

Comparative Phytochemical Profiling of Three Turkish Viburnum Species Collected from Different Altitudes

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ABSTRACT

Viburnum species are valuable to pharmaceutical and food industries owing to their rich polyphenols and traditional medicinal use. In this study, *Viburnum opulus* (870 m), *V. orientale* (1300 m), and *V. lantana* (1850 m), collected from different altitudes, were analyzed for volatile profiles by GC-MS, phenolic and flavonoid composition by HPLC, and antioxidant capacity. The species exhibited distinct chemical profiles: dominant volatiles were D-limonene in *V. orientale* leaves, methyl 3-methylbutanoate in *V. opulus* fruit, and benzaldehyde in *V. lantana* fruit. HPLC confirmed species-specific signatures, with *V. opulus* rich in (+)-catechin and chlorogenic acid, *V. orientale* in rutin, and *V. lantana* in L-ascorbic acid. *V. opulus* showed the highest total phenolic and flavonoid contents and antioxidant capacity. Compound accumulation was associated with both species identity and collection altitude. These signatures indicate that the three taxa are differentially rich sources of bioactive compounds, with potential for targeted pharmaceutical and nutraceutical applications.

Keywords: Antioxidant Activity, Essential Oils, Phenolic, *Viburnum*

Farklı Rakımlardan Toplanan Üç Türk Viburnum Türünün Karşılaştırmalı Fitokimyasal Profillemesi

ÖZ

Viburnum türleri, zengin polifenolik içerikleri ve geleneksel tıptaki kullanımları nedeniyle farmasötik ve gıda endüstrileri için değerlidir. Bu çalışmada farklı rakımlardan toplanan *Viburnum opulus* (870 m), *V. orientale* (1300 m) ve *V. lantana* (1850 m) türlerinin uçucu yağ profilleri GC-MS, fenolik ve flavonoid bileşimleri HPLC ile analiz edilmiş ve antioksidan kapasiteleri değerlendirilmiştir. Türler belirgin kimyasal profiller sergilemiştir: baskın uçucu bileşenler *V. orientale* yaprağında D-limonen, *V. opulus* meyvesinde metil 3-metilbutanoat, *V. lantana* meyvesinde benzaldehit olarak belirlenmiştir. HPLC analizleri türe özgü imzaları doğrulamış; *V. opulus* yüksek (+)-kateşin ve klorojenik asit, *V. orientale* yüksek rutin, *V. lantana* ise belirgin L-askorbik asit içeriğiyle öne çıkmıştır. *V. opulus* en yüksek toplam fenolik, flavonoid ve antioksidan kapasite değerlerini göstermiştir. Bileşik birikiminin hem tür kimliği hem de toplama rakımıyla ilişkili olduğu belirlenmiştir. Bu fitokimyasal imzalar, üç türün farklı biyoaktif bileşik sınıfları açısından zengin kaynaklar olduğunu ve hedefe yönelik farmasötik ile nötrösötik uygulamalar için potansiyel taşıdığını göstermektedir.

Anahtar Kelimeler: Antioksidan Aktivite, Esansiyel Yağlar, Fenolik, *Viburnum*

INTRODUCTION

The genus *Viburnum* (Adoxaceae) comprises approximately 160-230 species of shrubs and small trees that hold significant importance both as ornamental plants and for their applications in traditional and modern medicine [1]. These species are widely distributed across temperate regions of the Northern Hemisphere, with particular abundance in Asia and Central America. In the Southern Hemisphere, *Viburnum* populations are more restricted, occurring mainly in the mountainous regions of Southeast Asia, with some isolated species in southern South America [2].

The biomedical importance of *Viburnum* species is attributed to their abundance of bioactive compounds, particularly flavonoids and phenolic acids in fruits, leaves, and bark, which demonstrate potent antioxidant activity. Within the Turkish flora, four species have been identified: *V. lantana*, *V. opulus*, and *V. orientale*, which are considered native, and *V. tinus*, which is characteristic of the Mediterranean coast and is not regarded as endemic in some sources. *V. orientale* is generally distributed in Eastern and North-Eastern Anatolia [3]. Among these, *V. opulus*, commonly known as "gilaburu" in Turkey, has traditionally been used for the treatment of various ailments, ranging from gastric spasms to kidney stones. Its antimicrobial, antioxidant, anti-inflammatory, and potential neuroprotective effects have been confirmed in

several scientific studies [4]. In Turkish folk medicine, *V. lantana* species is utilized as an analgesic [5]. Chlorogenic acid, a phytochemical component found in the fruits, leaves, and branches of *V. orientale*, has been demonstrated to possess antioxidant and enzyme-inhibiting properties [6]. Research on *Viburnum* species has evolved through several stages over time. Initial studies predominantly focused on basic morphological and anatomical traits. These included fruit number, size, shape, weight, and density [7, 8], as well as detailed examination of anatomical structures such as the endocarp [9, 10]. Following these foundational characterizations, more recent studies have concentrated on the functional compound content and antioxidant potential of *V. lantana*, and *V. opulus*, *V. orientale*. For instance, fruit juices of *V. opulus* have been extensively analyzed across different genotypes using various antioxidant assays such as DPPH, ABTS, FRAP, ORAC, and the Folin–Ciocalteu method [11]. Similarly, aqueous extracts from the fruit, leaf, and branch parts of *V. opulus* and *V. lantana* have been evaluated for their DPPH and superoxide anion scavenging activities, revealing significant variability in antioxidant capacity depending on the plant part used [12]. Beyond antioxidant capacity, several other parameters, such as phenolic and anthocyanin content, volatile oil composition, ascorbic acid (vitamin C) levels, fatty acid profiles, and mineral contents, have been assessed in genotypes collected from various geographical regions [4, 13-15]. These analyses have contributed to a more comprehensive understanding of how environmental factors influence the phytochemical composition of these species. Research on *V. orientale* has

