

Article

A Comparative Analysis between the Phenolic Content, Key Enzyme Inhibitory Potential, and Cytotoxic Activity of *Arum italicum* Miller in Two Different Organs

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Abstract: The present study assessed two different plant parts (leaves and tubers) of *Arum italicum* species growing in Northeast Algeria for their phytochemical composition and pharmacological effects. The phytochemical content was determined using ultra-high-performance liquid chromatography coupled with a diode array detector and an electrospray mass spectrometer (UHPLC-DAD-ESI/MS). The results revealed that the tuber extract was rich in lignans with a fraxiresinol glycoside as the major compound. In contrast, the leaf extract was rich in flavonoid glycosides, described for the first time in the aerial part of this species. The extract's inhibitory activity against key enzymes was linked to hyperglycemia, α -glucosidase, and α -amylase, and their ability to inhibit the growth of human gastric carcinoma (AGS) and lung carcinoma (A549) cancer cell lines was also assessed. A cell line morphology study was also conducted with the most effective extract. The chromatin status of the cells was evaluated using DAPI, while the cytoplasmic morphology was evaluated using phalloidin. The tuber extract generally inhibited α -glucosidase and α -amylase enzymes more efficiently than the leaf extract. Its inhibition effect against the α -glucosidase was significantly higher when compared to the standard acarbose. The tuber extract also caused more viability loss of AGS and A549 cancer cells than the leaf extract in the cytotoxicity assay. In conclusion, our findings show that, compared to the leaf extract, the tuber extract exhibited more pronounced biological effects. The strong inhibitory potential of the tuber extract against the α -glucosidase enzyme should also be highlighted, which suggests it is a good candidate for discovering new antidiabetic agents.

Keywords: *Arum italicum*; UHPLC-DAD-ESI/MS profile; enzyme inhibition; cytotoxicity; leaves; tubers



Citation: Rechek, H.; Haouat, A.; Pinto, D.C.G.A.; Pereira, D.M.; Pereira, R.B.; Válega, M.S.G.A.; Cardoso, S.M.; Silva, A.M.S. A Comparative Analysis between the Phenolic Content, Key Enzyme Inhibitory Potential, and Cytotoxic Activity of *Arum italicum* Miller in Two Different Organs. *Int. J. Plant Biol.* **2023**, *14*, 520–532. <https://doi.org/10.3390/ijpb14020041>

Academic Editor: Adriano Sofo

Received: 10 May 2023

Revised: 31 May 2023

Accepted: 6 June 2023

Published: 7 June 2023



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1. Introduction

Cancer and diabetes are two diseases that represent a heavy social and economic burden on public health. Type 2 diabetes is associated with a risk of developing specific cancerous pathologies [1]. The connection is likely complex, with hormonal and inflammatory alterations playing a significant role [2].

Since antiquity, medicinal plants have been decisive in preserving human health. In several regions of the world, these traditional practices are still used. The secondary metabolites produced by these plants contribute to their beneficial health properties. Furthermore, many of these plants are known to have marked biological effects on preventing

various diseases associated with oxidative stress, such as cancer and diabetes. Thus, there is a growing interest in studying their bioactive compounds' natures and benefits. Although much needs to be explored and established about the plants' biological effects, several studies showing their anticancer potential [3–5], including against human gastric carcinoma (AGS) and lung carcinoma (A549) cancer cell lines [6,7], have been published. Previous reports have also described plants used to treat diabetes, particularly their potential to inhibit key enzymes such as α -glucosidase and α -amylase, involved in the absorption of glucose produced from starch, as a strategy to manage hyperglycemia [8].

Arum italicum Miller is a plant belonging to the Araceae family, generally native to North Africa, West and South Europe, the Balkans, Turkey, and Iraq [9]. In Algeria and depending on the region, this plant is called Ayerni or Avequq. Their tubers are traditionally used in culinary preparations; although the fresh plant can be toxic, the cooked tubers can be eaten and used as traditional medicine as a prophylactic for influenza, angina, sore throats, physical pain, and colds [10]. Additionally, it is recognized that species from the *Arum* genus have demonstrated high medicinal potential [11]. Nevertheless, it is a genus that requires more studies; several species showed anticancer activity, and only a few species were investigated for their antidiabetic activity [11]. *A. italicum* is among the less studied species, and its antidiabetic potential still needs to be explored.

