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Mediterranean Asteraceae as Natural Sources of Antioxidants and Enzyme Inhibitors: A Case Study on *Urospermum dalechampii* and *Andryala integrifolia* From Tunisia

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ABSTRACT

This study investigated the phenolic composition, antioxidant capacity, and key enzyme-inhibitory activities of hydroethanolic leaf extracts from two Mediterranean Asteraceae, *Urospermum dalechampii* and *Andryala integrifolia*, from Tunisia. *U. dalechampii* exhibited higher total phenolic content (62.84 mg GAE/g crude extract), whereas *A. integrifolia* showed higher flavonoids (34.55 mg QE/g crude extract). The UHPLC-DAD-MS analysis revealed caffeoylquinic acid isomers, flavonols (quercetin and kaempferol types), and flavones (luteolin derivatives) in both species, while cynarine was detected in *U. dalechampii*. Some compounds were specific, such as caftaric acid, which was detected only in *U. dalechampii* leaf extract. Both species exhibited notable antioxidant capacities, with *U. dalechampii* showing stronger radical-scavenging activity (DPPH: EC_{50} = 0.95 mg/mL; ABTS: EC_{50} = 0.72 mg/mL), whereas *A. integrifolia* displayed higher reducing power (FRAP: EC_{50} = 5.40 mg/mL) and copper-chelating activity (EC_{50} = 1.72 mg/mL). *A. integrifolia* showed marked inhibition of tyrosinase (IC_{50} = 0.54 mg/mL), α-glucosidase (IC_{50} = 1.9 mg/mL), and lipase (IC_{50} = 9.7 mg/mL). Both leaf extracts from studied species inhibited AChE, while only *A. integrifolia* inhibited BChE (IC_{50} = 8.63 mg/mL). No cytotoxicity was detected at 100 µg/mL in human hepatocarcinoma (HepG2), neuroblastoma (SH-SY5Y), embryonic kidney (HEK-293), and murine melanoma (B16) cells. These results highlight these two plants as a promising source of multifunctional bioactive compounds and demonstrate that unexploited wild plants could offer new prospects for pharmaceutical and nutraceutical applications.

1 | Introduction

Global demand for plant-based products has grown steadily in recent years, driven by the search for safer, naturally derived alternatives for health promotion, disease prevention, and therapeutic applications [1, 2]. Consumer preference for plant ingredients is also reinforced by the undesirable effects and long-term safety of synthetic compounds, leading to increased scientific interest in natural bioactive molecules [3, 4].

This trend aligns with the rising prevalence of chronic diseases associated with oxidative stress and metabolic imbalance,

including neurodegenerative disorders, diabetes, obesity, and skin hyperpigmentation. Reactive oxygen species (ROS) driven oxidative damage plays a central role in the onset and progression of these pathologies [5, 6], highlighting the need for multifunctional natural agents capable of both counteracting oxidative stress and modulating disease-related biological pathways.

Within this context, medicinal and edible plants are recognized as valuable sources of active metabolites with antioxidant and enzyme-inhibitory properties [7, 8]. The Asteraceae family, one of the largest and most chemically diverse angiosperm families, is particularly known for its richness in phenolic acids, flavonoids, and sesquiterpene lactones supporting its traditional use in both folk medicine and nutrition [9, 10]. These metabolites are frequently associated with free-radical scavenging, metal-chelating capacity, and the inhibition of key enzymes linked to neurodegeneration (AChE, BChE), carbohydrate metabolism (α-glucosidase), lipid digestion (lipase), and melanogenesis/food browning (tyrosinase) [11].

In the Mediterranean region, Asteraceae species are traditionally used for nutritional and medicinal purposes and constitute an important component of regional ethnobotany. *Andryala integrifolia* and *Urospermum dalechampii* are two Mediterranean species that remain largely underexplored, despite reports on their nutritional relevance and preliminary phenolic content [10–12].

Andryala integrifolia is widely distributed across Mediterranean habitats, occupying sandy or rocky soils, agricultural margins, and ruderal environments [13]. *Urospermum dalechampii* is native to southern Europe and North Africa, growing in dry grasslands, roadsides, and disturbed soils up to 1,200 m altitude [14]. Their ability to thrive in nutrient-poor and stress-prone environments suggests enhanced production of defense-related secondary metabolites, including phenolic compounds, features of interest for bioprospecting and natural product discovery.

These species were selected due to their traditional dietary relevance, ecological adaptability, and accessibility, combined with the absence of comprehensive phytochemical and bioactivity data compared with other Asteraceae commonly investigated in phytochemistry [9]. However, no previous study has performed integrated phenolic profiling combined with antioxidant testing, enzyme inhibition, and cytotoxicity evaluation of these species.

Therefore, the aim of this study was to investigate the phenolic composition, antioxidant activity, and enzyme inhibitor potential of leaf extracts of *Andryala integrifolia* and *Urospermum dalechampii*. For this, hydroethanolic leaf extracts of these species were analyzed using spectrophotometric and UHPLC-DAD-MS assays. The antioxidant properties were investigated by four complementary assays, and the inhibitory activities against cholinesterases, α-glucosidase, lipase, and tyrosinase, as well as preliminary cytotoxicity tests, were conducted. This work provides the first comprehensive biochemical characterization of underexplored Mediterranean taxa.

2 | Results and Discussion

2.1 | Phytochemical Analysis

The phytochemical composition of the hydroethanolic leaf extracts was evaluated by determining their extraction yield and total contents of polyphenols and flavonoids (Figure 1).

The hydroethanolic extract of *U. dalechampii* showed the highest extraction efficiency (22.10%) and the greatest total phenolic content (62.84 mg GAE/g CE), indicating a higher overall abundance of phenolic constituents in this species. In contrast, *A. integrifolia* accumulated the highest total flavonoid content (34.55 mg QE/g CE), despite its lower extraction yield (16.75%).

Lower total phenolic and flavonoid contents were previously reported for these species using aqueous extracts of roots and aerial parts [12]. These differences can be mainly attributed to the extraction solvent and the plant organ analyzed. Hydroethanolic solvents are known to enhance polyphenol extraction compared with absolute organic solvents, while phenolic yields can vary substantially among plant organs due to tissue-specific metabolite distribution [15].

Indeed, an aqueous extract of *U. dalechampii* leaves collected in Italy showed a polyphenol content of 143 mg GAE/100 g dry weight, which is lower than the value reported in the present study (62.84 mg GAE/g dry extract). Similarly, the hydroethanolic extract of *Urospermum picroides* aerial parts exhibited lower phenolic and flavonoid contents (TPC: 35.22 mg GAE/g DW; TFC: 24.67 mg QE/g DW) [9] compared with the present results. Compared with other species within the Asteraceae family, *Centaurea stapfiana* showed a moderate phenolic content (32.17 mg GAE/g extract) in ethanol/water extracts [16]. Organ-specific analyses of *Cynara cardunculus* revealed TPC values ranging from 7 to 14.8 mg GAE/g dry matter [17], while ethanol leaf extracts of *Cichorium intybus* presented TPC values between 21.01 and 25.93 mg GAE/g extract weight [18].

These differences may arise from variations in genetic background, solvent systems, and extraction methodologies; nevertheless, they highlight the richness of these Mediterranean taxa in bioactive compounds [19]. The consistently high accumulation of polyphenols and flavonoids across different Asteraceae genera supports their relevance as natural sources of antioxidant and health-promoting metabolites [9]. Phenolic compounds and flavonoids are major classes of plant secondary metabolites widely recognized for their redox-active properties. Owing to these properties, plants represent a major source of natural antioxidants capable of counteracting free radical–mediated damage to essential cellular biomolecules, including lipids, proteins, and DNA, which is closely associated with the development of chronic degenerative diseases [11, 20].