primarily focused on investigating its neuroprotective potential, inspired by its use in folk medicine. It was reported that the leaf, fruit, and branch extracts of this species exhibited strong inhibition of acetylcholinesterase and tyrosinase enzymes, along with pronounced antioxidant activity [6]. These findings suggest that *V. orientale* could be a promising candidate for preventing neurodegenerative disorders. Comparative studies have also characterized the histological and phytochemical profiles of *V. opulus* and *V. lantana*. The pericarp structure of both species was examined, and in that particular study, *V. opulus* specimens were reported to possess higher levels of carotenoids, flavonoids, steroids, and pectin compared to *V. lantana* samples examined, though such comparisons may vary across different populations and sampling conditions [16].

Most of the existing literature has focused on either fruit or leaf extracts alone, or has been limited to a single analytical perspective such as antioxidant capacity or total phenolic content. However, comprehensive and integrative studies comparing multiple plant parts and species (particularly *V. opulus*, *V. orientale*, and *V. lantana*) remain scarce. Therefore, the present study was designed to simultaneously evaluate both fruit and leaf tissues of *V. opulus*, *V. orientale*, and *V. lantana* in terms of volatile oil profiles, phenolic and flavonoid composition, and antioxidant capacity, with the aim of addressing this gap in the literature.

Material and Methods

Plant Materials

Samples of *V. opulus*, *V. orientale*, and *V. lantana* were harvested from various sites across Trabzon and Artvin provinces in Turkey throughout the blooming season (June–September, 2023–2025). Samples were collected from the same individual populations at each site across all sampling years; however, inter-annual variation in phytochemical composition was not assessed in the present study and may be considered in future research. Furthermore, since each species was collected from a single locality at a distinct altitude, the independent contributions of species identity and collection altitude to the observed phytochemical differences cannot be statistically disentangled. This represents an inherent limitation of the study design and should be considered when interpreting altitude-related comparisons throughout this work. *V. lantana* specimens originated from Velat Plateau forest clearings in Şavşat (Artvin) at an elevation of 1850 m, *V. orientale* from forest margins in Düzköy (Trabzon) at 1300 m, and *V. opulus* from riparian zones within Altındere Valley National Park, Maçka (Trabzon) at 870 m (Table 1). Taxonomic identification was performed by Dr. Nurşen AKSU KALMUK, with herbarium vouchers deposited at the Medical and Aromatic Plants Application and Research Center, Artvin Çoruh University. Geographic coordinates and collection site information are provided in Table 1. Following harvest, fruits and leaves were subjected to shade-drying under ambient conditions (23±2°C), mechanically pulverized using a laboratory grinder (Retsch GM200, Germany), and preserved at -20°C prior to subsequent analyses.

Table 1. Collection details of the examined *Viburnum* taxa

Species Name	Locality	Coordinates	Voucher	Altitude	Date
<i>V. lantana</i>	Şavşat, Velat Plateau, forest clearing	41°14'12.0"N 42°20'45.0"E	Aksu 465	1850 m	01.09.2023
<i>V. orientale</i>	Trabzon, Düzköy, forest edge	40°59'00"N 39°45'00"E	Aksu 381	1300 m	24.06.2024
<i>V. opulus</i>	Trabzon, Maçka, within Altındere Valley National Park, streamside	40°38'41.2"N 39°39'13.7"E	Aksu 430	870 m	30.06.2025

Methanolic Extracts

Methanol was selected for its capacity to solubilize a broad spectrum of polar and moderately nonpolar phytochemicals, ensuring comprehensive bioactive compound recovery, in accordance with previously reported extraction protocols [17, 18]. Dried plant powder (20 g) was extracted with 200 mL HPLC-grade methanol (Merck, Germany) at a 1:10 (w/v) ratio. Extraction commenced with ultrasonication (Fisherbrand, UK) at 600 W for 30 min to facilitate cellular disruption, followed by orbital shaking (Heidolph Unimax 1010, Germany) at 150 rpm for 24 h at room temperature in darkness to prevent photodegradation. Extracts underwent sequential filtration: initially through Whatman No. 1 filter paper, then through 0.45 μm PTFE syringe filters (MilliporeSigma, USA) for complete particle removal. Clarified extracts were stored at 4°C until phenolic and antioxidant analyses. This integrated approach combining sonication, prolonged agitation, and dual filtration optimizes phytochemical transfer efficiency.

Headspace Solid Phase Microextraction Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS)

Powdered samples were placed in 20 mL vials (one-third capacity) and hermetically sealed with PTFE/silicone septa (Supelco). Prior to extraction, the SPME fiber underwent thermal conditioning at 250°C for 30-60 min to remove residual contaminants. Samples were equilibrated at 45°C for 15 min before fiber insertion into the vial headspace for 40 min to capture volatile metabolites. Subsequently, the fiber was introduced into the GC injector where thermal desorption occurred for 20 min, releasing adsorbed volatiles directly into the analytical system. Compound identification employed retention indices calculated against a C_7 - C_{30} n-alkane standard mixture.