Earlier studies describing the phytochemical composition of *A. italicum* are limited and only described the tuber part of the species. None included the study of the whole plant organs. As far as we know, information concerning the inhibitory enzyme potential of this species needs to be obtained. Agalar et al. [12] reported that some fractions from *A. italicum* tubers showed promising cytotoxic activities against MCF-7 and A549 cancer cell lines. Although this previous report describes the anticancer properties of *A. italicum* [12], this still needs to be explored, and further studies are required. In addition, some species growing in Algeria showed interesting activity in inhibiting enzymes linked to hyperglycemia [13,14].

Therefore, this study was carried out to provide better knowledge about the chemical composition and the bioactivity of two different parts (leaves and tubers) of this species growing in Algeria and used by the local population as food and medication for some health problems.

Two different morphological parts of this plant were evaluated for their enzyme inhibition, cytotoxic effects, and chemical composition to provide scientific evidence of the plant's traditional uses.

2. Materials and Methods

2.1. Chemicals

α -Glucosidase from *Saccharomyces cerevisiae*, 4-nitrophenyl α -D-glucopyranoside (pNPG), α -amylase from porcine pancreas, and acarbose and potato starch were purchased from Fluka (Bucharest, Romania) and Fisher (Pittsburgh, PA, USA), respectively. Sodium nitroprusside, sulfanilamide, and 3,5-dinitrosalicylic acid (DNS) were obtained from Acros Organics (Hampton, NH, USA). HPLC-grade solvents were purchased from Panreac (Barcelona, Spain). Standard compounds used in UHPLC-ESI-MS/MS analysis were obtained from EXTRASYNTHESE (GenayCedex, France). Dulbecco's Modified Eagle Medium (DMEM), penicillin/streptomycin solution (penicillin 10,000 Units/mL and streptomycin 10,000 μ g/mL), fetal bovine serum (FBS), Trypsin-EDTA (0.25%), and PrestoBlue™ were purchased from Invitrogen (Grand Island, NE, USA). Phalloidin (CF543) and Z-VAD-FMK were obtained from Biotium (Fremont, CA, USA) and Santa Cruz (Heidelberg, Germany), respectively.

2.2. Extract Preparation

The aerial parts (leaves) and the tubers of *Arum italicum* were collected in Jijel (North-east of Algeria) in April 2019. The leaves and the tubers were both shade-dried at room temperature (25 °C) and were then ground into a fine powder. A total of 10 g of the powder of both leaves and tubers was extracted three times via maceration, using 200 mL of ethanol in each cycle. The maceration was performed for 48 h in dark conditions. At the end of the

process, vegetable material was removed via filtration, and a dried extract was obtained after ethanol evaporation at reduced pressure.

2.3. UHPLC-DAD-ESI/MS Characterization of *A. italicum* Extracts

The plant extracts were analyzed in UHPLC Ultimate 3000 (Dionex Co., San Jose, CA, USA) equipment, coupled to a UV detector (Dionex Co., San Jose, CA, USA) and a Thermo LTQ XL mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with an electrospray ionization interface (ESI). The elution was carried out with a mixture of 0.1% (*v/v*) of formic acid in water (A) and acetonitrile (B). The column system was a Hypersil Gold (Thermo Scientific, USA) C18 (100 mm length; 2.1 mm i.d.; 1.9 μ m particle diameter; end-capped). The default flow was kept at 0.2 mL/min. The injection volume was 10 μ L (1 mg of extract in 1 mL of ethanol). UV-Vis spectral data were recorded in the range of 200–500 nm, while the chromatogram profiles were recorded at 280 nm. The instrument was operating in the negative-ion mode and with the ESI needle voltage set at 5.00 kV and an ESI capillary temperature of 275 °C. The full scan covered the mass range from *m/z* 100 to 2000. Nitrogen (purity > 99%) was used with gas pressure set at 520 kPa (75 psi). CID-MS/MS and MS² experiments were simultaneously acquired for precursor ions using helium as the collision gas with collision energy of 25–35 arbitrary units.

Phenolic compounds were identified by comparing their retention time and MS data with those of pure standards injected in the same conditions or by comparing MS and MS/MS spectra with those reported in the literature. Quantification was performed based on the areas of the corresponding chromatographic peaks using the external standard method. Calibration curves were obtained via the injection of known concentrations of structurally related standard compounds.