To obtain a more detailed characterization of the phenolic profile, the extracts were further analyzed using UHPLC-DAD-MS (Table 1, supplementary material in Figure S). A total of 31 metabolites were identified at [M+Cl]- including 26 in *A. integrifolia* and 28 in *U. dalechampii*. Both species were dominated by hydroxycinnamic acid derivatives, particularly caffeoylquinic

TABLE 1 | Putative identification of metabolites in *A. integrifolia* and *U. dalechampii* by UHPLC-DAD-MS. The presence of compounds was categorized as −, + or ++ to indicate absent, low, or high abundance, respectively.

No.	RT (min)	Putative ID	m/z	Formula	Adduct	Library screening similarity	*A. integrifolia*	*U. dalechampii*
1	1.0842	Sucrose	377.0842	$C_{12}H_{22}O_{11}$	[M+Cl]−	0.982	+	++
2	2.9953	Gentisic acid 5-O-beta-glucoside	315.0710	$C_{13}H_{16}O_9$	[M−H]−	0.955	+	++
3	3.3474	Gentisic acid 5-O-beta-glucoside isomer	315.0710	$C_{13}H_{16}O_9$	[M−H]−	0.869	+	++
4	3.701	Neochlorogenic acid	353.0863	$C_{16}H_{18}O_9$	[M−H]−	0.995	++	+
5	3.8721	Caftaric acid isomer	311.0401	$C_{13}H_{12}O_9$	[M−H]−	0.894	—	+
6	3.9687	Caffeic acid 3-glucoside	341.0863	$C_{15}H_{18}O_9$	[M−H]−	0.962	+	++
7	4.6111	Cryptochlorogenic acid	353.0857	$C_{16}H_{18}O_9$	[M−H]−	0.914	+	++
8	5.0673	Chlorogenic acid	353.0867	$C_{16}H_{18}O_9$	[M−H]−	0.964	+	++
9	5.4587	Cynarine	515.1191	$C_{25}H_{24}O_{12}$	[M−H]−	0.992	—	+
10	5.4596	Coumaroyl quinic acid	337.0920	$C_{16}H_{18}O_8$	[M−H]−	0.769	+	++
11	5.5876	Isoschaftoside	563.1401	$C_{26}H_{28}O_{14}$	[M−H]−	0.921	+	—
12	6.1931	Rutin	609.1452	$C_{27}H_{30}O_{16}$	[M−H]−	0.981	+	++
13	6.361	Chicoric acid	473.0709	$C_{22}H_{18}O_{12}$	[M−H]−	0.934	+	++
14	6.3628	Caftaric acid	311.0401	$C_{13}H_{12}O_9$	[M−H]−	0.943	+	++
15	6.3719	Isoquercitrin	463.0849	$C_{21}H_{20}O_{12}$	[M−H]−	0.982	+	++
16	6.4451	Quercetin 3-O-glucuronide	477.0677	$C_{21}H_{18}O_{13}$	[M−H]−	0.947	+	++
17	6.5201	Luteolin 7-glucuronide	461.0715	$C_{21}H_{18}O_{12}$	[M−H]−	0.942	++	+
18	6.7023	Isochlorogenic acid C	515.1178	$C_{25}H_{24}O_{12}$	[M−H]−	0.984	+	++
19	6.7473	Quercetin 3-O-malonylglucoside	549.0876	$C_{24}H_{22}O15$	[M−H]−	0.911	—	+
20	6.8517	Avicularin	433.0761	$C_{20}H_{18}O_{11}$	[M−H]−	0.987	—	+
21	6.8575	Astragalin	447.0923	$C_{21}H_{20}O_{11}$	[M−H]−	0.955	+	++
22	6.9882	1,4-Dicaffeoylquinic acid	515.1174	$C_{25}H_{24}O_{12}$	[M−H]−	0.976	++	+
23	7.2044	3,4-Dicaffeoylquinic acid	515.1189	$C_{25}H_{24}O_{12}$	[M−H]−	0.996	++	+
24	7.2341	Flavone O-glucuronide	445.0772	$C_{21}H_{18}O_{11}$	[M−H]−	0.998	+	—
25	7.2791	Kaempferol 3-O-beta-D-xyloside	417.0821	$C_{20}H_{18}O_{10}$	[M−H]−	0.977	—	+
26	7.7799	3,4-Dicaffeoylquinic acid isomer	515.1184	$C_{25}H_{24}O_{12}$	[M−H]−	0.997	++	+
27	7.8765	Torachrysone 8-glucoside	407.1349	$C_{20}H_{22}O_9$	[M−H]−	0.625	+	—
28	7.8901	Caffeoyl–sinapoyl ester	559.1448	$C_{27}H_{28}O_{13}$	[M−H]−	0.991	+	—

(Continues)

TABLE 1 | (Continued)

No.	RT (min)	Putative ID	m/z	Formula	Adduct	Library screening similarity	*A. integrifolia*	*U. dalechampii*
29	8.5765	3,4,5-Tricaffeoylquinic acid	677.1487	$C_{34}H_{30}O_{15}$	[M−H]−	0.979	++	+
30	8.7757	Quercetin	301.0347	$C_{15}H_{10}O_7$	[M−H]−	0.950	—	+
31	8.8025	Luteolin	285.0395	$C_{15}H_{10}O_6$	[M−H]−	0.949	++	+

acids such as chlorogenic (8), neochlorogenic (4), and isochlorogenic acids (18). The *A. integrifolia* extract displayed a broad set of caffeoylquinic acid isomers, including mono-, di- and tricaffeoyl derivatives (23, 25, 28), whereas *U. dalechampii* showed a similar pattern but was distinguished by the exclusive presence of cynarine (9), a dicaffeoylquinic acid derivative. Caftaric acid isomer was also detected only in *U. dalechampii*, suggesting its potential use as a species-specific marker.

In terms of flavonoids, both extracts contained predominantly flavonol and flavone derivatives, mainly from quercetin, kaempferol, and luteolin. *A. integrifolia* was particularly rich in kaempferol 7-O-glucoside (24), luteolin 7-glucuronide (17), and luteolin (32), and also contained isoschaftoside (12) and a flavone O-glucuronide (24), indicating diversification into C-glycosylated and glucuronidated structures. In *U. dalechampii*, quercetin and kaempferol glycosides such as rutin (12), avicularin (20), astragalin (21), and kaempferol 7-O-glucoside (25) were prominent.

These differences indicate that each extract possesses a distinct spectrum of biological activity, driven by the diversity and relative abundance of phenolic acids and flavonoids. Plant-derived phenolic compounds are products of secondary metabolism and represent an important source of bioactive molecules with a wide range of biological effects, supporting their applications in food, cosmetic, and pharmaceutical contexts [21, 22]. In particular, caffeic acid derivatives, including caffeic acid 3-glucoside, 4-O-caffeoylquinic, cryptochlorogenic, chlorogenic, and neochlorogenic acids, which were abundant in the analyzed leaf extracts, have been associated with neuroprotective effects and potential relevance in Alzheimer's disease-related pathways [21, 23]. Similarly, flavonoids such as quercetin, isoquercetin, kaempferol, and rutin, identified in the studied extracts, are well

