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RESEARCH ARTICLE

Chemical Composition and Biological Activities of *Malus domestica* Burkh. 'Ak Sakı' Cultivar Growing in Erzincan

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ABSTRACT

The chemical composition and biological activities of *Malus domestica* 'Ak Sakı' fruits cultivated in Erzincan, Türkiye, were analyzed. The aroma profile of the fruits was investigated for the first time. This study aimed to identify the major volatile and phenolic compounds and determine their antioxidant, antimicrobial, and cytotoxicity properties to investigate the potential health benefits of this cultivar. GC-MS analysis identified major volatile compounds, including 1-hexanol (20.54%), benzaldehyde (13.49%), and 1-octanol (6.68%). LC-MS/MS analysis of phenolic compounds in the ethyl acetate fraction revealed high levels of chlorogenic acid (12 802.67 μg/g), catechin (8601.52 μg/g), and quercetin (805.96 μg/g). Among the tested extracts, the ethyl acetate extract exhibited the highest antioxidant activity (47.18 ± 0.92 μg/mL), total phenolic content (70.17 ± 0.28 mg GAE/g extract), and reducing power (90.17 ± 0.71 mg TE/g extract), followed by butanol and diethyl ether extracts. Antimicrobial tests demonstrated varying inhibition zones, with volatile oil being the most effective. In addition, extracts exhibit cytotoxicity potential. The diethyl ether fraction shows the most potent selective cytotoxicity against A549 lung cancer cells. The findings indicate that the 'Ak Saki' cultivar contains diverse bioactive compounds with strong antioxidant, cytotoxicity, and antimicrobial properties. This fruit may contribute to the nutritional and health value of the daily diet.

1 | Introduction

Apples (*Malus domestica* Borkh.) are among the most popular fruits worldwide and are valued for their sensory qualities, nutritional value, and the presence of bioactive compounds, including phenolics, flavonoids, and volatile constituents [1–4]. Beyond sugars, organic acids, vitamins, and dietary fiber, apples represent a major dietary source of phytochemicals—particularly phenolic acids, flavonoids, procyanidins, and related polyphenols—whose biological activities have been associated with antioxidant, antimicrobial, antiproliferative, and anti-inflammatory effects, as well as potential benefits in cardiometabolic health and carcinogenesis-related pathways [5–10]. The health-promoting properties of apples are therefore largely attributed to these bioactive constituents and their ability to modulate oxidative status, inflammatory responses, and cell signaling processes [11].

Among apple phytochemicals, phenolic compounds constitute one of the most widely studied groups due to their high content and broad bioactivity. Typical apple phenolics include hydroxycinnamic acids (e.g., chlorogenic acid), flavan-3-ols (catechin, epicatechin, and procyanidins), flavonols (quercetin glycosides), and dihydrochalcones (e.g., phloridzin). The relative distribution of these compounds is strongly tissue-dependent; the fruit skin

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generally contains higher concentrations of flavonols, while the flesh contains higher proportions of hydroxycinnamic acids, and this distribution is influenced by genotype, agronomic conditions, and postharvest factors. Recent cultivar-focused studies confirm that chlorogenic acid, catechin/epicatechin, and quercetin derivatives are among the major contributors to apple phenolic profiles, while traditional or local varieties can contain higher polyphenol concentrations than conventional cultivars [12].

This compositional variability has practical implications for both functional food development and bioactivity-guided utilization. Apple matrices and extracts with high phenolic content have been repeatedly associated with strong in vitro antioxidant capacity, but their biological relevance also includes the modulation of cellular oxidative stress and inflammation-related responses, in addition to radical scavenging. In addition, the recovery and selectivity of phenolic subclasses depend heavily on solvent polarity and extraction strategy, which is critical when aiming to relate chemical composition to biological effects. Comparative studies on apple matrices (including pomace) indicate that the extraction method and solvent system significantly influence the phenolic composition and concentration, with specific conditions favoring higher concentrations of chlorogenic acid, quercetin glycosides, and dihydrochalcones [13].

In parallel with non-volatile polyphenols, apple quality and acceptability are closely associated with volatile organic compounds (VOCs) that define aroma and flavor. Apple volatiles comprise a complex mixture of esters, alcohols, aldehydes/ketones, and terpenoid-related constituents, and their sensory characteristics depend on both their concentration and chemical composition. Large profiling studies and reviews report that esters account for the largest proportion of total volatiles in many cultivars, contributing fruity notes, while alcohols and aldehydes are associated with "green/fresh" sensory attributes; odor-active compounds commonly include C6 alcohols and aldehydes, together with characteristic esters such as ethyl butanoate and ethyl 2-methylbutanoate [14].

Importantly, volatile constituents are not only relevant for sensory properties: several VOCs and associated apple-derived phytochemicals have been implicated in antimicrobial effects, indicating potential value for natural preservation and health-oriented applications. Previous studies have shown that apple polyphenols can inhibit the growth of human pathogens, with activity often associated with flavan-3-ols and phenolic acids. Previous studies on apple skin polyphenols have reported inhibitory activity against bacterial pathogens, indicating that cultivar-dependent differences in phenolic composition can be associated with measurable antimicrobial effects [15]. Recent mechanistic and applied studies have also focused on specific apple polyphenols (e.g., phloretin) and their antibacterial and inflammation-related effects, supporting continued interest in apple-derived compounds as multifunctional bioactives [16].

Beyond antioxidant and antimicrobial activities, apple phytochemicals have been reported to exhibit cytotoxic/antiproliferative and chemopreventive potential. Multiple investigations have reported that apple extracts (varying by cultivar and extraction method) can modulate cell viability and inflammatory signaling in cell-based models, while comprehensive cultivar-focused studies have demonstrated cytotoxic or antiproliferative activity across different cancer cell lines, together with anti-inflammatory effects [17]. While these effects depend on dose, matrix, and composition, they provide a rationale for profiling cultivar-specific extracts and testing them against relevant model systems such as lung cancer cell lines.