Chromatographic separation utilized an Agilent GC system equipped with an HP-5MS ultra-inert column (30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness). Ultra-high purity helium ($\geq 99.99\%$) flowed at 1.0 mL/min with injector temperature at 250°C. The temperature gradient consisted of: 50°C for 2 min, ramped at 2.5°C/min to 150°C (held 5 min), then increased at 6.5°C/min to 250°C (held 1 min). Mass spectrometric detection employed electron impact ionization at 70 eV across m/z 35–500. The protocol was adapted from [19].

HPLC-DAD Quantification of Phenolic Compounds Chemicals and Reagents

HPLC-gradient acetonitrile and methanol were sourced from Sigma-Aldrich (St. Louis, MO, USA) and Merck KGaA (Darmstadt, Germany), respectively. Phenolic reference standards were obtained from Sigma-Aldrich.

Chromatographic Conditions

Phenolic profiling employed an Agilent 1260 Infinity II HPLC equipped with diode array detection, utilizing protocols adapted from [19, 20]. Two distinct methods were established:

Method A: Chromatographic separation employed an ACE 5 C18 column (250 $\times$ 4.6 mm, 5 μm) for analysis of ascorbic acid, gallic acid, catechin, epicatechin, trans-caffeic acid, vanillic acid, rutin, p-coumaric acid, rosmarinic acid, and quercetin. Mobile phases: (A) acetonitrile; (B) 1.5% aqueous acetic acid. Gradient profile: 0 min, 15% A; 29 min, 40% A. Additional parameters: 0.7 mL/min flow rate; 10 μL injection; 35°C; 250, 270, 320 nm detection.

Method B: Quantification of pyrogallol, chlorogenic acid, resveratrol, hesperetin, and baicalein using identical stationary phase. Elution system: (A) methanol and (B) 1.5% aqueous acetic acid; gradient profile: 10% A/90% B (initial), 40% A/60% B (29 min), 60% A/40% B (29-40 min), 90% A/10% B (40-53 min). Detection at 280, 290, 320, 370, and 535 nm with equivalent chromatographic conditions.

Calibration and Sample Processing

Six-point calibration curves (25-300 $\mu\text{g mL}^{-1}$) were constructed for each analyte to establish linearity and retention parameters. Plant samples (5 g) underwent extraction with 50 mL solvent mixture—comprising acetonitrile and 1% acetic acid (9:1, v/v) combined with methanol (1:1, v/v)—using ultrasonication (30 min) followed by 24 h dark incubation at ambient temperature. Clarified extracts were analyzed directly by HPLC-DAD.

Total Phenolic and Flavonoid Content

All absorbance measurements for total phenolic content, total flavonoid content, and antioxidant capacity assays (FRAP and CUPRAC) were performed using an Agilent Cary 3500 UV-Vis spectrophotometer equipped with multicell holder and multizone temperature control (Agilent Technologies, Santa Clara, CA, USA).

Total phenolic content (TPC) was quantified via the Folin-Ciocalteu assay [21] against gallic acid calibration standards (0.03125–1 mg mL^{-1}). Briefly, extract or standard solution (20 μL at 1 mg mL^{-1}) was combined with 400 μL Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) (0.5 N) and 680 μL distilled water. Following 3 min equilibration, 400 μL sodium carbonate (Merck Chemical Co. in Darmstadt, Germany) (10% Na_2CO_3) was added and the mixture incubated for 2 h at 25°C. Absorbance readings at 760 nm were converted to mg gallic acid equivalents (GAE) per g dry weight.

Total flavonoid content (TFC) employed the aluminum chloride colorimetric method [22]. Reaction mixtures containing extract or quercetin standards (0.03125–1.0 mg mL^{-1}) with aluminum chloride (Sigma-Aldrich, St. Louis, MO, USA) reagent were incubated for 30 min before spectrophotometric measurement at 415 nm.

Results were expressed as mg quercetin equivalents (QE) per g dry weight.

Antioxidant Capacity

Ferric reducing antioxidant power (FRAP) was evaluated [23] by monitoring Fe^{3+} -TPTZ (Sigma Chemical Co. in St. Louis, MO, USA) reduction to its ferrous form under acidic conditions. Sample or blank (100 μL) was combined with FRAP reagent (3 mL), and absorbance monitored at 593 nm for 4 min at 25°C. Reducing capacity was calculated from a $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Merck, Germany) calibration curve (100–1000 $\mu\text{mol L}^{-1}$) and expressed as $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O}$ equivalents per g dry weight.

Cupric reducing antioxidant capacity (CUPRAC) was assessed [24] by measuring Cu(II) reduction to Cu(I) in the presence of neocuproine (Merck Chemical Co. in Darmstadt, Germany) chromogenic reagent. The reaction system comprised 10 mM CuCl_2 , 7.5 mM neocuproine, and 1 M ammonium acetate (Merck Chemical Co. in Darmstadt, Germany) buffer (pH 7.0; 1 mL each), supplemented with 0.2 mL sample and 0.9 mL distilled water. Following 60 min incubation, absorbance was recorded at 450 nm and results reported as mM Trolox® (Sigma Chemical Co. in St. Louis, MO, USA) equivalents using a 0.1–1.0 mM standard series.