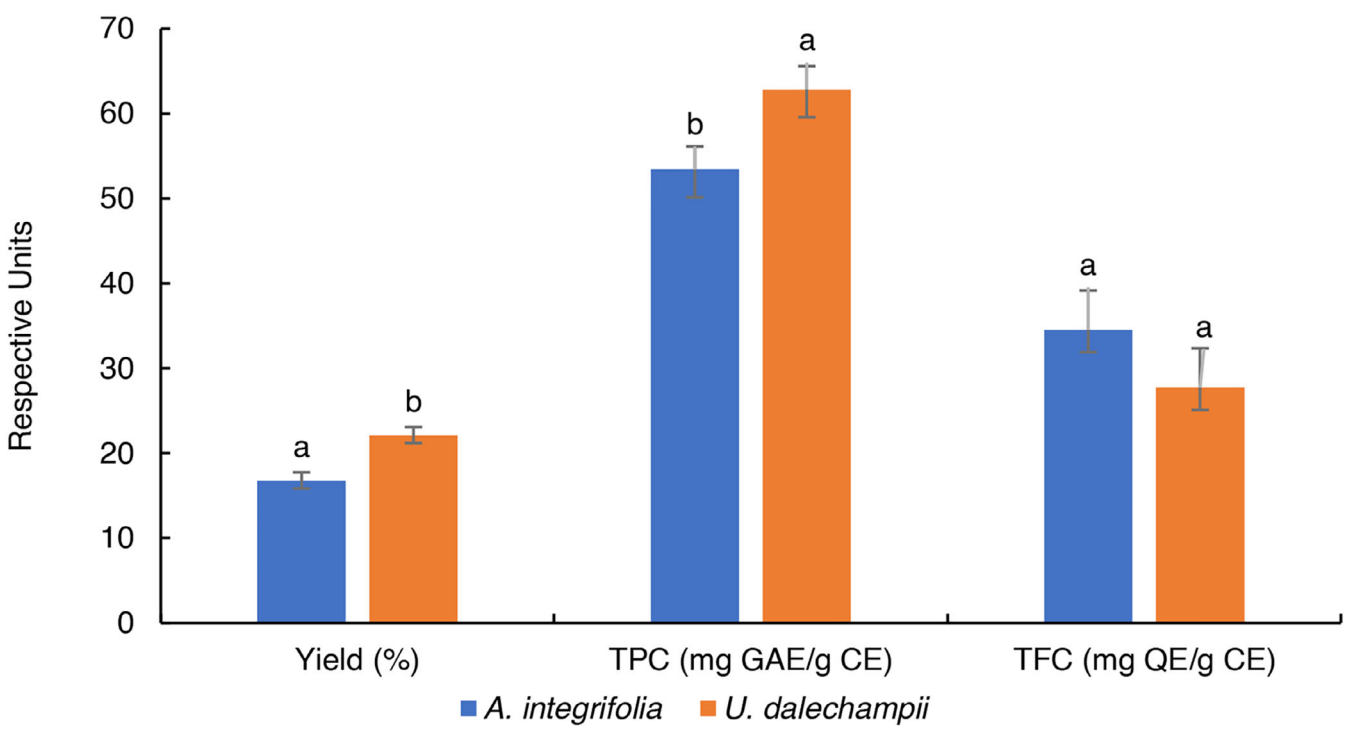

FIGURE 1 | Yield of polyphenols, total polyphenols (TPC), and flavonoids (TFC) contents of *A. integrifolia* and *U. dalechampii* hydroethanolic leaf extracts. Results represent the mean ± standard error of the mean (SEM) of three triplicates (n = 3). For each assay, bars marked with different letters (a-b) indicate a significant difference at $p < 0.05$ according to the Student's *t*-test. GAE: gallic acid equivalent; QE: Quercetin equivalent; CE: crude extract.

TABLE 2 | Antioxidant activity of *U. dalechampii* and *A. integrifolia* hydroethanolic leaf extract. Results are expressed as EC_{50} values (mg/mL).

	DPPH	ABTS	FRAP	ICA	CCA
U. dalechampii	0.95 ± 0.01 [b]	0.72 ± 0.00 [b]	7.16 ± 0.00 [a]	31.94 ± 1.09 [a]	2.06 ± 0.02 [a]
A. integrifolia	1.21 ± 0.10 [a]	0.90 ± 0.00 [a]	5.40 ± 0.00 [c]	29.08 ± 0.07 [b]	1.72 ± 0.03 [b]
BHT*	—	0.42 ± 0.01 [c]	0.57 ± 0.03 [a]	—	—
EDTA*	—	—	—	0.06 ± 0.00 [c]	0.1 ± 0.00 [c]
Ascorbic acid*	0.45 ± 0.01 [c]	—	—	—	—

Values represent the mean ± standard error of the mean (SEM) of 9 triplicates (n = 9). Within the same column, different letters (a–c) indicate significant differences according to Tukey's multiple comparisons test ($p < 0.05$)

*Positive control; -: not tested.

known for their antidiabetic and anti-inflammatory activities [24].

2.2 | Antioxidant Activity

The antioxidant capacity of the hydroethanolic leaf extracts of *A. integrifolia* and *U. dalechampii* were assessed using five complementary in vitro assays (DPPH, ABTS, FRAP, ICA, and CCA; Table 2).

Both extracts demonstrated the ability to neutralize free radicals in the DPPH and ABTS assays, which evaluate hydrogen-atom donation and single-electron transfer, respectively [6]. *U. dalechampii* showed the strongest radical-scavenging effect, with the lowest EC_{50} values in both assays (DPPH: 0.95 mg/mL; ABTS: 0.72 mg/mL), followed by *A. integrifolia* (1.21 and 0.90 mg/mL, respectively). In the FRAP assay, which assesses the reducing capacity of antioxidants through their ability to convert Fe^{3+} to Fe^{2+}, the opposite trend was observed. *A. integrifolia* exhibited the strongest reducing power (EC_{50} = 5.40 mg/mL), whereas *U. dalechampii* showed a lower activity (EC_{50} = 7.16 mg/mL), indicating that *A. integrifolia* contains compounds with a greater electron-donating ability under these assay conditions. Regarding metal-chelating activity, both species showed comparable iron-chelating effects, with EC_{50} values of 29.08 mg/mL for *A. integrifolia* and 31.94 mg/mL for *U. dalechampii*. In contrast, *A. integrifolia* showed stronger copper-chelating activity (EC_{50} = 1.72 mg/mL) than *U. dalechampii* (EC_{50} = 2.06 mg/mL).

To the best of our knowledge, this is the first study evaluating the antioxidant potential of crude leaf extracts of *U. dalechampii* and *A. integrifolia*. However, antioxidant activity has been previously reported in other species from the same genus and family, indicating that members of Asteraceae commonly accumulate phenolic compounds with relevant redox properties. Polyphenols, particularly phenolic acids, stabilize reactive species through hydrogen- or electron-donation mechanisms, while flavonoids possess additional structural features that enhance radical-scavenging and metal-chelating capacities. Variations in their abundance reflect species-specific metabolic profiles and provide an initial indication of the chemical functionality of the extracts. The antioxidant activity observed in the present study is primarily attributed to the phytochemical composition of the extracts, particularly phenolic acids and flavonoids, which act by interrupting free-radical chain reactions [6]. Antioxidant efficacy is strongly influenced by structural features such as the number and position of hydroxyl groups, degree of conjugation, and glycosylation patterns [25]. Hydroxycinnamic acid derivatives identified in the extracts including 4-*O*-caffeoylquinic, chlorogenic, cryptochlorogenic, neochlorogenic, isochlorogenic, and coumaroylquinic acids contain multiple hydroxyl groups that enhance radical-scavenging capacity through hydrogen donation [21]. The presence of these compounds is consistent with the strong DPPH and ABTS antioxidant activities observed.

Similarly, flavonoids such as quercetin, rutin, and kaempferol, also detected in the analyzed leaf extracts, are well known for their potent radical-scavenging activity, attributed to their hydroxylated structures [25]. In addition to direct radical scavenging, metal-chelating properties, particularly attributed to hydroxycinnamic acids, can further reduce oxidative reactions by inhibiting metal-catalyzed radical generation [26], in line with the chelating effects observed on iron and copper.

Overall, the antioxidant efficiency of the extracts reflects not only the relative abundance of phenolic compounds but also their specific chemical structures, which collectively determine their redox properties [7, 15, 18].

2.3 | Enzymatic Inhibitory Activity

The enzymatic inhibitory capacity of the hydroethanolic leaf extracts was evaluated against four key enzymes implicated in major human disorders, namely acetyl- and butyrylcholinesterase (neurodegeneration), pancreatic lipase (obesity and acne), and tyrosinase (hyperpigmentation and food oxidation). The results are summarized in Table 3.

Overall, *A. integrifolia* consistently showed the strongest and broadest inhibitory activity across the tested enzymes, with low IC_{50} values for lipase (9.70 mg/mL), tyrosinase (0.50 mg/mL), α-glucosidase (1.9 mg/mL), and both cholinesterases (AChE: 4.61 mg/mL; BChE: 8.63 mg/mL). In contrast, *U. dalechampii* displayed weaker inhibition, as reflected by IC_{50} values of 20.10 and 14.01 mg/mL for lipase and tyrosinase inhibition, respectively, and a moderate α-glucosidase inhibitory effect on α-glucosidase (IC_{50} = 4.08 mg/mL).