2.4. Inhibition of Enzymatic Activities

2.4.1. Inhibition of α -Glucosidase Activity

The α -glucosidase inhibitory activity was measured as described by Pereira et al. [15] with slight modifications. In total, 15 μ L of 4-nitrophenyl α -D-glucopyranoside (PNPG) solution was added to 50 μ L aliquots of each extract concentration. One hundred microliters of the α -glucosidase enzyme solution was then added to the mixture. The absorbance at 405 nm was recorded every min for 20 min at 37 °C. Acarbose was used as a standard reference. The α -glucosidase inhibitory activity was expressed as percentage inhibition and calculated using the following formula:

$$\text{Inhibition\%} = (A_c - A_s) / A_c \times 100$$

where A_c corresponds to the control absorbance (enzyme and buffer); A_s corresponds to the sample absorbance (enzyme and inhibitor).

The inhibition of α -glucosidase activity was expressed as IC₅₀, which is the concentration of inhibitor required to inhibit 50% of enzyme activity.

2.4.2. Inhibition of α -Amylase Activity

The α -amylase inhibition activity was conducted according to the method of Pereira et al. [15] with slight modifications. In total, 200 μ L of each extract concentration was mixed with 400 μ L of a 0.8% (*w/v*) starch solution and was incubated for 5 min at 37 °C. After that, 200 μ L of α -amylase solution was added and the reaction was started. Then, 200 μ L of the mixture was mixed with 600 μ L of 3,5-dinitrosalicylic acid (DNS) reagent. A second aliquot of 200 μ L of the first mixture was collected 15 min later and mixed with DNS reagent. After 10 min of boiling, 250 μ L of each mixture was transferred to each well in a 96-well microplate, and the absorbance was recorded at 450 nm. Acarbose was used as a reference compound. The α -amylase inhibitory activity was expressed as percentage inhibition and was calculated using the following formula:

$$\text{Inhibition\%} = (A_c - A_s) / A_c \times 100$$

where A_c corresponds to the control absorbance (enzyme and buffer); A_s corresponds to the sample absorbance (enzyme and inhibitor).

The inhibition of α -amylase activity was expressed as the IC_{50} , which is the concentration of inhibitor required to inhibit 50% of enzyme activity.

2.5. Cytotoxic Activity

2.5.1. Viability Assessment

The cytotoxic activity of the ethanol extracts of both the leaf and tuber parts of the *A. italicum* species was determined against human gastric carcinoma (AGS; ATCC, LGC Standards SLU, Spain), lung carcinoma (A549, ATCC, LGC Standards SLU, Spain), and human keratinocytes (HaCaT, ATCC). The cytotoxic activity was assessed using a resazurin-based method according to the method previously described by Teixeira et al. [16]. Cell lines were cultured in DMEM (Gibco) supplemented with 1% streptomycin/penicillin and 10% FBS (Gibco) and were kept at 37 °C in an atmosphere containing 5% CO₂. Cells were plated at a density of 1.5×10^4 cells/well for AGS and HaCaT cells and 1.0×10^4 cells/well for A549 cells and incubated for 24 h, after which, they were exposed to the tuber and leaf extracts of the *A. italicum* species (at 250 µg/mL; maximum DMSO concentration: 0.5%) for 24 h. A commercial solution of resazurin was then added (1:10, final volume: 200 µL), and the plates were incubated for 30 min. Finally, fluorescence was read at 560/590 nm in a microplate reader (Cytation™ 3, BioTek, Winooski, VT, USA).

2.5.2. Morphological Assessment

Morphology experiments were conducted on the most effective extract. In the presence of the plant extracts under research, A549 cells were grown in 96-well plates at the same density utilized for viability studies. Following incubation, cells were rinsed with HBSS and fixed for 30 min at room temperature in a 10% formalin solution. Cells were stained for 25 min at room temperature with CF543 (5 U/mL) and DAPI (0.25 g/mL), then rinsed with HBSS.

Pictures were captured with an inverted Eclipse Ts2R-FL (Nikon, Tokyo, Japan) equipped with a camera (Retiga R1) and an S Plan Fluor ELWD 20x DIC N1 objective (Nikon, Tokyo, Japan). Fiji [17] was used to analyze the obtained images.

2.5.3. Involvement of the RIP1 Kinase

AGS and A549 cells were pre-incubated with 9 µM of necrostatin-1 for 1 h, after which, the plant extract was added, followed by incubation for another 24 h and the subsequent evaluation of viability, as described above.