Statistical Analysis

Data represent mean $\pm$ standard deviation from triplicate determinations. Statistical evaluation employed one-way ANOVA using IBM SPSS Statistics version 27, with post-hoc comparisons conducted via Duncan's multiple range test. Significance threshold was set at $p < 0.05$.

Results and Discussion

Essential oil profiles of *Viburnum* species

HS-SPME-GC-MS analysis of volatile compounds from fruit and leaf tissues of *V. orientale*, *V. opulus*, and *V. lantana* revealed distinct species-specific profiles (Tables 2-7).

D-Limonene was the predominant volatile in *V. orientale*, comprising 34.16% of leaf and 24.18% of fruit volatiles (Tables 2, 3). This monoterpene exhibits documented antioxidant, anti-inflammatory, antimicrobial, and neuroprotective activities. Fruit tissue additionally contained substantial ester fractions: ethyl 3-methylbutanoate (32.60%) and methyl 3-methylbutanoate (7.89%). Leaf tissue exhibited greater chemical diversity, including 2,3,6-trimethyl-1,5-heptadiene, o-cymene, β -pinene, methyl salicylate, and furan derivatives,

suggesting organ-specific differences in volatile compound accumulation between fruit and leaf tissues, consistent with previously reported tissue-dependent variation in *Viburnum* species [3].

V. opulus fruit displayed a highly specific volatile profile dominated by three esters: methyl 3-methylbutanoate (61.29%), ethyl 3-methylbutanoate (29.33%), and 3,5-dimethyl-1H-pyrazole (9.38%) (Table 4). This chemotaxonomic signature contrasts sharply with the diverse leaf composition, where hexanal (24.95%) and γ -terpinene (21.01%) predominated alongside cyclofenchene, isophorone, dihydroedulan II, and methyl salicylate (Table 5).

V. lantana fruit was characterized by benzaldehyde (39.43%), 2-ethyloxetane (24.56%), and 3-methylbutanal (10.76%) (Table 6). The exclusive presence of (E)-2-hexenal isomers, a rare cyclic ether, represents a potential chemotaxonomic marker. Leaf tissue contained hexanal (24.52%) and 2-hexenal isomers (24.45% and 7.46%), along with cyclofenchene (12.55%), cyclohexanol derivatives, and tetramethylcyclopentene (Table 7).

Volatile composition differences may reflect both species-specific metabolic pathways and altitude-associated environmental parameters. The stark contrast between our *V. opulus* samples (Altındere, 870 m) dominated by esters (methyl 3-methylbutanoate 61.29%, ethyl 3-methylbutanoate 29.33%) and previously reported Trabzon-Düzköy samples (higher elevation) rich in sesquiterpenes and diterpenes, α -cadinol (4.8%), phytol (7.8%), γ -cadinene (4.7%), trans- β -damascenone (4.9%) [3], suggests possible altitude-associated metabolic differences. This pattern suggests that *V. opulus* populations at lower elevations may preferentially allocate resources toward ester biosynthesis, whereas higher-altitude populations enhance terpenoid production as an adaptive response to increased UV radiation and temperature stress. Similarly, substantial geographic variation in organic acids and esters, suggesting that environmental factors may influence volatile profiles even within species. The elevated terpenoid content in *V. lantana* collected from Velat Plateau (1850 m)—the highest sampling site—further supports the observation that altitude may contribute to volatile diversity through modulation of ecological factors including temperature gradients, humidity, and light intensity. These findings suggest that volatile oil composition in *Viburnum* species may reflect the combined influence of species-specific genetic background and altitude-associated environmental factors, though further studies are needed to clarify the relative contribution of each.

Table 2. Chemical composition of the essential oils in fruit of the *Viburnum orientale*

No	RT (min)	RI	Name of the compound	Content [%]
1	3.63	689	2-methylbutanal	4.74
3	5,58	774	methyl 3-methylbutanoate	7.89
4	6.17	802	Hexanal	4.38
5	7.88	854	Ethyl 3-methylbutanoate	32.60
7	12.39	971	Benzaldehyde	6.46
7	13.75	1003	Sulcatone	6.67
8	15.63	1044	1-ethyl-2,3-dimethylbenzene	5.20
9	15.84	1049	D-Limonene	24.18
10	49.25	1865	Cadalene	1.28

Rt -Retention times on an HP-5MS UI column, RI - Experimentally determined retention indices on an HP-5MS UI column

Table 3. Essential oil composition of *Viburnum orientale* leaves

No	RT (min)	RI	Name of the compound	Content [%]
1	3.55	687	Isovaleraldehyde	0.50
3	3.66		α -methylbutanal	0.57
4	4.126	702	2-ethylfuran	2.855
5	5.45	767	Methyl 3-methylbutanoate	2.08
6	6.18	800	Hexanal	3.19
7	7.76	850	(E)-2-Hexenal	6.40
8	9.058	890	Methyl β -methylvalerate	0.25
9	10.93	937	Sabine ketone	1.46
10	11.18	943	α -Pinene	1.59
11	11.86	959	Camphene	1.39
12	12.19	967	6-methyl-2-Heptanone	0.24
13	12.41	972	Benzaldehyde	0.430
14	13.75	1003	2,6-Dimethyl 2,6-octadiene (cis)	1.67
15	13.94	1007	β -Pinene	5.55
16	14.46	1019	2,3,6-Trimethyl-1,5-heptadiene	9.75
17	14.78	1026	Cyclodecene	0.60
18	14.93	1029	m-Dichlorobenzene	0.44
19	15.22	1035	α -Terpinene	2.41
20	15.64	1044	o-Cymene	5.71
21	15.85	1049	D-Limonene	34.16
22	16.36	1060	β -Ocimene	2.94
23	16.63	1066	Phthalan	0.810
24	16.90	1072	β -cis-Ocimene	2.27
25	17.45	1084	γ -Terpinene	2.40