Concerning cholinesterases, *U. dalechampii* exhibited minor inhibitory activity against AChE and no detectable inhibition of

TABLE 3 | Enzymatic inhibitory properties of *U. dalechampii* and *A. integrifolia* hydroethanolic leaf extract. Results are expressed as IC_{50} values (mg/mL).

	Lipase	Tyrosinase	α-Glucosidase	AChE	BChE
U. dalechampii	20.10 ± 0.80[a]	14.01 ± 0.30[a]	4.08 ± 0.13[a]	10.25 ± 0.43[a]	n.a.
A. integrifolia	9.70 ± 0.10[b]	0.54 ± 0.01[b]	1.90 ± 0.03[b]	4.61 ± 0.06[b]	8.63 ± 0.03[a]
Orsilat*	0.12 ± 0.00[c]	—	—	—	—
Arbutin*	—	0.32 ± 0.00[c]	—	—	—
Acarbose*	—	—	0.29 ± 0.00[c]	—	—
Galantamine*	—	—	—	0.08 ± 0.15[c]	0.32 ± 0.10[a]

Values represent the mean ± standard error of the mean (SEM) of 9 triplicates (n = 9). Within the same column, different letters (a–c) indicate significant differences according to Tukey's multiple comparisons test ($p < 0.05$)

*: positive control; -: not tested; n.a.: not active.

BChE. Overall, these results indicate that *A. integrifolia* possesses stronger multitarget enzyme inhibitory potential, whereas *U. dalechampii* shows selective and lower activity.

Due to the various side effects of synthetic drugs, plants are increasingly being investigated for their enzyme inhibitory potential. In the present study, the ability of *A. integrifolia* and *U. dalechampii* leaf extracts to modulate the activity of enzymes associated with Alzheimer's disease (AChE and BChE), anti-obesity (lipase), type 2 diabetes (α-glucosidase), and skin hyperpigmentation (tyrosinase) was assessed, the results being summarized in Table 3. The observed inhibitory effects of the extracts are linked to the presence of specific phytochemical compounds, which may act synergistically to enhance enzyme inhibition.

Due to the adverse effects of many anti-obesity drugs, there is growing interest in herbal alternatives. Pancreatic lipase, a key enzyme in triglyceride digestion and lipid absorption, represents an important target for obesity management [27]. The phenolic acids and flavonoids identified in this study are known as pancreatic lipase inhibitors, confirming their biological significance. Consistently, several studies have reported strong lipase inhibitory activity in Asteraceae plant extracts. In fact, Spínola & Castilho [28] demonstrated that methanolic leaves extracts from 10 plants exhibited significant inhibitory activity against key digestive enzymes linked to type II diabetes and obesity, with caffeoylquinic acids, the dominant compounds, identified as the main hypoglycemic and anti-glycation agents. In aqueous extracts, phytochemicals known to inhibit pancreatic lipase, such as galloyl molecules or caffeoylquinic acids detected in *Chrysanthemum morifolium*, *Grindelia camporum*, and *Hieracium pilosella* showing notable pancreatic lipase inhibitory activity [29]. The methanolic extract of *Baccharis trimera* considerably inhibited the lipase activity by about 78% [30].

Lee et al. [31] evaluated the antilipase activity of ethanolic fruit extracts of *Acer ginnala* and have reported an IC_{50} values between 30 and 50 mg/mL. The ethanolic extract of branches and leaves of *Acer mono* also showed lipase inhibitory activity, with an IC_{50} value less than 10 μg/mL [32]. Collectively, these findings support the role of phenolic acids and flavonoids, particularly caffeoylquinic acid derivatives and flavonols, as bioactive compounds involved in the modulation of lipid digestion.

Tyrosinase is an essential enzyme in melanogenesis, catalyzing the conversion of L-tyrosine to L-DOPA, then to o-dopaquinone, ultimately leading to melanin synthesis [33]. Overproduction of melanin can lead to dermatological disorders such as hyperpigmentation and melasma, underlining the importance of tyrosinase inhibitors in skin lightening strategies. While synthetic inhibitors such as hydroquinone are effective, their toxicity and side effects limit their use, prompting interest in natural alternatives. Phenolic compounds and flavonoids, including chalcones, flavanones, gallic acid, caffeoylquinic acid, and rosmarinic acid, have been widely described as natural tyrosinase inhibitors [34]. These compounds can inhibit the enzyme by hydrogen bonding at the active site and potentially by antioxidant mechanisms [23].

A. integrifolia extract is a promising potent inhibitor of tyrosinase compared with other plant extracts, such as *Vernonia cinerea* (70% ethanol) [35] and *Vernonia anthelmintica* [36] (50% methanol), with IC_{50} of 29.89 and 30.35 mg/mL, respectively. This can be explained by the high content of flavonoids and phenolic components, as well as the presence of caffeoylquinic and dicaffeoylquinic acids. The number of caffeoyl groups correlated with tyrosinase inhibitory activity, and flavonol rhamnosyl glucoside showed potent activity on tyrosinase activity with an IC_{50} = 113.7 μM in the extract of the aerial part of *Conyza fliaginoides* [37] and in the methanol-based leaf extract of *Inula conyza* with an IC_{50} of 1.55 [23]. Collectively, the results indicate that *U. dalechampii* and *A. integrifolia* are promising natural sources of tyrosinase inhibitors and may have potential uses in the development of safe herbal depigmenting agents.

The anti-diabetic potential of plant extracts has been extensively researched through the inhibition of α-amylase and α-glucosidase; two enzymes essential to carbohydrate metabolism. α-Amylase is involved in the hydrolysis of polysaccharides into oligosaccharides, while α-glucosidase catalyzes the subsequent conversion of disaccharides into absorbable monosaccharides [38]. Targeted inhibition of these enzymes effectively reduces postprandial glycemic variations, offering a validated approach

to glycemic control in the management of diabetes [39]. Noting that oxidative stress, resulting from free radicals, contributes to metabolic disorders such as type 2 diabetes (T2D) [40]. The studied species showed strong antioxidant activity, attributed to various phytochemical compounds that inhibit α-glucosidase, with IC50 values of 1.9 mg/mL for *A. integrifolia* and 4.80 mg/mL for *U. dalechampii*. Phenolic compounds, in particular, reduce glucose absorption by forming complexes with starch, thus reducing its enzymatic hydrolysis [41].

The α-glucosidase inhibitory activity of plant extracts correlates with their phenolic and flavonoid content [29, 38]. For instance, the aqueous extract of *Nelumbo nucifera* exhibited strong α-glucosidase inhibitory activity (IC_{50} = 13.17 μg/mL) [41], likely attributable to its phenolic acid and flavonoid content. While, neochlorogenic acid from Houttuynia cordata demonstrated superior α-glucosidase inhibition (IC_{50} = 0.81 mg/mL) compared to the standard acarbose (IC_{50} = 2.73 mg/mL). Flavonoids such as quercetin, isoquercetin, kaempferol, and rutin are well documented α-glucosidase inhibitors [24], which is consistent with the inhibitory activity observed in *A. integrifolia* leaf extracts, where isoquercetin was identified.

In *A. integrifolia*, high levels of neochlorogenic, 1,4-dicaffeoylquinic and 3,4-dicaffeoylquinic acids likely contribute to its stronger activity relative to *U. dalechampii*. Additionally, the exclusive presence of 2,3,4',5-tetrahydroxystilbene-2-glucoside in *A. integrifolia* may further enhance its anti-diabetic potential [42]. These findings support the value of plant-derived α-glucosidase inhibitors as potentially safer alternatives to synthetic agents such as acarbose.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are responsible for the hydrolysis of acetylcholine, a neurotransmitter essential for cholinergic transmission and cognitive functions such as learning and memory [43]. Their excessive activity leads to reduced levels of acetylcholine in the brain, contributing to cognitive decline [44]. Inhibition of these enzymes thus remains a major therapeutic approach, although the limitations of current drugs have encouraged the search for new plant-based cholinesterase inhibitors.