Türkiye is an important region for apple cultivation and hosts many local cultivars that grow under specific ecological and agronomic conditions. Such local genetic resources are increasingly regarded as promising candidates for functional food and nutraceutical research, as genotype–environment interactions can result in characteristic metabolic profiles. In this context, the 'Ak Sakı' cultivar grown in Erzincan, Türkiye, represents a traditional variety with local consumption and potential for value-added applications. However, although apples have been widely studied and global interest in cultivar-based bioactive profiling has increased, integrated datasets that combine (i) detailed volatile characterization, (ii) phenolic quantification, and (iii) a multi-assay biological activity panel (antioxidant, antimicrobial, and cytotoxicity/selectivity) are still limited for many local cultivars.

Accordingly, the present study focuses on the 'Ak Sakı' cultivar for two main reasons. First, aroma profiling is essential to characterize its sensory-related VOC composition and to compare it with patterns reported in other cultivars. Second, coupling phenolic profiling with biological assays provides an opportunity to interpret composition–activity relationships and supports future consideration of functional food applications, natural antimicrobial agents, or bioactivity-guided product development. Therefore, the present study aimed to provide an integrated investigation of the chemical composition and biological activities of *Malus domestica* 'Ak Sakı' fruits cultivated in Erzincan, Türkiye. Volatile constituents were characterized by GC–MS, and phenolic compounds in different solvent fractions were quantitatively profiled by LC–MS/MS. In addition, antioxidant capacity was determined using complementary assays, while antimicrobial activity was tested against selected bacterial strains, and cytotoxic activity was investigated in a lung cancer model (A549) alongside a non-cancerous cell line to determine selectivity. These results contribute to the scientific characterization of the 'Ak Sakı' cultivar as a source of bioactive compounds with relevance to food and health-related research.

2 | Results and Discussion

2.1 | GC-MS Profile of Aroma Content of 'Ak Sakı' Cultivar

The volatile composition of the *Malus domestica* 'Ak Sakı' cultivar was characterized using gas chromatography–mass spectrometry (GC–MS), revealing a complex and cultivar-specific aroma profile. The identified compounds, together with their retention times, retention indices, and relative contents, are presented in Table 1, and the corresponding GC–MS chromatogram is provided in Figure S1. In total, 31 volatile compounds were identified, accounting for 94.97% of the total chromatographic area. This finding is consistent with previous reports indicating that apple cultivars typically contain around 20–50 detectable volatile

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TABLE 1 | Aroma profile of the 'Ak Sakı' cultivar of *Malus domestica*.

Comp. No.	RT	RI[a]	RI[b]	Component	% Composition[c]
1	5.09	867	870	**1-Hexanol**	**20.54 ± 0.29**
2	7.34	963	960	**Benzaldehyde**	**13.49 ± 0.33**
3	7.63	973	970	1-Heptanol	0.80 ± 0.06
4	8.32	990	994	Butanoic acid, butyl ester	0.62 ± 0.05
5	10.49	1075	1073	**1-Octanol**	**6.68 ± 0.24**
6	11.41	1108	1102	Nonanal	3.21 ± 0.03
7	13.91	1192	1191	Butanoic acid, hexyl ester	3.24 ± 0.04
8	15.16	1234	1236	Hexyl 2-methylbutanoate	2.50 ± 0.09
9	15.74	1260	1262	9-Decen-1-ol	1.00 ± 0.08
10	15.85	1288	1285	7-Decenal	0.92 ± 0.04
11	16.23	1295	1297	Nonanoic acid	0.60 ± 0.06
12	18.25	1538	1535	Nerolidol	1.10 ± 0.05
13	19.13	1582	1383	Hexanoic acid, hexyl ester	4.49 ± 0.21
14	22.47	1622	1620	2-Hydroxy-tetradecanoic acid	0.86 ± 0.07
15	22.79	1725	1727	(2E,6E)-Farnesal	1.07 ± 0.08
16	23.27	1744	1740	Cis-farnesol	1.83 ± 0.11
17	23.88	1778	1771	Octyl octanoate	3.41 ± 0.15
18	26.95	1796	1796	Tetradecanoic acid ethyl ester	1.34 ± 0.10
19	27.03	1808	1803	**(Z,Z)-2,6-Farnesol**	**6.21 ± 0.31**
20	30.85	1824	1821	3,7,11-Trimethyl-dodeca-2,6,10-trienoic acid	0.85 ± 0.06
21	30.96	1858	1853	Nerolidol-epoxy acetate	0.79 ± 0.05
22	31.84	1973	1977	Hexadecanoic acid	0.64 ± 0.04
23	32.53	2000	2000	Eicosane	1.10 ± 0.08
24	34.37	2100	2100	Heneicosane	1.81 ± 0.11
25	35.09	2144	2141	9-Octadecenoic acid	2.32 ± 0.14
26	36.13	2159	2155	Ethyl linoleate	2.34 ± 0.07
27	37.82	2178	2177	9,12-Octadecadienoic acid methyl ester	0.55 ± 0.04
28	38.75	2200	2200	Docosane	1.50 ± 0.11
29	38.83	2300	2300	Tricosane	0.85 ± 0.07
30	39.44	2400	2400	Tetracosane	0.64 ± 0.05
31	43.22	2800	2800	**Octacosane**	**7.67 ± 0.20**
Alcohols					29.02
Esters					18.49
Aldehydes					17.62
Terpenes					11.00
Hydrocarbons					18.84
Total identified					94.97

[a]Retention index determined on an HP-5MS column using the homologous series of n-alkane (C8–C30).
[b]Retention index values obtained from the NIST webbook.
[c]Major compounds (> 5%) highlighted in bold.

compounds, depending on genotype, analyzed tissue, maturity stage, and analytical methodology [14, 18]. The volatile profile of 'Ak Sakı' consisted mainly of alcohols (29.02%), followed by aldehydes (17.62%) and esters (18.49%), with additional contributions from terpenes (11.00%) and hydrocarbons (18.84%). This distribution is consistent with biochemical processes involved in apple aroma formation, including lipid oxidation associated with the lipoxygenase system and enzymatic reduction and esterification reactions [19–21]. In contrast, the proportion of alcohols relative to esters in 'Ak Sakı' is different from that reported for many

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widely cultivated apple cultivars, in which profiles with a high ester content are commonly observed at full ripeness [14, 19].

