21
BHT	10.59±0.81	9.19±0.94
Askorbic acid	9.88±0.91	8.22±0.37

The total number and location of hydroxyl groups on the aromatic rings of phenolic subtances significantly influence their antioxidant activity. Resveratrol outperformed Trolox in the ABTS tests (IC_{50} = 6.84 ± 0.47 µg/mL), possibly due to its ability to participate in multi-step electron transfer pathways. Gülçin (2010) reported that the para-hydroxyl group in conjunction with the conjugated stilbene backbone enhances stabilization of the resulting phenoxyl radical via resonance, thereby improving radical scavenging efficiency in aqueous–organic systems (Gülçin, 2010).

The obtained resveratrol showed an IC_{50} value of 17.33 ± 1.22 µg/mL in the DPPH experiment. Despite showing relatively less activity than BHA and Trolox, this value is nevertheless quite competitive when compared to numerous naturally occurring stilbenoids. The difference between the DPPH and ABTS values is in accordance with earlier findings (Gözcü & Atmaca, 2025; Samad et al., 2014). According to Floegel et al. (2011), the DPPH test can be more affected by steric accessibility and structural limitations surrounding the radical site, while the ABTS assay is sensitive to both hydrophilic and lipophilic antioxidants (Floegel et al., 2011).

Notable is the isolated resveratrol's higher activity in the ABTS system when compared to ascorbic acid (IC_{50} = 8.22 µg/mL) and BHT (IC_{50} = 9.19 µg/mL). According to these results, grape seeds grown in the Erzincan area could be a good source of bioactive trans-resveratrol. According to earlier research on Vitis vinifera cultivars, environmental stressors, such as those found in high-altitude areas like Eastern Anatolia, can promote the production of secondary metabolites as a defense mechanism for plants, which may increase the phytochemical content and activity (Coklar, 2017).

Resveratrol's antioxidant activity can be attributed to its capacity to operate via both the Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) pathways. According to Truong et al. (2018), the 4'-hydroxyl group is the most reactive site. Its strong activity across various in vitro antioxidant models is facilitated by its synergistic interaction with the 3,5-dihydroxy (resorcinol) moiety, which effectively neutralizes reactive oxygen species (ROS) (Kovacic & Somanathan, 2010).

Conclusion and Recommendations

In this study, trans-resveratrol was successfully isolated from grape seeds obtained in Erzincan and structurally confirmed by ^{1}H and ^{13}C NMR spectroscopy, with the large olefinic coupling constant (J = 16.3 Hz) clearly indicating the trans configuration. The isolated compound demonstrated significant *in vitro* antioxidant activity, particularly in the ABTS assay, where its potency was comparable to BHA and superior to Trolox, BHT, and ascorbic acid, while also exhibiting competitive activity in the DPPH assay. The variation between assay results suggests the involvement of both hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms, supported by the structural features of resveratrol, especially the 4-hydroxyl group and conjugated stilbene system that enhance radical stabilization. Overall, these findings indicate that grape seeds from the Erzincan region represent a promising natural source of bioactive trans-resveratrol with considerable antioxidant potential for nutraceutical and pharmaceutical application

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