In the present study, *A. integrifolia* leaf extract was distinguished by their significant inhibitory activity against AChE and BChE activities, particularly AChE enzyme. This inhibitory activity is likely attributable to the presence of caffeoylquinic acids, compounds previously reported to inhibit acetylcholinesterase [23] demonstrated that neochlorogenic acid has potential therapeutic effects against Alzheimer's disease by attenuation blood-brain barrier dysfunction in a zebrafish model of the disease. These findings support our results, as neochlorogenic acid was identified in the studied leaf extract, particularly in higher amounts in *A. integrifolia*, which corresponds to its stronger AChE inhibitory activity compared to *U. dalechampii*. In addition, chlorogenic acid has also been shown to inhibit AChE activity in *Actinidia arguta* fruit, underlining its potential as a natural enzyme inhibitor [45].

Apigenin, luteolin, quercetin, phloretin, and cyanidin were found to be the most potent BuChE inhibitors among the flavonoids [46, 47]. This is consistent with our findings, as only *A. integrifolia* leaf extract showed BuChE inhibitory activity and contained higher levels of luteolin than *U. dalechampii* leaf extract. Moreover, 2,3,4',5-Tetrahydroxystilbene 2-glucoside, detected exclusively *in A. integrifolia* leaf extract, is natural polyhydroxylated stilbene and known by its nephroprotective action [48], likely contribute to this selective inhibition. Furthermore, cynarin, identified both in our extracts and in other Asteraceae species (e.g., *Cynara scolymus* L), has demonstrated neuroprotective effects in rat models [46].

All enzymatic activities examined and differences observed among the studied leaf extracts correlate with the qualitative composition of phenolic acids and flavonoids, as summarized in Figure 2.

2.4 | Cytotoxicity

The cellular viability of the hydroethanolic leaf extracts was assessed at 100 μg/mL in four mammalian cell lines: human hepatocarcinoma (HepG2), human neuroblastoma (SH-SY5Y), human embryonic kidney (HEK 293), and mouse melanoma (B16 4A5) (Figure 3).

Both extracts maintained high cell viability across all models, with *A. integrifolia* showing values above 88% and *U. dalechampii* consistently above 93%. According to ISO 10993-5, materials are considered non-cytotoxic when cell viability exceeds 80%; therefore, the values obtained in this study clearly indicate that neither extract exerted cytotoxic effects at 100 μg/mL, and both can be classified as non-cytotoxic under the tested conditions.

Evaluating the cytotoxicity of plant extracts is essential for determining their safety and supporting potential biological applications. The absence of cytotoxic effects observed in this study is consistent with findings of other Asteraceae species. The absence of cytotoxic effects observed in the present study is in line with results obtained for other species. For example, non-cytotoxic responses in HepG2, SH-SY5Y, and HEK-293 cells have been reported for extracts of *Limonium algarvense* [48] and *Crithmum maritimum* [49], suggesting that phenol-rich extracts from this family often exhibit good cellular tolerance. The lack of cytotoxicity observed for *A. integrifolia* and *U. dalechampii* therefore confirms their potential safety for other biological applications, in particular their proven antioxidant and enzyme-inhibiting properties. At this stage, however, the cytotoxicity data should not be extrapolated to other biological effects; rather, the main conclusion is that both extracts present a favorable in vitro safety profile.

2.5 | Heatmap Analysis

To thermographic analysis was applied on all data including phenolic compounds and studied biological activity of leaf extract of *A. integerifolia* and *U. dalechampii* to display the relative abundance in each species (Figure 4). The leaf extract of *A. integerifolia* was distinguished by high total flavonoids content (TFC), Isoschaftoside, Neochlorogenic acid, Luteolin 7-glucuronide, 1,4-Dicaffeoylquinic acid, Kaempferol 7-O-glucoside, 3,4-Dicaffeoylquinic acid, Flavone O-glucuronide, 3,4-Dicaffeoylquinic acid, 2,3,4',5-Tetrahydroxystilbene

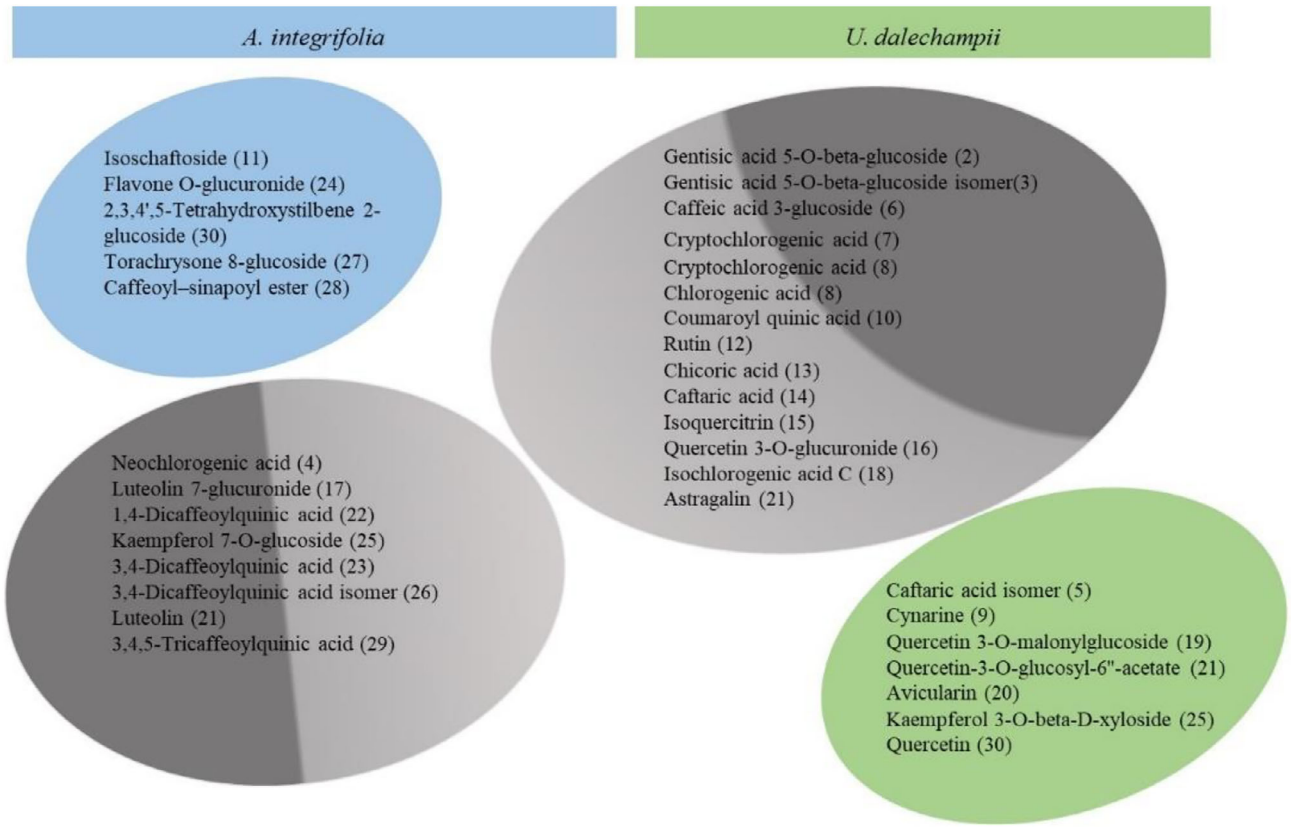

FIGURE 2 | Abundance and specific phenolic compounds of *A. integrifolia* and *U. dalechmapii* leaf extracts (blue color identified in *A. integrifolia*; green color identified in *U. dalechmapii*; the intensity of grey color is related to the abundance).