Among the identified volatiles, 1-hexanol represented 20.54% of the total volatile fraction. C6 alcohols such as 1-hexanol are widely associated with fresh, green, and grassy sensory attributes in apples and are consistently reported among the volatile compounds identified across numerous cultivars [14, 19, 22]. The proportion of 1-hexanol observed in 'Ak Sakı' is consistent with the presence of lipid-derived volatiles and with fresh and green sensory attributes in the overall aroma profile. The presence of other alcohols, including 1-octanol (6.68%), further contributes to aroma complexity and supports previous findings that alcohol-rich profiles are cultivar- and condition-dependent [21, 23, 24].

Aldehydes constituted 17.62% of the total volatile fraction, with benzaldehyde representing 13.49% of this group. Benzaldehyde has been described as having an almond-like aroma and has been reported in several apple cultivars, generally at lower relative concentrations than aliphatic aldehydes such as hexanal and (E)-2-hexenal [14, 19]. The elevated benzaldehyde level observed in 'Ak Sakı' therefore represents a distinguishing feature of this cultivar and provides a chemical basis for its almond-like and floral sensory notes. In addition to its aroma-related attributes, benzaldehyde has been reported to show antimicrobial and antioxidant activity in vitro; however, the detection of this compound does not indicate a direct biological effect of the fruit [25, 26].

Esters represented 18.49% of the total volatile composition and are commonly associated with fruity and sweet aroma attributes in apples. Major esters identified included hexanoic acid, hexyl ester (4.49%), butanoic acid, hexyl ester (3.24%), hexyl 2-methylbutanoate (2.50%), and octyl octanoate (3.41%). These compounds are frequently reported as important aroma-active esters in apples and are associated with pleasant fruity and sweet notes [14, 27, 28]. When compared with cultivars such as "Fuji" or "Gala," the ester content of 'Ak Sakı' shows a different relative proportion, with fruity and sweet aroma attributes reported together with green and aromatic characteristics, consistent with reports indicating that ester biosynthesis is influenced by cultivar genetics and ripening conditions [19, 29–31].

Terpenoid compounds constituted 11.00% of the volatile fraction and included (Z,Z)-2,6-farnesol (6.21%), cis-farnesol (1.83%), (2E, 6E)-farnesal (1.07%), and nerolidol (1.10%). Although terpenes are typically minor constituents of apple aroma, they contribute floral, woody, and spicy nuances and are often associated with cultivar-specific characteristics [19, 32, 33]. Farnesol and its derivatives have also been reported to exhibit antimicrobial and biofilm-inhibitory activities in microbiological studies, suggesting that their presence may add functional interest to the 'Ak Sakı' cultivar, although such effects cannot be inferred directly from volatile composition data alone [34–37].

Hydrocarbons constituted part of the 'Ak Sakı' volatile profile, including long-chain n-alkanes, particularly octacosane (7.67%). Eicosane, heneicosane, docosane, tricosane, tetracosane, and octacosane have been reported as components of apple cuticular wax, and their detection suggests the presence of peel-derived compounds in the analyzed fraction [38–40]. Long-chain hydrocarbons and fatty acid derivatives such as hexadecanoic acid and unsaturated C18 acids have been associated with surface protection, reduced water loss, and postharvest behavior of apples, suggesting that these compounds may influence storage performance and fruit quality [41, 42].

The volatile composition of 'Ak Sakı' includes alcohols associated with fresh and green sensory attributes, benzaldehyde and terpenes associated with almond-like and floral attributes, and esters associated with fruity sweetness. Compared with widely cultivated commercial apples such as "Honeycrisp" and "Fuji," differences are reported in the relative composition of volatile classes, with ester- and aldehyde-rich profiles described for "Honeycrisp" and higher proportions of acetate esters reported for "Fuji" [43, 44]. Large-scale comparative studies across multiple apple cultivars report that, while certain volatiles are ubiquitous, the relative proportions of aromatic aldehydes, terpenes, and wax-associated hydrocarbons vary among cultivars [14, 33].

Overall, the volatile composition of the 'Ak Sakı' apple includes alcohols, aldehydes, esters, terpenes, and long-chain hydrocarbons. 1-Hexanol and benzaldehyde were detected together with farnesol-related sesquiterpenes and wax-associated aliphatic compounds in this cultivar. These characteristics highlight its potential value for aroma-driven product differentiation and provide a strong foundation for future studies integrating sensory analysis, peel–pulp differentiation, and bioactivity investigations.

2.2 | Phenolic Profile of 'Ak Sakı' Cultivar

The LC-MS/MS analysis of hot water extract fractions of the 'Ak Sakı' cultivar revealed considerable variations in phenolic composition depending on the extraction solvent. Table 2 presents a quantitative analysis of these extracts, while Figure S2 shows the LC-MS/MS TIC chromatograms of the 'Ak Sakı' cultivar. The EtOAc fraction exhibited the highest phenolic content, with chlorogenic acid (12 802.67 μg/g extract), epicatechin (11 672.23 μg/g extract), catechin (8601.52 μg/g extract), and quercetin (805.96 μg/g extract) detected. The ButOH fraction, while showing lower overall phenolic concentrations, contained detected levels of chlorogenic acid (6191.45 μg/g extract), rutin (856.23 μg/g extract), quercetin (440.42 μg/g extract), and apigenin (194.99 μg/g extract). In contrast, the DEE fraction contained lower levels of phenolic compounds and included *p*-coumaric acid (2983.52 μg/g extract) and ferulic acid (614.43 μg/g extract). Salicylic acid, hesperidin, rosmarinic acid, ellagic acid, pinocembrin, chrysin, and galangin, classified in flavonoids and phenolic acids, were not detected in all fractions. Interestingly, protocatechuic acid, gentisic acid, and syringic acid were found at moderate levels, particularly in the EtOAc fraction. The observed differences suggest that EtOAc is the optimal solvent for recovering flavonoids and organic acids. These findings showed that the 'Ak Sakı' apple is a valuable natural source of polyphenols, which may be used in functional foods and nutraceuticals.