26	19.67	1132	Cyclofenchene	2.57
27	20.17	1143	2-methylbutyl 3-methylbutanoate	0.81
28	21.301	1167	Cosmene	0.52
29	21.94	1181	allo-Ocimene	0.49
30	24.78	1244	Methyl salicylate	0.890
31	27.08	1295	(3Z)-hex-3-en-1-yl pentanoate	0.502
32	27.38	1330	hexyl 3-methylbutanoate	0.450

Rt -Retention times on an HP-5MS UI column, RI - Experimentally determined retention indices on an HP-5MS UI column

Table 4. Chemical composition of the essential oils in fruits of the *Viburnum opulus*

Rt -Retention times on an HP-5MS UI column, RI - Experimentally determined retention indices on an HP-5MS UI column

No	RT (min)	RI	Name of the compound	Content [%]
1	5.61	775	methyl 3-methylbutanoate	61.29
2	7.24	834	3,5-dimethyl-1H-pyrazole	9.38
3	7.92	855	ethyl 3-methylbutanoate	29.33

Table 5. Chemical composition of the essential oils in leaves of the *Viburnum opulus*

Rt -Retention times on an HP-5MS UI column, RI - Experimentally determined retention indices on an HP-5MS UI column

No	RT (min)	RI	Name of the compound	Content [%]
1	4.07	699	2-ethylfuran	6.34
2	6.15	801	Hexanal	24.52
3	7.37	838	(E)-2-Hexenal	7.46
4	7.63	846	1,2,4,4-Tetramethylcyclopentene	2.13
5	7.85	853	(E)-2-Hexenal	24.45
6	8.11	861	cis-3,3,5-trimethylcyclohexanol	5.05
7	10.84	934	α -Pinene	5.24
8	11.18	943	Cyclofenchene	12.55
9	12.41	972	Benzaldehyde	9.76

Table 6. Chemical composition of the essential oils in fruit of the *Viburnum lantana*

No	RT (min)	RI	Name of the compound	Content [%]
1	3.03	674	2-ethyloxetane	24.56
2	3.51	686	Isovaleraldehyde	10.76
3	6.041	797	Hexanal	7.64
4	7.00	827	furan-2-carbaldehyde	2.65
5	7.27	835	3-methylbutanoic acid	4.98
6	10.63	929	α -Pinene	1.50
7	11.69	955	Benzaldehyde	39.43
8	13.06	987	2-Amylfuran	1.74
9	15.36	1238	Salicylaldehyde	3.05

Rt -Retention times on an HP-5MS UI column, RI - Experimentally determined retention indices on an HP-5MS UI column

Table 7. Chemical composition of the essential oils in leaves of the *Viburnum lantana*

No	RT (min)	RI	Name of the compound	Content [%]
1	5.66	778	Methyl α -methylbutanoate	4.11
2	6.21	803	Hexanal	24.95
3	9.35	899	2-Heptanone	2.29
4	9.68	907	Nonane	1.23
5	9.78	909	Heptanal	2.20
6	11.03	939	α -Pinene	6.18
7	11.20	943	γ -Terpinene	21.01
8	12,21	967	6-methyl-2-Heptanone	3.30
9	12.43	972	Benzaldehyde	1.52
10	13,98	1008	2-pentyl-furan	3.50
11	14.79	1026	α -Pinene (1R)	1.16
12	14.90	1028	3-Carene	7.04
13	15.88	1050	β -Terpinene	1.91
14	16.18	1056	1,1,3-Trimethyl-2-cyclohexanone	2.28
15	16.45	1062	3-Octen-2-one	2.36
16	17.51	1085	Isophorone	3.49
17	19.94	1138	Nonanal	5.36
18	24.78	1244	Methyl salicylate	1.66
19	29.80	1259	Dihydroedulan II	2.58

Rt -Retention times on an HP-5MS UI column, RI - Experimentally determined retention indices on an HP-5MS UI column

Content of Phenolic Compounds in *Viburnum* species

Since the structure of phenolic compounds directly influences their biological activity, identifying and quantifying individual phenolics is crucial. HPLC-DAD analysis of methanolic extracts from fruits and leaves of *V. opulus*, *V. lantana*, and *V. orientale* (Table 8) revealed statistically significant differences in phenolic profiles between species and tissues (one-way ANOVA, Duncan's multiple range test, $p < 0.05$).

Ascorbic acid (AsA) concentration was highest in *V. lantana* leaves (20.53 mg g⁻¹ DW), significantly exceeding all other samples. Among fruits, *V. opulus* exhibited the highest AsA level (12.22 mg g⁻¹ DW), followed by *V. orientale* (11.61 mg g⁻¹ DW). The elevated AsA content in leaves versus fruits likely reflects its role in neutralizing reactive oxygen species during photosynthesis. The pronounced AsA accumulation in *V. lantana* leaves from high-altitude Velat Plateau (1850 m) may represent an adaptive response to enhanced UV radiation and oxidative stress. Similarly, fruits from higher elevations have been shown to contain elevated vitamin C concentrations [25].