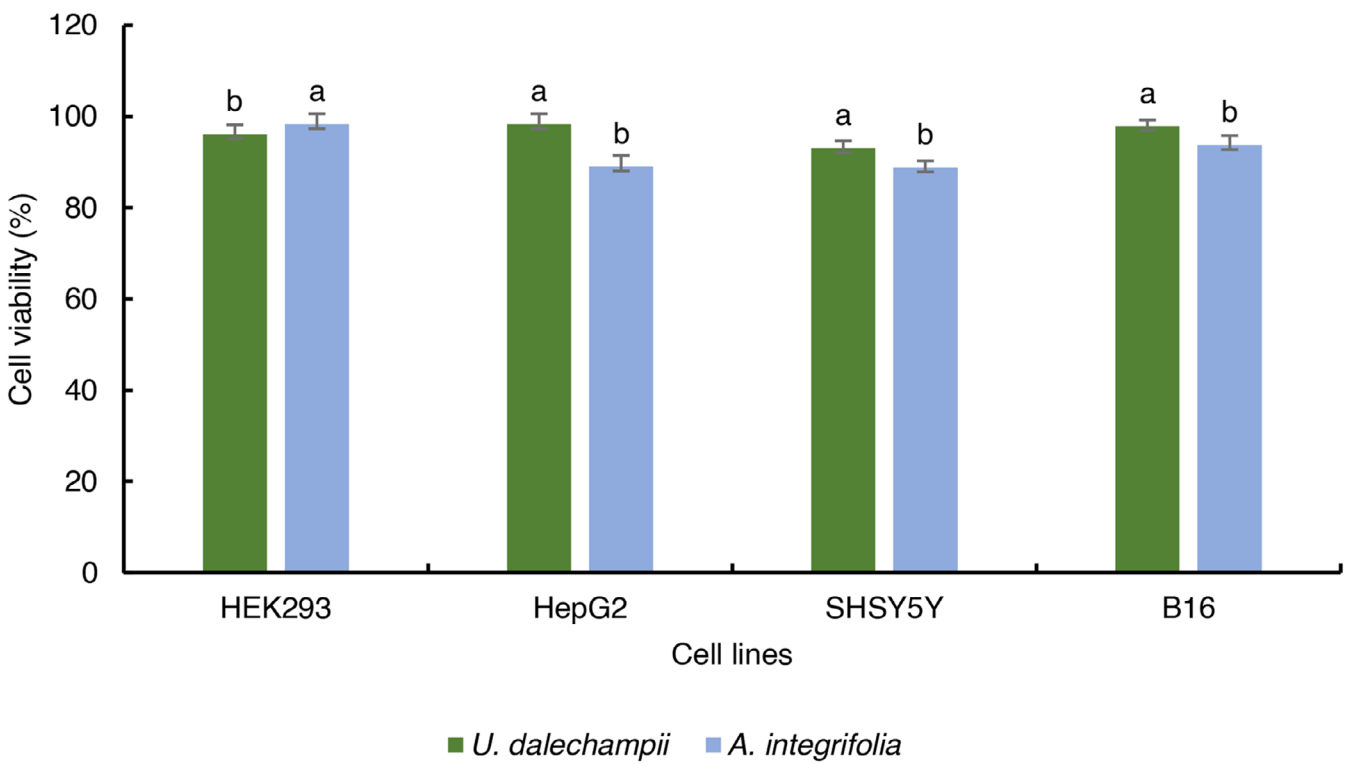

FIGURE 3 | Cytotoxicity of *U. dalechampii* and *A. integrifolia* hydroethanolic leaf extract against four mammalian cell lines: HepG2 (human hepatocellular carcinoma), SH-SY5Y (human neuroblastoma), HEK 293 (human embryonic kidney, nontumor), and B16 4A5 (murine melanoma). Results are expressed as mean ± SEM (n = 6). For each cell line, bars marked with different letters (a-b) indicate a significant difference at $p < 0.05$ according to the Student's *t*-test.

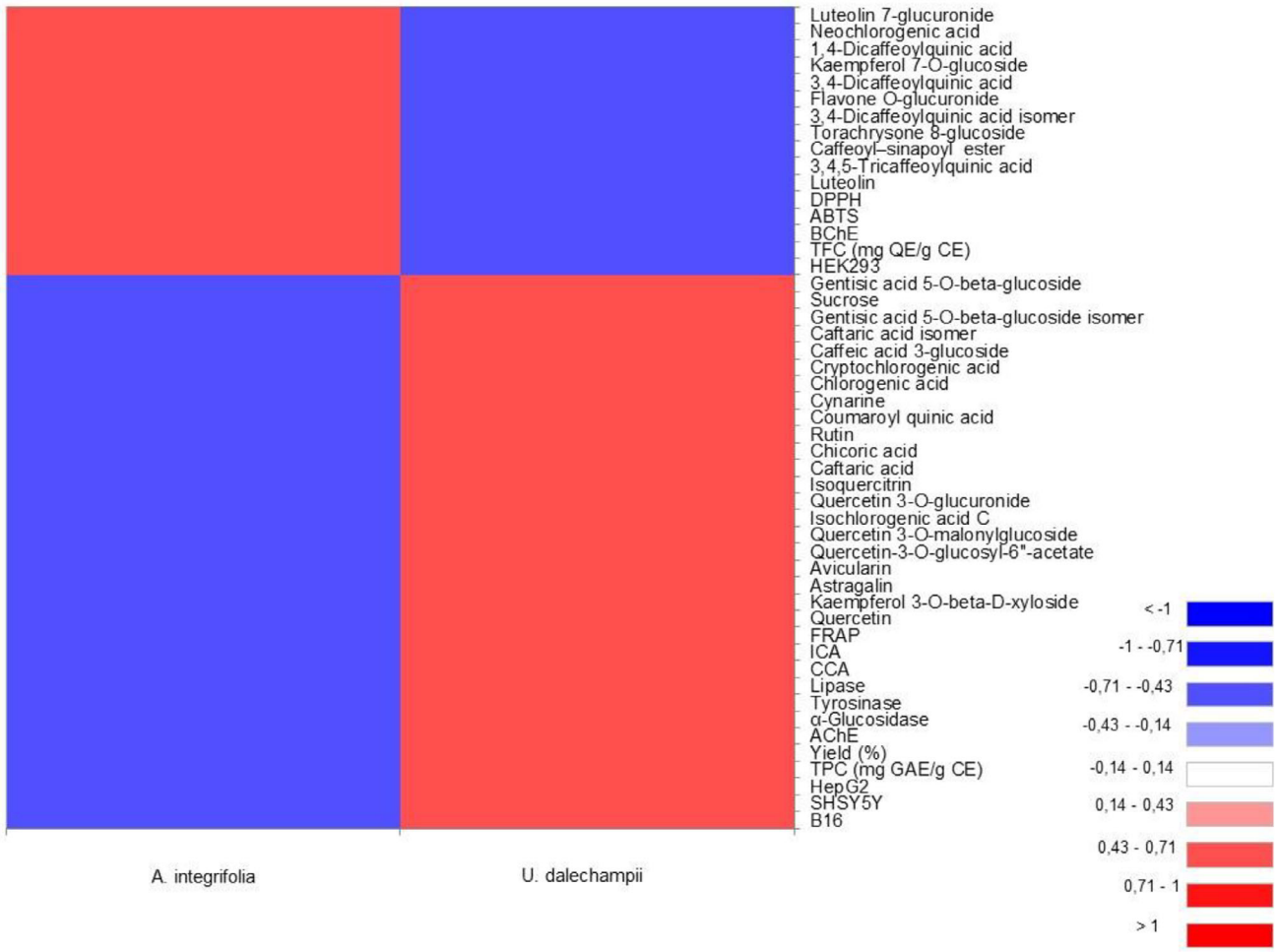

FIGURE 4 | Heatmap of thermographic analysis based on phenolic compounds and biological activities of the leaves of *Andryala integrifolia* and *Urospermum delachampii* (TPC: total polyphenols content; TFT: total flavonoids content; GAE: gallic equivalent acid; QE: quercetin equivalent; the scale showed the intensity color of red as high and blue as lower values).