A comparison with the literature highlights similarities and differences in polyphenol distribution. Similarly, recent LC–MS/MS-based studies have reported that chlorogenic acid, catechin derivatives, and flavonoids are among the major phenolic

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TABLE 2 | LC-MS/MS profile of fractions of the 'Ak Sakı' cultivar of *Malus domestica*.

No	Compound name	µg Phenolics/g Extract		
		DEE fraction	EtOAc fraction	ButOH fraction
1	Gallic acid	549.49	173.74	10.79
2	Protocatechuic acid	812.90	344.31	30.82
3	Protocatechuic aldehyde	105.68	10.25	< LOQ
4	Gentisic acid	34.59	258.46	< LOQ
5	Catechin	4421.36	8601.53	153.05
6	Chlorogenic acid	4733.14	12 802.67	6191.45
7	Epicatechin	4653.64	11 672.23	436.54
8	Caffeic acid	80.62	825.74	40.86
9	Vanillin	NF	NF	NF
10	Syringic acid	< LOQ	552.94	129.91
11	*p*-Coumaric acid	2983.52	120.56	< LOQ
12	Taxifolin	439.01	121.45	< LOQ
13	Ferulic acid	614.43	25.33	< LOQ
14	Salicylic acid	NF	NF	NF
15	4-Hydroxybenzoic acid	NF	NF	NF
16	Hesperidin	NF	NF	NF
17	Rosmarinic acid	NF	NF	NF
18	Luteolin-7-*O*-glucoside	< LOQ	70.49	212.41
19	Rutin	< LOQ	125.46	856.23
20	Ellagic acid	NF	NF	NF
21	Naringenin	< LOQ	46.25	80.02
22	Quercetin	< LOQ	805.96	440.43
23	Luteolin	< LOQ	52.12	102.26
24	Apigenin	< LOQ	120.78	194.99
25	Pinocembrin	NF	NF	NF
26	Chrysin	NF	NF	NF
27	Galangin	NF	NF	NF

Abbreviations: < LOQ, below the limit of quantification; NF, not found.

constituents of bioactive plant extracts and are associated with their antioxidant and cytotoxic properties, supporting the phytochemical profile observed in the present study [45, 46]. Chlorogenic acid, a major hydroxycinnamic acid found in apples, is present at a high concentration in 'Ak Sakı' (12 802.67 µg/g extract), in agreement with reported values in other cultivars, where concentrations have been reported between 13.6 and 79.5 mg/100 g fresh weight (FW), particularly in organically grown apples [1]. The concentrations of epicatechin (11 672.23 µg/g extract) and catechin (8601.52 µg/g extract) in 'Ak Sakı' are significantly higher than those reported for other apple cultivars, where epicatechin has been reported between 120.00 and 1,740.00 µg/g extract and catechin between 140.00 and 3,210.00 µg/g [4]. Similarly, the concentrations of rutin (856.23 µg/g) and apigenin (194.99 µg/g) in 'Ak Sakı' are consistent with those reported for other apple cultivars, such as "Kirkitchoo" and "Prima" [4].

A distinguishing feature of 'Ak Sakı' is its high p-coumaric acid concentration (2983.52 µg/g extract), which is higher than the concentrations reported for other apple cultivars (5.43–70.00 µg/g extract). Additionally, ferulic acid (614.43 µg/g extract) is present in 'Ak Sakı,' although information on its distribution in apples is limited. Interestingly, phloridzin was not detected among the analyzed standards, although it has been reported as a major dihydrochalcone in other apple cultivars, with concentrations reported between 5.0 and 25.20 mg/100 g FW [1].

In addition, several polyphenols, including salicylic acid, hesperidin, rosmarinic acid, ellagic acid, pinocembrin, chrysin, and galangin, were not detected in any of the analyzed fractions. The presence of protocatechuic acid, gentisic acid, and syringic acid, particularly in the EtOAc fraction, indicates the importance of solvent selection in phenolic extraction.

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TABLE 3 | Antioxidant activity of the 'Ak Sakı' cultivar of *Malus domestica*.

Sample	DPPH˙ scavenging IC_{50} (μg/mL)	Total phenolic (mg GAE/g extract)	Total flavonoids (mg QE/g extract)	Reducing power (mg TE/g extract)
DEE fraction	95.11 ± 0.79[d]	47.06 ± 0.17[b]	7.81 ± 0.30[b]	63.14 ± 0.78[e]
EtOAc fraction	47.18 ± 0.92[c]	70.17 ± 0.28[a]	20.48 ± 0.46[a]	90.17 ± 0.71[d]
ButOH fraction	179.71 ± 1.15[e]	29.13 ± 1.17[c]	3.57 ± 0.25[c]	38.90 ± 0.94[f]
Trolox	8.50 ± 0.77[a]	—	—	—
BHA	8.04 ± 0.69[a]	—	—	338.57 ± 0.31[b]
BHT	10.70 ± 0.73[b]	—	—	257.80 ± 1.24[c]
Ascorbic acid	9.91 ± 0.87[ab]	—	—	394.17 ±0.98[a]

Abbreviations: GAE, gallic acid equivalent; QE, quercetin equivalent; TE, trolox equivalent.
[a–f]Different letters in the same column indicate statistical difference ($p < 0.05$).

Overall, the high catechin and epicatechin concentrations, together with the high chlorogenic acid and *p*-coumaric acid concentrations, indicate a distinctive phenolic profile for 'Ak Sakı' compared with other commonly studied apple cultivars. These findings highlight its potential as a valuable natural source of bioactive polyphenols with promising applications in functional foods, nutraceuticals, and pharmaceutical formulations.