V. opulus fruit demonstrated the highest total phenolic (46.02 mg g⁻¹ DW) and flavanol (62.97 mg g⁻¹ DW) contents among examined species. (+)-Catechin (62.21 mg g⁻¹ DW) was the dominant compound in *V. opulus* fruit from Altındere Valley (870 m), with chlorogenic acid (43.02 mg g⁻¹ DW) ranking second. Previous studies reported substantially lower values on a fresh or dry weight basis: (+)-catechin (28.5–35.2 mg 100 g⁻¹ FW) and chlorogenic acid as 8–10-fold lower in fresh Erzincan fruit [8]; chlorogenic acid (204 mg 100 g⁻¹ FW) and (+)-catechin (29 mg 100 g⁻¹ FW) in commercial juice [26]; and chlorogenic acid (7.53 mg g⁻¹ DW) in Polish dried fruit [4]. Direct numerical comparison with the present study should be interpreted with caution due to differences in extraction methodology and geographic origin; however, the relatively higher concentrations observed here likely reflect: (i) enhanced extraction efficiency via ultrasonication (600 W, 30 min) combined with prolonged orbital shaking (24 h), (ii) genotypic variation across geographically distinct populations (Trabzon coastal vs. inland Erzincan and European sites), and (iii) altitude-associated metabolic differences, as moderate environmental stress at 870 m may facilitate

greater carbon allocation toward phenolic biosynthesis. Phenolic composition in *Viburnum* fruits has been demonstrated to be influenced by environmental and climatic factors [27].

(-)-Epicatechin was detected exclusively in *V. orientale* leaves from mid-altitude forest margins (1300 m) at high concentration (19.43 mg g⁻¹ DW), indicating species-specific accumulation. *V. opulus* fruit exhibited the

highest pyrogallol content (2.82 mg g⁻¹ DW), while *V. lantana* leaves contained the greatest resveratrol concentration (3.18 mg g⁻¹ DW). Notably, p-coumaric acid was absent in *V. opulus* leaves. These findings indicate that phenolic profiles are governed by both genetic determinants and environmental collection conditions, including altitude, UV exposure, and microclimate.

Table 8. Content of phenolic compounds in dried fruit and leaves of *Viburnum* species. Values are expressed as mean ± SD (n = 3) in mg g⁻¹ DW.

No	Compounds	<i>V. opulus</i>		<i>V. lantana</i>		<i>V. orientale</i>	
		Fruit	Leaf	Fruit	Leaf	Fruit	Leaf
Vitamin							
1	L-Ascorbic acid	12.22	5.61	6.78	20.53	11.61	11.09
Phenolics and related polyphenolic compounds							
2	Gallic acid	0.03	0.36	N/D	N/D	N/D	N/D
3	Vanillic acid	0.001	N/D	0.03	N/D	N/D	N/D
4	P-Coumaric Acid	N/D	N/D	1.26	N/D	0.19	0.48
5	Trans-Caffeic acid	0.10	N/D	N/D	N/D	N/D	N/D
6	Rosmarinic acid	0.06	0.07	0.17	0.07	0.11	0.08
7	Pyrogallol	2.82	0.05	0.56	N/D	0.41	N/D
8	Chlorogenic acid	43.02	25.56	N/D	27.11	31.91	16.51
9	Resveratrol	-	0.35	N/D	3.18	N/D	N/D
Flavonoids							
10	(+)-Catechin	62.21	1.03	48.25	0.46	0.82	24.84
11	(-)-Epicatechin	0.24	N/D	4.73	0.70	1.22	19.43
12	Rutin	0.11	N/D	2.79	0.16	40.70	15.52
13	Quercetin	0.41	0.40	0.40	0.40	0.42	N/D
14	Hesperetin	N/D	N/D	N/D	N/D	N/D	0.55
15	Baicalein	N/D	0.17	N/D	0.17	N/D	0.17

N/D: Not Detected

TPC and TFC in *Viburnum* species

Analysis of TPC and TFC contents in fruit and leaf tissues of different *Viburnum* species (Table 9) revealed statistically significant differences between species and plant parts (one-way ANOVA, Duncan's multiple range test, $p < 0.05$). *V. opulus* dried fruits exhibited the highest TPC value (20.72 ± 1.33 mg GAE g^{-1}), while the lowest TPC was found in *V. lantana* leaf extract (1.93 ± 0.37 mg GAE g^{-1}), indicating that *V. opulus* possesses a substantially richer phenolic and flavonoid composition compared to the other examined species.

The TPC values observed in the present study are considerably higher than those previously reported for *V. opulus* fruit extracts: 6.80–8.29 mg GAE g^{-1} [27] and 3.25 mg GAE g^{-1} [28]. This discrepancy likely reflects differences in extraction methodology, with our ultrasound-assisted extraction (600 W, 30 min) combined with prolonged orbital shaking (24 h) achieving superior phenolic recovery compared to conventional methods. Substantial phenolic variation has been documented among *V. opulus* genotypes, with reported values ranging from 5.47–10.61 mg GAE g^{-1} in fruit juice depending on cultivar [13], further demonstrating genotypic and methodological influence on phenolic quantification. Geographic origin and altitude may contribute additional variation to the observed phytochemical differences [25, 27]; however, since each species was collected from a single locality in the present study, the relative contributions of altitude, geographic origin, and genotypic background to phenolic accumulation cannot be statistically disentangled, and the mechanistic basis of these differences remains to be clarified in future comparative studies.