2.6. Statistical Analysis

All calculations were performed in MINITAB software (version 16, State College, PA, USA). The data recorded as the mean $\pm$ standard deviation ($m \pm SD$) were submitted to the analysis of variance (ANOVA) followed by a post hoc honestly significant difference (HSD) Tukey's test at $p < 0.05$. For the cytotoxicity test, the Shapiro–Wilks normality test was performed with the data to ensure that it followed a normal distribution. Comparisons were carried out using Student's *t*-test. Analyses were achieved using GraphPad Prism 7.0 or ggplot/R software, and values were considered statistically significant at $p < 0.05$.

3. Results

3.1. UHPLC-DAD-ESI/MS Characterization of *A. italicum* Extracts

The phenolic constituents of both leaves and tubers extracts were determined, and the results show different chemical profiles (Figure 1) and different amounts in each extract (Table 1). In total, 16 and 17 specialized metabolites were identified in the leaf and tuber extracts, respectively. Compounds' identification was assigned based on their retention time, MS/MS fragmentation patterns, direct comparison with standards injected in the same conditions, or comparison with data from the literature.

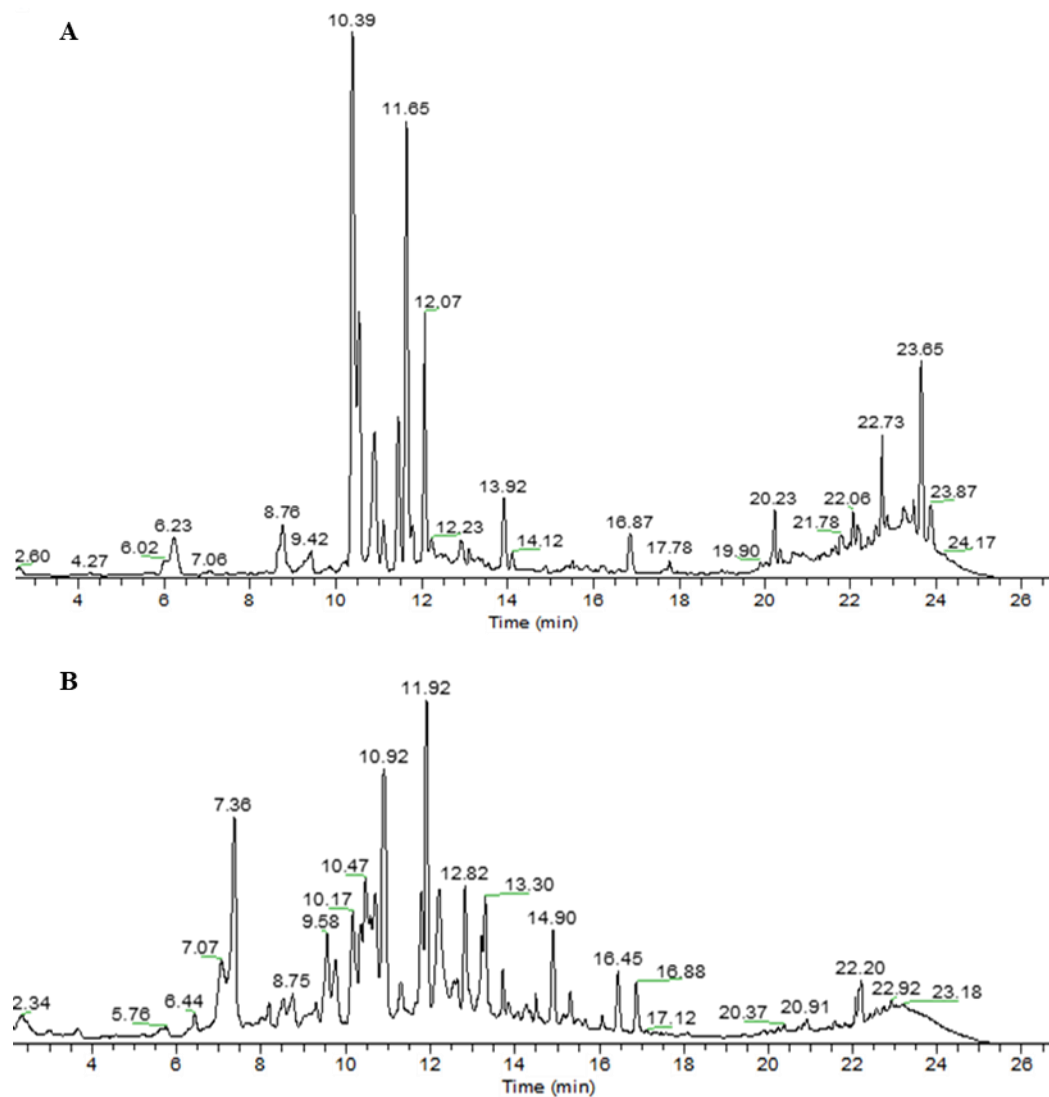


Figure 1. UHPLC chromatogram of the leaf (A) and tuber (B) extracts of *A. italicum*, recorded at 280 nm; green lines point the peak to which the retention time is indicated.

Table 1. Chemical composition of the ethanol extracts from different parts of *A. italicum* via UHPLC-DAD-ESI/MS.

Peak	Rt	[M–H] [–]	MS ²	Identified Compound	Quantity
Leaf extract					
1	8.76	179	135	Caffeic acid [13]	0.41 ± 0.06
2	10.39	593	283, 311, 341, 431, 473, 503	Apigenin 6-C-glucoside-7-O-glucoside [18,19]	11.38 ± 1.63
3	10.54	563	353, 383, 443, 455, 473, 503	Apigenin 6-C-arabinosyl-8-C-glucoside * [20]	4.81 ± 0.64
4	10.91	563	353, 383, 443, 455, 473, 503	Apigenin 6-C-glucosyl-8-C-arabinoside * [20,21]	3.84 ± 1.23
5	11.11	593	293, 413	Isovitexin 2''-O-glucoside [20,22]	1.03 ± 0.15
6	11.46	593	297, 325, 383, 413, 455, 473, 503	Apigenin 6,8-di-C-glucoside [21]	2.59 ± 0.40