2.3 | Antioxidant Potential of 'Ak Sakı' Cultivar

The results indicate that, presented in Table 3, the EtOAc fraction of the 'Ak Sakı' cultivar exhibits the highest antioxidant activity among the tested fractions, as demonstrated by its lowest DPPH IC_{50} value (47.18 μg/mL), highest total phenolic content (70.17 mg GAE/g), highest flavonoid content (20.48 mg QE/g), and strongest reducing power (90.17 mg TE/g). These values differed significantly from those of the other fractions ($p < 0.05$). These findings suggest that the active compounds responsible for antioxidant activity, such as phenolics and flavonoids, are concentrated in the EtOAc fraction. The ButOH fraction shows moderate antioxidant activity, while the DEE fraction has the lowest activity, as reflected in its higher DPPH IC_{50} value (179.71 μg/mL) and lower phenolic, flavonoid, and reducing power values. Although the fractions obtained from the hot water extract of the 'Ak Saki' cultivar of *Malus domestica* exhibit significant antioxidant potential, their activity is lower than that of synthetic antioxidants such as Trolox, BHA, BHT, and ascorbic acid, which exhibit lower IC_{50} values and higher reducing power. These results suggest that the EtOAc fraction could serve as a valuable natural source of antioxidants, potentially useful in food, cosmetic, or pharmaceutical applications.

A strong correlation was observed between LC-MS/MS phenolic composition and antioxidant activity. The EtOAc fraction, which exhibited the highest antioxidant activity, contained phenolic compounds such as chlorogenic acid, epicatechin, and catechin. These compounds have been reported in the literature for their antioxidant activity [47, 48] and are frequently included in antioxidant assays. Similarly, the ButOH fraction, with moderate antioxidant activity, contains significant but lower concentrations of phenolics like *p*-coumaric acid and ferulic acid. In contrast, the DEE fraction exhibited lower antioxidant activity and lower phenolic content, including chlorogenic acid and epicatechin. The presence of quercetin and rutin in the EtOAc and ButOH fractions also contributes to their antioxidant capacities. Overall, the data indicate that the antioxidant activity observed in the EtOAc fraction is associated with its phenolic composition, including chlorogenic acid, epicatechin, and catechin. Comparable findings have recently been reported for phenolic-rich plant extracts, in which fractions containing higher concentrations of phenolic acids and flavonoids exhibited stronger antioxidant activity than the other fractions [45].

The antioxidant activity of different apple cultivars has attracted considerable interest due to the health benefits associated with the widespread consumption of apples worldwide. The antioxidant potential of different apple cultivars has been widely investigated due to their health benefits and bioactive properties. Traditional and local apple varieties generally exhibit higher phenolic content and antioxidant capacity than recently commercialized cultivars [49]. This observation is further supported by research indicating that ancient apple cultivars often show higher phenolic content and antioxidant activity than standard commercial varieties, such as "Golden Delicious" [50]. In addition, certain local genotypes, especially red-fleshed varieties, exhibit significant antioxidant activities due to their high phenolic content and high anthocyanin content [51]. Similarly, the 'Ak Sakı' apple, a local variety, demonstrates an antioxidant capacity comparable to other traditional and commercial apple cultivars [52–54]. While our LC-MS/MS analysis focused on phenolic compounds, anthocyanins may also be present due to the red pigmentation of the 'Ak Sakı' apple. Anthocyanins, particularly cyanidin derivatives, are known for their strong radical-scavenging properties and their potential contribution to the antioxidant activity of the 'Ak Sakı' cultivar. Future studies incorporating anthocyanin profiling could provide a more comprehensive understanding of its bioactive potential.

2.4 | Antimicrobial Activity of Essential Oil and Extracts

The antimicrobial activity of fractions obtained from the 'Ak Sakı' cultivar of Malus domestica was determined based on inhibition zones (mm) measured by the disk diffusion method and minimum inhibitory concentration (MIC) values (μg/mL) obtained for sensitive strains. The inhibition zone diameters and

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TABLE 4 | The in vitro results on the antibacterial activity of the 'Ak Sakı' cultivar.

Bacteria	Essential oil	DEE fraction	EtOAc fraction	ButOH fraction	Imipenem[a]
P. aeruginosa	14 ± 1.3	11 ± 0.3	12 ± 0.6	7 ± 0.4	32 ± 2.2
E. coli	12 ± 0.2	10 ± 1.0	13 ± 1.2	ni	26 ± 1.5
S. enterica	11 ± 0.8	9 ± 0.3	9 ± 0.9	7 ± 0.5	32 ± 1.7
B. cereus	16 ± 1.6	9 ± 0.4	9 ± 0.7	7 ± 0.7	35 ± 1.4
S. aureus	12 ± 0.5	ni[b]	12 ± 0.6	7 ± 0.6	33 ± 1.3

[a]Positive control (Imipenem), inhibition zone diameters in mm (mean ± SD, $n = 3$).
[b]ni: no inhibition.

TABLE 5 | MIC (μg/mL) values of the 'Ak Sakı' cultivar.

Bacteria	Essential oil	DEE fraction	EtOAc fraction	ButOH fraction	Imipenem
P. aeruginosa	62.5	250	125	nt[a]	<3.81
E. coli	125	250	125	nt	3.81
S. enterica	250	nt	nt	nt	<3.81
B. cereus	31.25	nt	nt	nt	<3.81
S. aureus	250	nt	125	nt	<3.81

[a]nt: not tested (due to the absence of an inhibition zone in the preliminary disc diffusion assay).

MIC values of the fractions and essential oil are presented in Tables 4 and 5, respectively. The EtOAc fraction exhibits the broadest spectrum of activity, with inhibition zones ranging from 9 mm (*S. enterica* and *B. cereus*) to 13 mm (*E. coli*), and minimum inhibitory concentration (MIC) values of 125 μg/mL for *P. aeruginosa*, *E. coli*, and *S. aureus*. The essential oil demonstrates the strongest overall activity, particularly against *B. cereus* (16 mm, MIC: 31.25 μg/mL) and *P. aeruginosa* (14 mm, MIC: 62.5 μg/mL), followed by moderate activity against *E. coli* (12 mm, MIC: 125 μg/mL), *S. enterica* (11 mm, MIC: 250 μg/mL), and *S. aureus* (12 mm, MIC: 250 μg/mL).