The highest TFC value was found in *V. opulus* leaf extract (3.04 ± 0.06 mg quercetin g^{-1}), while the lowest was recorded in *V. lantana* leaves (0.15 ± 0.02 mg quercetin g^{-1}). Comparative studies have reported that total flavonoid content in *V. opulus* fruits (0.1 – 0.5 mg 100 g^{-1}) exceeds that in *V. lantana* fruits (0.05 – 0.2 mg 100 g^{-1}) [27, 29], consistent with our findings. The presence of specific flavonoid classes, including quercetin, flavones, and catechins with demonstrated antioxidant activity, has been confirmed in both species [29, 30]. Flavonoids in fruit tissues serve critical photoprotective functions, mitigating UV-B-induced oxidative damage to photosynthetic systems and reinforcing cuticle layer integrity [31]. The elevated flavonoid accumulation in *V. opulus* compared to *V. lantana* may reflect species-specific adaptive strategies, with *V. opulus* consistently showed higher flavonoid accumulation across tissues compared to the other examined species [27, 29], whereas *V. lantana* at high altitude (1850 m) was associated with elevated ascorbic acid levels rather than flavonoids, suggesting species-specific differences in the predominant antioxidant compound class that may reflect both genetic background and environmental conditions [25].

Antioxidant Capacity in *Viburnum* species

Antioxidant capacities of fruit and leaf extracts from *V. opulus*, *V. orientale*, and *V. lantana* were evaluated using FRAP and CUPRAC methods, revealing clear species and tissue-dependent differences (Table 9). FRAP analysis demonstrated that *V. opulus* exhibited the highest ferric reducing capacity in both leaves (7.60 ± 0.16 μ mol Fe^{2+} g^{-1}) and fruits (7.61 ± 0.21 μ mol Fe^{2+} g^{-1}), significantly exceeding *V. orientale* (leaf: 7.23 ± 0.14 ; fruit: 7.19 ± 0.13 μ mol Fe^{2+} g^{-1}) and *V. lantana* (leaf: 6.47 ± 0.27 ; fruit: 6.76 ± 0.18 μ mol Fe^{2+} g^{-1}). CUPRAC assays yielded consistent results, with *V. opulus* showing the highest values in both leaves (0.08 mmol Trolox g^{-1}) and fruits (0.09 mmol Trolox g^{-1}), whereas *V. orientale* exhibited 0.03 mmol Trolox g^{-1} in leaves and 0.02 mmol Trolox g^{-1} in fruits, and *V. lantana* showed 0.02 mmol Trolox g^{-1} in both tissues.

These results suggest that *V. opulus* possesses higher reducing capacity and phenolic content compared to the other examined species, as assessed by FRAP and CUPRAC assays, consistent with previous studies employing online HPLC-UV-ABTS radical scavenging methods to characterize antioxidant components in *V. opulus* [32]. Comprehensive analyses comparing flower, bark, and fruit of *V. opulus* identified exceptionally high flavonoid content in bark (up to 2250 mg 100 g^{-1}) with correspondingly elevated FRAP and CUPRAC reducing activities [33].

Antioxidant efficacy is determined not only by quantity but also by structural characteristics of phenolic and flavonoid compounds. The superior reducing activity observed in *V. opulus* directly correlates with its rich phenolic profile and is consistent with previous comparative studies within the genus. Notably, the highest ferric reducing capacity was observed in the species collected from the lowest altitude (870 m), contradicting the common assumption that higher elevations invariably yield stronger antioxidant profiles. This suggests that intrinsic genetic capacity for phenolic accumulation may supersede altitude-driven induction. Similarly, variations in antioxidant activity among Turkish hazelnut cultivars were shown to depend more on genotype than altitude, with elevation not serving as a significant determinant independently [34]. Our results indicate that reducing capacity among *Viburnum* species is primarily determined by quantitative and qualitative differences in phenolic profiles, with altitude exerting a secondary modulatory role. This phenomenon reveals that while polyphenol and ascorbic acid enhancement may be anticipated in high-altitude environments to manage UV stress, certain species such as *V. opulus* develop superior ferric and cupric reducing capacity even under low-altitude conditions, potentially through constitutive rather than stress-induced biosynthetic pathways. Plants from all three localities exhibited significant reducing capacity within their respective ecological contexts: flavonoid accumulation in *V.*

orientale foliage and the rich ascorbic acid content of *V. lantana* (20.53 mg g⁻¹ DW in leaves) represent unique defense mechanisms adapted to their specific altitudinal and environmental conditions.

High-altitude solar radiation has been reported to increase reactive oxygen species production in plants, thereby stimulating the synthesis of antioxidant compounds [25]. The antioxidant profiles observed in *V. lantana* and *V. orientale* extracts were associated with differential accumulation of phenolic antioxidants and vitamins across collection altitudes [25]; however, whether these patterns represent direct biochemical responses to altitudinal stress or primarily reflect inherent interspecific variation cannot be determined from the present study design, as genotypic background has been shown to modulate altitude-dependent phytochemical responses in other species [34]. Altitude and local environmental conditions play important roles in shaping secondary metabolite levels and biological activities in *Viburnum* species; however, the extent and direction of these effects depend on each species' genetic background, resulting in species-specific phytochemical

accumulation patterns that differ in both composition and magnitude. The observation that *V. opulus* from low altitude exhibits the highest antioxidant capacity while *V. lantana* from high altitude prioritizes ascorbic acid accumulation demonstrates that distinct phytochemical accumulation patterns are associated with different altitudinal and environmental conditions across taxa, rather than reflecting a uniform directional response to elevation, consistent with observations reported for other plant species where the relationship between altitude and secondary metabolite accumulation was shown to be species- and genotype-dependent [25, 34].