7	11.65	431	311, 341	Isovitexin or vitexin [18,19,23]	7.46 ± 1.22
8	11.78	431	311, 341	Isovitexin or vitexin isomer 1 [18,19]	0.75 ± 0.15
9	12.07	431	311, 341	Vitexin or isovitexin [18,19,23]	4.41 ± 0.75
10	12.23	431	311, 341	Isovitexin or vitexin isomer 2 [18,19]	0.41 ± 0.27
11	13.92	447	284	Kaempferol 3-O-glucoside [19,23]	0.32 ± 0.18
12	14.12	343	171, 209, 229, 253, 267, 289, 307, 325	Furofuranolignan derivative [14]	NQ
13	16.87	317	193, 289, 299	Myricetin [24]	0.56 ± 0.08
14	17.78	299	284	Chrysoeriol [13]	0.21 ± 0.06

Table 1. Cont.

Peak	Rt	[M–H] [–]	MS ²	Identified Compound	Quantity
15	20.23	359	299, 317, 341	Trimethylmyricetin [25–27]	0.61 ± 0.07
16	22.73	297	111, 136, 171, 223, 268	Unknown	NQ
17	23.65	279	261	Unknown	NQ
18	23.87	297	251, 253, 279	3,8-Dihydroxy-1-methylanthraquinone [28]	NQ
Tuber extract					
19	7.36	387	164, 207, 369	Medioresinol [20]	NQ
20	9.58	447 *	269, 401	Apigenin O-pentoside ** [23]	1.94 ± 0.44
21	9.76	593	353, 383, 473, 503, 575	Apigenin 6,8-di-C-hexoside [21]	NQ
22	10.17	563	353, 383, 413, 425, 443, 473, 503, 545	Apigenin 6-C-glucosyl-8-C-arabinoside * [20,21]	1.93 ± 0.79
23	10.47	563	353, 383, 413, 425, 443, 473, 503, 545	Apigenin 6-C-glucosyl-8-C-arabinoside * [21]	4.08 ± 2.71
24	10.71	563	353, 383, 413, 425, 443, 473, 503, 545	Apigenin 6-C-arabinosyl-8-C-glucoside * [20]	3.07 ± 1.21
25	10.92	563	353, 383, 413, 425, 443, 473, 503, 545	Apigenin 6-C-glucosyl-8-C-arabinoside * [21]	5.68 ± 1.19
26	11.30	563	353, 383, 425, 443, 455, 473, 503, 533, 545	Apigenin 6-C-glucosyl-8-C-arabinoside * [18]	0.57 ± 0.18
27	11.79	565	295, 373, 403	Fraxiresinol hexoside isomer 1 [29,30]	NQ
28	11.92	565	295, 355, 373, 385, 403	Fraxiresinol 1-O-glucoside [29,30]	NQ
29	12.22	436	145, 272, 316	Dicoumaroyl-spermidine isomer 1 [13]	NQ
30	12.82	436	145, 272, 316	Dicoumaroyl-spermidine isomer 2 [13]	NQ
31	13.22	403	161, 205, 367, 385	Fraxiresinol [29,30]	NQ
32	13.30	565	161, 205, 295, 373, 403	Fraxiresinol hexoside isomer 2 [29,30]	NQ
33	13.73	329	229, 314	Tricin [31]	0.42 ± 0.16
34	14.90	343	171, 177, 201, 217, 297, 289, 307, 325	Furofuranolignan derivative [12]	NQ