2-glucoside, Caffeoyl–sinapoyl ester, 3,4-Dicaffeoylquinic acid isomer, Luteolin. This extract was correlated with BChE activity that obtained only in this extract. In other hand, the leaf extract of *A. integerifolia* was correlated negatively with FRAP, ICA and CCA assays reflecting high potential to convert Fe^{3+} to Fe^{2+} and for metal-chelating activity, respectively. The IC50 of enzyme inhibitory activity were correlated negatively with this extract reflecting high activity of lipase, tyrosinase, α-glucosidase and AChE. Noting that the mentioned compounds were also correlated positively with high cell viability using HEK293.

Regarding, *U. dalechamppi* leaf extract, the thermographic maps showed high total polyphenols content (TPC) and phenolic acids and flavonoids compounds related to the high antioxidant activity through DPPH and ABTS assays reflected and high cell viability using HepG2, SHSY5Y, and B16 cell lines. The thermographic analysis constitutes a tool to investigate chemical compounds from plants as the bioactive compound contents and volatile profiles of products of *Aronia melanocarpa* L. fruits according different drying methods [50]. The effect of harvested date on phenolic content was also investigated through the thermographic analysis of *Rubus idaeus* L. fruits [51].

3 | Conclusion

The hydroethanolic leaf extracts of *Andryala integrifolia* and *Urospermum dalechampii* displayed a chemically rich profile, characterized by abundant phenolic acid and flavonoid compounds, including multiple caffeoylquinic acid derivatives, flavonols, and flavones identified by UHPLC-DAD-MS. This chemical composition is consistent with the antioxidant behavior observed across all assays, where both extracts exhibited relevant radical-scavenging, reducing, and metal-chelating capacities. Among the two species, *A. integrifolia* generally showed a stronger antioxidant response. This extract also displayed broad inhibitory activity against all tested enzymes, pancreatic lipase, tyrosinase, α-glucosidase, AChE, and BChE, while *U. dalechampii* presented weaker and more selective inhibition, reflecting differences in metabolite composition. Both extracts were non-cytotoxic at 100 µg/mL using crude extract in all tested mammalian cell lines, fulfilling ISO 10993-5 criteria and supporting their preliminary safety. Overall, the combination of a well-defined phenolic profile, relevant antioxidant properties, and selective enzyme inhibition highlights *A. integrifolia* and *U. dalechampii* as promising sources of natural bioactive

compounds, warranting further investigation for targeted pharmacological or nutraceutical applications. Further i*n vivo* studies and comprehensive toxicological evaluations are needed to confirm their safety and efficacy.

4 | Experimental Section

4.1 | Plant Material

Leaves of *U. dalechampii* and *A. integrifolia* (Asteraceae) were collected from a natural roadside population in northern Tunisia (36 48′28“N 10 6′4”E) in May 2023. The species were identified by doctor Hannachi, botanist at the Faculty of Science of Tunis based on Tunisia flora [52, 53]. The specimens of the two species were deposited in the herbarium of Faculty of Science of Tunis in the department of biology for voucher number assessment.

Samples were air-dried in the dark at room temperature for seven days, ground to a fine powder and stored at 4°C in the dark until analysis.

4.2 | Polyphenols Extraction

Polyphenols were extracted from leaf powder (20 g) using ultrasound-assisted extraction with 70% ethanol (plant:solvent ratio 1:10 w/v). Samples were sonicated in an ultrasonic bath for 30 min, and the resulting extracts were filtered through Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure using a rotary evaporator and stored at 4°C until analysis.

4.3 | Determination of Extract Yield

Extract yield (EY) was determined gravimetrically according to the following equation:

$$EY\,(\%) = \frac{W_2 - W_1}{W_0} \times 100$$

where W_2 is the weight of flask after evaporation, W_1 the weight of empty flask, and W_0 the mass of plant material.

4.4 | Phytochemical Analysis

4.4.1 | Determination of Total Phenolic Content (TPC)

The TPC was determined using the Folin-Ciocalteu method, as previously detailed in Hammami et al. [12]. Briefly, 63 µL of each extract was mixed with 63 µL of Folin- Ciocalteu reagent. After 5 min, 625 µL of 1 M sodium carbonate (Na2CO3) and 500 µL of distilled water were added. The mixture was incubated for 90 min at room temperature in the dark. Absorbance was measured at 765 nm using a microplate reader (Multiskan GO Spectrophotometer, Thermo Fisher Scientific, USA). Gallic acid was used as a standard to generate the calibration curve (y = 0.006x + 0.0857 with R2 = 0.9996), and TPC results were expressed as mg gallic acid equivalents per g crude extract (mg GAE/g CE).

4.4.2 | Determination of Total Flavonoid Content (TFC)

TFC was determined based on the formation of a flavonoid–aluminum complex with maximum absorbance at 430 nm, as reported before [12]. Briefly, 125 µL of each extract was mixed with 38 µL of 5% sodium nitrite (NaNO2). After 6 min, 75 µL of 10% aluminum chloride (AlCl3) were added. After a further 5 min, 250 µL of 1 M sodium hydroxide (NaOH) and 762 µL of distilled water were added. The reaction mixture was incubated at room temperature for 15 min, and absorbance was recorded at 430 nm using microplate reader. Quercetin was used as the standard to construct the calibration curve (y = 0.0079x + 0.0554 with R2 = 0.9993), and TFC were expressed as mg quercetin equivalents per g crude extract (mg QE/ g CE).

4.4.3 | Ultra-High Performance Liquid Chromatography Coupled With Diode Array Detection and Mass Spectrometry (UHPLC-DAD-MS) Analysis

UHPLC-DAD-MS analysis was performed on an x500r qToF mass spectrometer (SCIEX, Framingham, USA) coupled to a Nexara UHPLC system (Shimadzu, Kyoto, Japan). The dried extract was dissolved in 1 mL of methanol, and 2 µl of the dissolved extract was injected into a Luna Omega Polar C18 column (1.6 µm, 100 Å, 100 × 2.1 mm, Phenomenex, Aschaffenburg, Germany). The chromatographic separation was based on a system with mobile phase A containing 0.1% formic acid in water and mobile phase B containing 0.1% formic acid in acetonitrile. The flow was set to 0.3 mL/min, and the gradient was as follows: 5% B starting with a rise to 95% B in 20 min with a following isocratic condition at 95% B for 5 min. The electrospray ionization was performed at a temperature of 500°C and a capillary voltage of 4500 V or −4500 V, respectively, of the positive or negative ionization mode. MS2 spectra were recorded using 35 eV collision energy or −40 eV collision energy, respectively. MS spectra were analyzed with mzMine and matched against MS/MS databases (NIST2023, GNPS, MassBank) [49]. Relative abundance was calculated using a semi-quantitative conditional analysis performed in Microsoft Excel. For each detected compound, normalized peak areas (area/1000) were used to allow comparison across species. The lowest, the median, and the highest relative abundance values of each compound in the two samples are attributed as low (+), moderate (++), and high (+++), respectively.

4.5 | In Vitro Radical-Based Antioxidant Assays

4.5.1 | Radical Scavenging Activity (RSA) on DPPH Radical

The RSA of the extracts using the DPPH radical was assessed as previously described [22]. Samples (22 µL) at different concentrations (ranging from 0.6 to 20 mg/mL) were combined with 200 µL DPPH solution (120 µM in 96% methanol) and incubated in the dark for 30 min. The percentage inhibition was calculated in

comparison to the negative control (70% ethanol). Results were expressed as half maximal effective concentration (EC_{50}, mg/mL). Ascorbic acid was used as a positive control.

4.5.2 | RSA on ABTS Radical Cation

The RSA against the ABTS•+ radical was determined using the method detailed by Rodrigues et al. [50]. ABTS (7.4 mM) was mixed with potassium persulfate (2.45 mM) in 5 mL of distilled water to generate the ABTS•+ radical and incubated overnight in the dark at 4°C. The resulting solution was then diluted with ethanol to obtain an absorbance of approximately 0.7 at 725 nm. A volume of 10 μL of each sample at different concentrations (ranging from 0.6 to 20 mg/mL) was mixed with 190 μL of the ABTS•+ solution, followed by a 6-min incubation. Absorbance was recorded at 725 nm (EZ Read 400, Biochrom, Cambridge, UK). The percentage inhibition calculated in comparison to the negative control (70% ethanol), and results were expressed as EC_{50} values (mg/mL). The BHT was used as a positive control.