Table 9. Comparative Evaluation of TPC, TFC, and Antioxidant Capacities (FRAP and CUPRAC) of Leaves and Fruit Extracts from Three *Viburnum* Species. The values shown are means with SD (n= 3). Values are presented as mean $\pm$ standard deviation (n= 3). For each species, means followed by different lowercase letters are significantly different according to Duncan's multiple range test (p < 0.05).

Table 9. Comparative Evaluation of TPC, TFC, and Antioxidant Capacities (FRAP and CUPRAC) of Leaves and Fruit Extracts from Three *Viburnum* Species. The values shown are means with SD (n= 3). Values are presented as mean $\pm$ standard deviation (n= 3). For each species, means followed by different lowercase letters are significantly different according to Duncan's multiple range test (p < 0.05).

Species	Organ	TPC (mg GAE/g)	TFC (mg Quercetin/g)	FRAP (μ mol Fe ²⁺ /g)	CUPRAC (mmol Trolox/g)
<i>V. opulus</i>	Leaf	12.53 $\pm$ 0.53 ^b	3.04 $\pm$ 0.06 ^a	7.6 $\pm$ 0.16 ^a	0.08
<i>V. opulus</i>	Fruit	20.72 $\pm$ 1.33 ^a	2.09 $\pm$ 0.11 ^b	7.61 $\pm$ 0.21 ^a	0.09
<i>V. lantana</i>	Leaf	1.93 $\pm$ 0.37 ^d	0.64 $\pm$ 0.08 ^c	6.47 $\pm$ 0.27 ^c	0.02
<i>V. lantana</i>	Fruit	2.27 $\pm$ 0.16 ^d	0.15 $\pm$ 0.02 ^e	6.76 $\pm$ 0.18 ^c	0.02
<i>V. orientale</i>	Leaf	3.39 $\pm$ 0.22 ^c	0.57 $\pm$ 0.02 ^c	7.23 $\pm$ 0.14 ^b	0.03
<i>V. orientale</i>	Fruit	2.96 $\pm$ 0.19 ^d	0.46 $\pm$ 0.05 ^d	7.19 $\pm$ 0.13 ^b	0.02

TPC: Total Phenolic Content; TFC: Total Flavonoid Content; FRAP: Ferric Reducing Antioxidant Power; CUPRAC: Cupric Reducing Antioxidant Capacity; GAE: Gallic Acid Equivalent

CONCLUSION

This study demonstrates that phytochemical composition and reducing capacity of *V. opulus*, *V. orientale*, and *V. lantana* vary among species collected from different altitudes, revealing species-specific phytochemical profiles rather than uniform environmental responses.

V. opulus from low elevation exhibited the highest reducing capacity among the examined species, with (+)-catechin and chlorogenic acid as dominant phenolic compounds. These findings suggest that species-specific factors, rather than altitude-driven stress induction alone, may contribute to the elevated phytochemical levels observed in this species; however, the mechanistic basis of these differences cannot be concluded from the present data and requires dedicated investigation incorporating gene expression or enzyme activity analyses.

V. orientale from mid-altitude showed elevated rutin and exclusive (–)-epicatechin accumulation alongside D-limonene-dominated volatiles. These phytochemical characteristics suggest that this species warrants further investigation for potential bioactive applications; however, enzyme inhibition assays, cell culture studies, or clinical trials would be required before any specific therapeutic application could be proposed.

V. lantana from the highest elevation showed notable ascorbic acid accumulation and contained 2-ethyloxetane as a potentially distinctive volatile marker. Its phytochemical profile is consistent with patterns reported for plants growing under conditions of elevated UV radiation and oxidative stress, though whether this reflects a direct adaptive response or constitutive species-level variation requires further investigation.

These species-specific phytochemical signatures suggest that *V. opulus* fruit, *V. orientale* foliage, and *V. lantana*

leaves represent differentially rich sources of distinct bioactive compound classes, flavanols and hydroxycinnamic acids, flavonol glycosides, and ascorbic acid, respectively, with potential relevance to targeted pharmaceutical and nutraceutical applications. The biochemical and regulatory mechanisms underlying these compositional differences cannot be resolved on the basis of phytochemical profiling alone and require dedicated investigation through metabolomic, enzymatic, and transcriptomic approaches. Both species-specific genetic background and altitude-associated environmental factors may jointly influence secondary metabolite accumulation in *Viburnum* species. Since each species was sampled from a single altitude, the relative contributions of species identity and altitude to the observed differences could not be statistically separated in this study. Geographic and altitudinal parameters should therefore be considered when selecting *Viburnum* germplasm for phytochemical studies. Future research incorporating metabolomic, enzymatic, and bioactivity analyses across multiple populations and altitudes would provide a more comprehensive understanding of these patterns. Inter-annual variation across the same populations should also be investigated in future studies.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried.

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