Quantification values are expressed as µg/mg extract; retention time (Rt) is expressed in minutes; both [M–H][–] and MS² are in *m/z*; not quantified (NQ); ** detected as formic acid adduct. Compounds were identified based on MS² data and their comparison with standards or MS and UV data from literature reference sources. * These compounds can be interchanged because they are isomers. The identified compounds were quantified based on the areas of the corresponding chromatographic peaks, using the external standard method. Calibration curves were obtained by injection of known concentrations of structurally related standard compounds.

Even though the LC-MS analysis revealed that the phenolic composition was widely different between the leaf and the tuber extracts of *A. italicum*, several compounds were identified in both extracts. Both extracts have several apigenin glycosides, although the leaf extract is more affluent in flavonoid derivatives. In contrast, the tuber extract is also rich in lignan derivatives, from which fraxiresinol derivatives are the ones that present a higher area percentage (Figure 1; Table 1).

Based on their fragmentation patterns, several flavonoid glycosides (peaks 2–11) were identified in the leaf extract of the *A. italicum* species. These compounds were assigned to apigenin 6-C-glucoside-7-O-glucoside, apigenin 6-C-arabinosyl-8-C-glucoside, apigenin 6-C-glucosyl-8-C-arabinoside, isovitexin 2''-O-glucoside, apigenin 6,8-di-C-glucoside, isovitexin or vitexin, isovitexin or vitexin isomer 1, vitexin or isovitexin, isovitexin or vitexin isomer 2, and kaempferol 3-O-glucoside (Table 1). Albeit in lower amounts, flavonoid aglycones (peaks 13–15) were also found in this extract and were assigned to myricetin, chrysoeriol, and trimethylmyricetin (Table 1). Only one phenolic acid (peak 1) was detected in the leaf extract and was assigned to caffeic acid by direct comparison with the pure standard injected in the same conditions.

In the tuber extract, the most abundant group was lignan derivatives (peaks 19, 27, 28, 31, 32, and 34). These compounds were tentatively assigned to medioresinol, fraxiresinol hexoside isomer 1, fraxiresinol 1-O-glucoside, fraxiresinol, fraxiresinol hexoside isomer 2, and a furofuranolignan derivative (Table 1). Flavonoid glycosides (peaks 20–26) were also presented in this extract and were represented by apigenin O-pentoside, apigenin 6,8-di-C-hexoside, apigenin 6-C-glucosyl-8-C-arabinoside, apigenin 6-C-β-glucosyl-8-C-α-arabinoside, apigenin 6-C-arabinosyl-8-C-glucoside, apigenin 6-C-β-glucosyl-8-C-β-arabinoside, and apigenin 6-C-glucosyl-8-C-arabinoside. Other compounds were also detected in the tuber extract, namely dicoumaroyl-spermidine isomers 1 and 2 (peaks 29, 30) and tricetin (33) (Table 1).

3.2. Enzyme Inhibitory Activities

The inhibition of important key enzymes is the basis of many illness treatments. These enzymes are frequently employed to evaluate the bioactivity of natural substances. Among these, the metabolic enzymes α -glucosidase and α -amylase are used to test the antidiabetic capacities of substances. In this study, the enzyme inhibitory activity of the *A. italicum* ethanol extracts from leaves and tubers were tested regarding their ability to inactivate α -glucosidase and α -amylase. Results expressed as IC_{50} (μ g/mL) values (concentration allowing 50% of enzyme inhibition) or as a percentage of inhibition are presented in Table 2.

Table 2. Enzyme inhibitory activities of ethanol extracts from different parts of *A. italicum* against α -glucosidase and α -amylase.