The DEE fraction also exhibited antimicrobial activity against *P. aeruginosa* (11 mm, MIC: 250 μg/mL) and *E. coli* (10 mm, MIC: 250 μg/mL), while the ButOH fraction showed moderate activity against *P. aeruginosa*, *S. enterica*, *B. cereus*, and *S. aureus* (7 mm each; MIC values were not determined). Although antimicrobial activity was observed for these fractions and the essential oil, the antibiotic imipenem showed larger inhibition zones (26–35 mm) and lower MIC values (< 3.81 μg/mL for most bacteria). The findings suggest that the EtOAc fraction and essential oil of the 'Ak Sakı' cultivar contain bioactive compounds with both antimicrobial and antioxidant properties.

Numerous studies have reported the antimicrobial potential of apple extracts and essential oils, which are influenced by their polyphenolic composition, differences between cultivars, and storage conditions. Our findings are consistent with previous reports indicating that extracts and essential oils derived from apples exhibit antimicrobial activity. Apple seed extracts and essential oils have been reported to exhibit antibacterial and antifungal activities, indicating the potential of apple-derived oils as natural antimicrobial agents [55, 56]. The polyphenolic content of apples is closely linked to the antimicrobial properties of their extracts. Various apple cultivars contain polyphenolic compounds that contribute to the inhibition of microbial growth [57]. A positive correlation has been observed between phenolic content and antimicrobial activity across different apple varieties [58]. Our results support this correlation, as the EtOAc fraction, which contained the highest concentrations of chlorogenic acid (12 802.67 μg/g extract), epicatechin (11 672.23 μg/g extract), and catechin (8601.52 μg/g extract), exhibited the broadest antimicrobial activity among the solvent fractions, while the essential oil showed the strongest overall antimicrobial activity. Considering that the 'Ak Sakı' apple is commonly consumed by local populations, its antimicrobial activity suggests potential applications as a natural resource for the prevention or management of infections associated with these microorganisms.

2.5 | Cytotoxic Activity of the Fractions of 'Ak Sakı' Cultivar

The cytotoxic activity of 'Ak Sakı' extracts was determined based on IC_{50} values against A549 and Beas-2B cell lines (Table 6). The selectivity index (SI) was calculated as the ratio of the IC_{50} value in non-malignant cells to the IC_{50} value in cancer cells. An SI > 1 suggests preferential toxicity toward cancer cells, with higher values indicating increased selectivity and potential therapeutic relevance [59, 60].

The DEE fraction demonstrated the most potent cytotoxic effect against A549 cells, with an IC_{50} value of 12.19 μg/mL. The IC_{50} and SI values of the DEE fraction differed significantly from those of the other fractions ($p < 0.05$). In addition, its selectivity index (SI: 3.98) indicated a preferential cytotoxic effect toward cancer

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TABLE 6 | Cytotoxicity of the 'Ak Sakı' cultivar of *Malus domestica*.

Extracts	IC_{50} (μg/mL)[a]		$S_i^{(A549)}$[b]
	Beas-2B	A549	
ButOH fraction	97.32 ± 2.3[c]	171.98 ± 1.6[c]	0.56[b]
EtOAc fraction	173.92 ± 3.1[d]	364.78 ± 2.5[d]	0.47[c]
DEE fraction	48.53 ± 1.1[b]	12.19 ± 0.5[b]	3.98[a]
5-Flourouracil	18.11 ± 0.7[a]	6.64 ± 0.3[a]	2.72[a b]

[a]The test results were expressed as the mean of triplicate assays.
[b]SI (cell line): The selectivity index for the cell line.
[a–d] Different letters in the same column indicate statistical difference ($p < 0.05$).

cells. This selectivity was higher than that of 5-FU (SI: 2.72), suggesting a favorable ability to differentiate between cancerous and healthy cells, which is important for minimizing toxicity to healthy tissues during chemotherapy. The ButOH and EtOAc fractions exhibited moderate cytotoxicity, with IC_{50} values of 171.98 μg/mL and 364.78 μg/mL, respectively, and lower selectivity indices (SI: 0.56 and 0.47, respectively), reflecting non-selective cytotoxic effects. These findings indicate that the DEE extract of 'Ak Sakı' selectively inhibits A549 cancer cells and exhibits higher selectivity than 5-FU, supporting further investigation. Further studies may focus on cytotoxic mechanisms, including apoptosis-related processes, and on in vivo investigations of the extracts because conventional antineoplastic drugs are associated with severe side effects.

The cytotoxic activity (IC_{50}: 12.19 μg/mL) and selectivity (SI: 3.98) of the DEE fraction against A549 cells may be associated with its quantitative phenolic profile. According to the LC–MS/MS results, the DEE fraction contains chlorogenic acid (4,733.14 μg/g extract), epicatechin (4653.64 μg/g extract), catechin (4421.36 μg/g extract), and *p*-coumaric acid (2983.52 μg/g extract) as the major polyphenolic compounds. The concentration of p-coumaric acid was higher in the DEE fraction than in the EtOAc and ButOH fractions. Literature reports indicate that these major dietary phenolics and flavonoids exhibit pro-apoptotic and antiproliferative activities against A549 lung cancer cells. The presence of these monomeric catechins and hydroxycinnamic acids in the DEE fraction may be associated with its cytotoxic activity against cancer cells while showing lower toxicity toward non-malignant Beas-2B cells.

Recent studies have shown that the cytotoxicity of various apple cultivars is associated with their diverse phytochemical profiles and potential health benefits. Research indicates that specific cultivars exhibit different levels of cytotoxic effects on various cancer cell lines, which may be associated with differences in their polyphenol composition. In this context, extracts prepared from Costa Rican apple cultivars were found to exhibit significant cytotoxicity against tumor cell lines [61]. Similarly, the "Anna" cultivar of *Malus domestica* has been shown to exhibit significant cytotoxic effects against human breast and lung cancer cells, with IC_{50} values indicating effective doses as low as 33.12 μg/mL for MCF7 cell lines [62]. These reports and our findings are consistent with those of Lanzerstorfer et al., who reported that apple juice has strong chemopreventive effects on various human cancer cell lines [63]. The cytotoxic potential of apples is not limited to *Malus domestica*, as other apple species also exhibit promising antiproliferative properties. Previous studies have reported that red-fleshed apple hybrids (*Malus sieversii* f. *niedzwetzkyana*) exert cytotoxic effects against human cancer cell lines, with certain hybrids showing higher antiproliferative activity than commercial apple cultivars such as "Fuji" [51]. The therapeutic potential of bioactive compounds derived from apples is further highlighted by these findings, which also support the need for more research into their mechanisms of action and possible clinical applications.