4.6 | *In Vitro* Metal-Based Antioxidant Assays

4.6.1 | Copper Chelating Activity (CCA)

The CCA was assessed by titration of the chelates with pyrocatechol violet (PV) as an indicator, according to the method described previously by Rodrigues et al. [54]. A volume of 30 μL of each sample at different concentrations (ranging from 0.6 to 20 mg/mL) was mixed with 200 μL sodium acetate buffer (50 mM, pH 6), 6 μL PV (4 mM in the same buffer), and 100 μL copper sulfate (CuSO4, 50 μg/mL in water). Absorbance was recorded at 620 nm (EZ Read 400, Biochrom, Cambridge, UK). The percentage inhibition was calculated in comparison with the negative control (70% ethanol), and the results were expressed as EC_{50} values (mg/mL). The EDTA was used as a standard.

4.6.2 | Iron Chelating Activity (ICA)

The ICA was determined using the ferrous ion (Fe2+)-ferrozine complex formation method, as described by Rodrigues et al. [54]. A volume of 30 μL of each sample at different concentrations (ranging from 0.6 to 20 mg/mL) was mixed with 200 μL deionized water and 30 μL iron (II) chloride (FeCl2) solution (0.1 mg/mL in water). After 30 min of incubation, 12.5 μL ferrozine (40 mM in water) was added. Following a 10 min incubation in the dark at room temperature, absorbance was measured at 562 nm (EZ Read 400, Biochrom, Cambridge, UK). The percentage inhibition was determined relative to the negative control (70% ethanol), and results were presented as EC_{50} values (mg/mL). The EDTA was used as a positive control.

4.6.3 | Ferric Reducing Antioxidant Power (FRAP)

The FRAP was measured using the protocol of Rodrigues et al. [54]. A volume of 50 μL of each extract at various concentrations (ranging from 0.6 to 20 mg/mL) was mixed with 50 μL of distilled water and 50 μL of 1% potassium ferricyanide, then incubated at 50°C for 20 min. Subsquentely, 50 μL of 10% trichloroacetic acid (TCA) and 10 μL 0.1% of ferric chloride (FeCl3) were added, followed by an additional 10 min at 50°C. Absorbance was recorded at 700 nm. BHT was used as the standard. The percentage inhibition was calculated in relation to the positive control at 1 mg/mL, and results were presented as EC_{50} (mg/mL).

4.7 | Enzyme Inhibitory Activity

4.7.1 | Tyrosinase Inhibition Assay

The anti-tyrosinase activity was determined according to Lemoine et al. [49]. A volume of 70 μL of each sample at various concentrations (0.6 to 20 mg/mL) were combined with 30 μL of an enzyme solution (333 U/mL) prepared in 25 mM potassium phosphate buffer (pH 6.5). After a 5 min, 110 μL of L-tyrosine substrate (2 mM) was added, followed by incubation for a further 30 min at room temperature. Arbutin was used as a positive control. The absorbance was recorded at 492 nm, and the results were expressed as IC_{50} values (mg/mL).

4.7.2 | Lipase Inhibition Assay

The inhibitory activity on lipase was evaluated according to the method previously described by McDougall et al. [55] adapted to 96-well microplates. Samples (20 μL), at concentrations ranging from 2.5 to 40 mg/mL, were mixed with 200 μL of Tris-HCl buffer (100 mM, pH 8.2), 24 μL of the enzyme solution (20 mg/mL in Tris-HCl buffer), and 20 μL of the substrate (4-nitrophenyl dodecanoate, 5.1 mM in ethanol). After 10 min of incubation at 37 °C, absorbance was read at 410 nm. Orlistat was used as a positive control. Results were calculated as a percentage of inhibition in relation to the negative control (70% ethanol) and expressed as IC_{50} values (mg/mL).

4.7.3 | Cholinesterase Inhibition Assay

The neuroprotective potential of *U. dalechampii* and *A. integrifolia* leaf extracts was evaluated through the in vitro inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BchE), as described by Custódio et al. [56]. For both assays, 20 μL of each extract at various concentrations (0.6–20 mg/mL) was mixed with 140 μL of phosphate buffer (0.02 M, pH 8.0) and 20 μL of the corresponding enzyme solution (AChE or BChE, both at 0.28 U/mL in buffer). The mixtures were incubated for 15 min at room temperature.

The reactions were initiated by the addition of 10 μL of substrate (acetylthiocholine iodide for AChE; butyrylcholine chloride for BChE, both at 4 mg/mL in buffer) and 20 μL of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB, 1.2 mg/mL in ethanol). After a further 15-minute incubation at 25°C, absorbance was measured at 405 nm (AChE) and 412 nm (BChE). Galantamine was used as a positive control. The inhibitory activity was calculated as a percentage relative to a control containing 70% ethanol, and results were expressed as IC_{50} values (mg/mL).

4.7.4 | Alpha-glucosidase Inhibition Assay

The α-glucosidase inhibitory activity was evaluated according to the method described by Bendjedou et al. [20], with slight modifications. Briefly, 50 µL of the extract at different concentrations (0.6–20 mg/mL) was mixed with 100 µL of the enzyme solution (1.0 U/mL in 0.1 M buffer) and incubated for 10 min at 25°C. Subsequently, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG, in ethanol) was added, and the reaction mixture was incubated for 5 min at 25°C. Absorbance was measured at 405 nm. The inhibitory activity was expressed as IC_{50} values (mg/mL) based on the percentage of inhibition relative to a control containing 70% ethanol. Acarbose was used as a positive control.

4.7.5 | Cell Culture and Cell Viability Assessment

Human hepatocarcinoma HepG2 (RRID:CVCL_0027), human neuroblastoma SH-SY5Y (RRID:CVCL_0019), human embryonic kidney HEK 293 (RRID:CVCL_0045), and mouse B16 melanoma 4A5 (RRID:CVCL_4612) cell lines were cultured as previously detailed [48]. Cells were seeded into 96-well plates at a density of 5,000 cells per well and incubated overnight to allow for adherence. The following day, cells were treated with the extracts at a concentration of 100 µg/mL for 72 h. After treatment, 20 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 5 mg/mL in PBS) was added to each well, and plates were incubated for 2 h at 37°C. The supernatant was then carefully removed, and the resulting formazan crystals were dissolved in 150 µL of dimethyl sulfoxide (DMSO). Absorbance was measured at 590 nm. Cell viability was expressed as a percentage relative to the control group treated with 0.5% DMSO.

4.8 | Statistical Analysis

All experiments were performed in triplicate, with three independent assays, and results are expressed as mean ± SEM (standard error of the mean; $n = 9$). Differences between the two species were evaluated using Student's *t*-test. For multiple comparisons, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied. In all analyses, statistical significance was set at $p < 0.05$. Thermographic analysis was conducted to visualize the heatmap of all data obtained using polyphenols yield, total polyphenols content, total flavonoids content; phenolic acids, flavonoids compounds, and the IC50 of all antioxidant and enzyme activities. In this thermographic, the distribution of all data was shown at high value (red color) and lower Value (blue color) of experimental data. Statistical analyses were performed using XLSTAT software (version 2022, Addinsoft).

Author Contributions

Nourhen Hammami: conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft; **Maria João Rodrigues:** conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, writing – review and editing, visualization, supervision; **Eliana Fernandes:** formal analysis, methodology, data curation; **Thomas Stegemann:** formal analysis, methodology, data curation, software; **Wissal Saadellaoui:** formal analysis, methodology; **Hédia Hannachi:** conceptualization, investigation, methodology, writing – review and editing, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: cbdv71434-sup-0001-SuppMat.docx