3 | Conclusions

The findings of this study highlight the significant chemical and biological potential of the *Malus domestica* 'Ak Saki' cultivar grown in Erzincan, Türkiye. The comprehensive analysis of volatile and phenolic compounds demonstrates that this apple variety contains a diverse bioactive composition, with chlorogenic acid, catechin, and quercetin identified as the major phenolic compounds. The EtOAc fraction, in particular, exhibited the highest antioxidant capacity, suggesting its potential as a natural source of bioactive compounds. Additionally, the antimicrobial activity of the extracts and essential oils further supports their applicability in food preservation and natural therapeutics.

Considering the interest in functional foods and natural antioxidants in the food and pharmaceutical industries, the 'Ak Sakı' cultivar may have potential for dietary applications. Its strong antioxidant, antimicrobial, and selective cytotoxicity properties suggest that incorporating this apple variety into daily nutrition could contribute to overall health and well-being. Future studies focusing on the bioavailability and mechanistic pathways of its bioactive compounds would provide additional information on its potential use in nutraceutical and functional food formulations. The findings suggest that 'Ak Sakı' apples may have potential for inclusion in health-conscious dietary practices because of their bioactive composition.

4 | Experimental Section

4.1 | Plant Material

The apple samples (*Malus domestica* 'Ak Sakı') were collected in October 2024 from a local orchard in Elmaköy, Erzincan (39°47'12.7″ N and 39°21'21.0″ E), a region known for its historically high apple production. The fruits were harvested from 20-year-old trees belonging to a local producer. Since the plant material is a widely recognized cultivated variant, no wild voucher specimen number was deposited; however, the cultivar's authenticity was certified by Assoc. Prof. Dr. Halil İbrahim Öztürk. After collection, the apples were washed with tap water to remove surface contaminants and then stored at a temperature of +4°C until further processing.

4.2 | Preparation of Extracts and Essential Oils

The apples were sliced (2.5 kg) and subjected to hydrodistillation using a Neo-Clevenger apparatus for 3 h with 5 liters of distilled water. The collected essential oils were separated and properly

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stored. The methods used to extract bioactive compounds from apple cultivars are important for determining their bioactivity [64, 65]. Among these, liquid-liquid extraction offers several advantages, including high selectivity for target compounds, effective separation of metabolites with different polarities, and scalability for processing larger samples. Therefore, the remaining aqueous extract was cooled to room temperature, followed by liquid-liquid extraction using diethyl ether (DEE), ethyl acetate (EtOAc), and butanol (ButOH) (2 L each × 3), respectively. The solvents were then evaporated to dryness, and the resulting extracts were stored at +4°C until further analysis.

4.2.1 | GC/MS Analysis of Aroma Content

The analysis was performed using an Agilent 7890A GC system coupled to an Agilent 5975C mass selective detector with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) following previous reports [66]. Helium was used as the carrier gas at a 1.2 mL/min flow rate. The injector temperature was 250°C, and the oven temperature program was as follows: initial temperature set at 50°C (held for 2 min), ramped to 150°C at 5°C/min, then to 280°C at 10°C/min (held for 10 min). A 1 μL aliquot (5% in acetone) was injected in split mode (50:1). The mass spectrometer operated in electron ionization (EI) mode at 70 eV, with a scan range of 50–600 m/z. Ion source and interface temperatures were set at 280°C. Compound identification was performed using the NIST and Wiley spectral libraries, and retention indices were calculated with a homologous series of n-alkanes (C_8–C_{30}). The relative percentage of each compound was determined via peak area normalization, facilitating the identification of bioactive volatile constituents contributing to the characteristic odor of 'Ak Sakı' fruit and antimicrobial properties.

4.3 | Liquid Chromatography-Mass Spectrometry (LC-MS/MS) Analysis

The LC-MS/MS analysis was performed using a Dionex Ultimate 3000 LC system coupled to a Thermo Scientific TSQ Quantum Access Max triple quadrupole mass spectrometer according to the previous report [67]. The chromatographic separation was performed on an ODS HYPERSIL column (4.6 × 250 mm, 5 μm). The column oven operated at 30°C, and the mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (methanol). The flow rate was set at 0.7 mL/min, and the injection volume was 20 μL. The total run time, including the equilibration steps, was 34 min. A gradient elution program was used as follows: the mobile phase composition was 100% A and 0% B during the first minute, followed by a gradual transition to 5% A and 95% B at 22 min, with the same composition continuing until 25 min. At 30 min, the mobile phase composition changed to 0% A and 100% B to ensure optimal separation of the analytes.

Mass spectrometric detection was performed in both positive and negative heated electrospray ionization (H-ESI) modes. Data acquisition and processing were performed using Xcalibur software. The method was optimized for the identification and quantification of bioactive compounds present in the apple extracts. The optimized SRMs (selected ion monitoring), LOQ and LOD concentrations, and dose-response curves were given in our previous report [67].

4.4 | Antibacterial Activity

The antibacterial activity of the essential oil and extracts was determined using the disc diffusion method [68–70] and minimum inhibitory concentration (MIC) [71, 72] assays. Five bacterial strains were used: three Gram-positive [*Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 10876)] and three Gram-negative [*Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076)]. For disc diffusion, 100 μL of each bacterial suspension (10^8 CFU/mL) was inoculated onto PCA plates. Samples (5 mg/mL) dissolved in 10% DMSO (5 μL) were spiked on sterile discs. An imipenem disc was used as a positive control. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured with a digital caliper.

MIC values were determined using a resazurin-based 96-well microdilution method with slight modifications. Only the fractions/extracts showing significant antibacterial activity (inhibition zone diameter ≥ 10 mm) in the disc diffusion assay were selected for MIC determination. 50 μL of sterile Nutrient Broth was added to wells in rows two to ten. 100 μL of test sample was added to row one, followed by two-fold serial dilutions (final concentrations: 1000–1.95 μg/mL). Row eleven received 30 μL broth and 30 μL tetracycline (1000 μg/mL) as a positive control, while row 12 received 30 μL of broth and 30 μL of 10% DMSO as a negative control. Then, 10 μL of bacterial suspension (1×10^8 CFU/mL) and 30 μL of 0.02% resazurin were added to each well. Plates were incubated at 37°C for 6 h. MIC was recorded as the lowest concentration with no visible color change, indicating inhibition of bacterial growth.

4.5 | Cytotoxicity Assay

Adenocarcinoma human alveolar basal epithelial (A549, ATCC CCL-185) and non-malignant human bronchial epithelial (Beas-2B, ATCC CRL-9609) cell lines were used to determine the cytotoxic activity of the extracts. The cells were cultured in DMEM supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum, and 1% penicillin–streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. The cells were monitored using an inverted microscope and were subcultured at the appropriate confluency.

The cytotoxic activity of the extracts was determined using the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] [73]. Cells were added to 96-well microplates at a density of 5,000 cells per well and incubated for 24 h. The cells were then treated with increasing concentrations (10, 25, 50, 75, and 100 μg/mL) of the extracts for 24 h. After the treatment, the medium was removed from the wells, and MTT (1 mg/mL) was added before the cells were incubated at 37°C for 4 h. After incubation, the MTT solution was removed from the wells, and the formazan crystals were dissolved in DMSO. The optical density was measured at 550 nm using an ELISA reader. The absorbance of the control cells was considered to represent 100% viability. 5-FU was used as a positive control, applied at increasing concentrations (5, 10, 25, 50, 75, and 100 μg/mL). The IC_{50} values were calculated using CalcuSyn v.2.1 software based on time and dose [74].

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4.6 | DPPH Radical Scavenging Activity

The DPPH$^{\bullet}$ radical scavenging capacity of the extracts was determined following a previously described procedure [75–78]. Briefly, 3 mL solutions containing various concentrations (5-100 µg/mL) of test material were mixed with 1 mL of DPPH$^{\bullet}$ solution (0.26 mM). The mixtures were vortexed and incubated at RT for 30 min in the dark. The absorbance was measured at 517 nm using an ELISA reader. Radical scavenging activity was calculated as: RSA (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control reaction (without the test sample), and A_1 is the absorbance in the presence of the extracts or standards. The absorbance values were converted to % activity, and the IC_{50} (µg/mL) concentrations of the extracts were calculated. The results were compared with Trolox, butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), and ascorbic acid as a standard antioxidant [79]. All analyses were performed in triplicate, and results are expressed as mean ± standard deviation.

4.7 | Ferric Reducing Antioxidant Power (FRAP)

The FRAP of the extracts was determined following a previously described procedure [80–83]. Stock solutions of extracts (1 mg/mL) were prepared, and 250 µL was mixed with phosphate buffer (0.2 M, pH 6.6) to a total volume of 1.25 mL. Then, 1.25 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] was added, and the mixture was incubated at 50°C for 20 min. After incubation, 1.25 mL of 10% TCA solution and 0.25 mL of 0.1% FeC_3 solution were added to the reaction mixture. The absorbance of the final mixture was measured at 700 nm. The results were reported as mg Trolox equivalent (TE) activity/g extract, using a calibration curve at different Trolox concentrations (10–100 µg/mL) [84]. The results were compared with Trolox, BHT, BHA, and ascorbic acid as standard antioxidants. All analyses were conducted in triplicate, and the results are presented as mean ± standard deviation.

4.8 | Total Phenolic Content (TPC)

The total phenolic content of the extracts was determined spectrophotometrically using the Folin-Ciocalteu reagent [85–88]. 100 µl of extract and standard solutions (1 mg/mL) were briefly diluted with 4.5 mL of distilled water. Then, 100 µL of Folin-Ciocalteu reagent was added to the mixtures, followed by 300 µL of 2% Na_2CO_3. After vortexing, the final mixtures were incubated for 120 min at room temperature. The absorbances were measured at 760 nm using a spectrophotometer. The calibration curve ($y = 0.117x - 0.011$) was generated using various concentrations of gallic acid (1–1000 µg/mL) [89]. The results, expressed as mg gallic acid equivalents (GAE) per gram of extract, were obtained from analyses performed in triplicate and are presented as mean ± standard deviation.

4.9 | Total Flavonoid Content (TFC)

The total flavonoid content of the extracts was determined following a previously described procedure [71, 84, 90, 91]. 100 µL of extract and standard solutions (1 mg/mL) were briefly mixed with 4.7 mL of methanol. Then, 100 µL of NH_4CH_3COO (1 M) solution and 100 µL of $AlCl_3$ (10%) were added. The mixtures were incubated for 45 min at room temperature after being vortexed. The absorbances were measured at 415 nm after the incubation. Results were expressed as mg quercetin equivalents (QE) per gram of extract [92, 93], using a calibration curve drawn with varied quercetin concentrations (1–1000 µg/mL). All tests were performed in triplicate, and the results are presented as mean ± standard deviation.

4.10 | Statistical Analysis

Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to compare the fractions, followed by Duncan's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

Author Contributions

Samed Şimşek: investigation, writing – review and editing, formal analysis, writing – original draft. **Büşra Demir Çetinkaya**: investigation, writing – review and editing, formal analysis. **Zeynep Akşit**: investigation, formal analysis, writing – review and editing. **Emircan Aydemir**: formal analysis, writing – review and editing. **Ömer Kayır**: investigation, writing – review and editing, formal analysis. **Hüseyin Akşit**: supervision, conceptualization, methodology, writing – original draft.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

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