Sample	Enzyme Inhibitory Activity			
	α -Glucosidase		α -Amylase	
	IC_{50} (μ g/mL)	% of Inhibition	IC_{50} (μ g/mL)	% of Inhibition
Leaves	ND	$46.53 \pm 3.55^{A,a}$	ND	$32.64^{A,b}$
Tubers	170.87 ± 4.75^B	-	ND	$35.49^{A,c}$
Acarbose (μ g/mL)	405.77 ± 34.83^B	-	ND	-

^A Because of the very high concentrations required to produce stronger inhibitions, it was not possible to determine the IC_{50} value. ^B IC_{50} (μ g/mL) values (concentration allowing 50% of enzyme inhibition. ND = not determined.

^a Determined at the concentration of 7.5 mg/mL of the extract. ^{b,c} Determined at the concentration of 2.5 mg/mL of the extract.

Statistical differences were observed between the extracts, highlighting that the ethanol tuber extract has more remarkable enzyme inhibitory activities when tested against both α -glucosidase and α -amylase enzymes. It is essential to emphasize that the tuber ethanol extract with an IC_{50} value of 170.87 ± 4.75 μ g/mL had a significantly higher inhibition effect against the α -glucosidase enzyme when compared to acarbose used as a positive control ($IC_{50} = 405.77 \pm 34.83$ μ g/mL).

3.3. Cytotoxic Activity

The cytotoxic activity of the ethanol extracts of leaves and tubers of the *A. italicum* species was determined using the resazurin method against human gastric carcinoma (AGS) and lung carcinoma (A549). The data are presented as the average percentage values of cell viability (Figure 2).

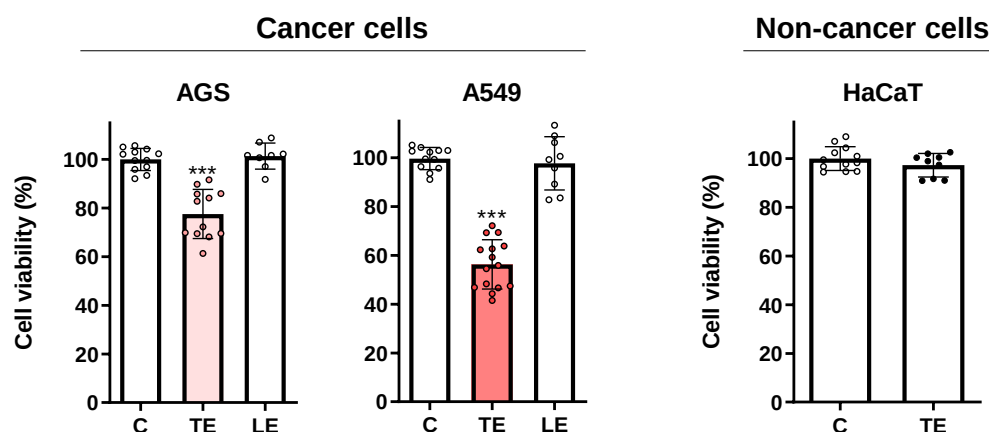


Figure 2. Viability of AGS, A549, and HaCaT cells exposed to tuber extract (TE) and leaf extract (LE) of *A. italicum* species (250 μ g/mL) and control (C). Cells were incubated for 24 h, after which, viability was evaluated. *** $p < 0.001$.

The ethanol extract from the tubers demonstrated higher in vitro cytotoxic activity with a viability loss of ca 20% and over 40% against the AGS and A549 cell lines, respectively.

The ethanol extract from the leaf part was almost devoid of any toxicity (Figure 2). No impact on cell viability was detected for non-cancer cells, specifically human keratinocytes.

A549 cells treated with 250 µg/mL of *A. italicum* tuber extract were imaged to evaluate their cell morphology. The chromatin status of the cells was evaluated using DAPI, while the cytoplasmic morphology was evaluated using phalloidin, which has the ability to bind to actin filaments. From the image (Figure 3), we can notice a clear decrease in cell density, as well as an increase in chromatin condensation compared to the control conditions. Aiming to shed more light on the mechanistic events responsible for the loss of cell viability, cells were co-incubated with necrostatin-1 (nec-1), a serine/threonine kinase RIP1 inhibitor. The inhibition of RIP1/RIP3 did not result in any significant increase in cell viability when exposed to the extract (data not shown).

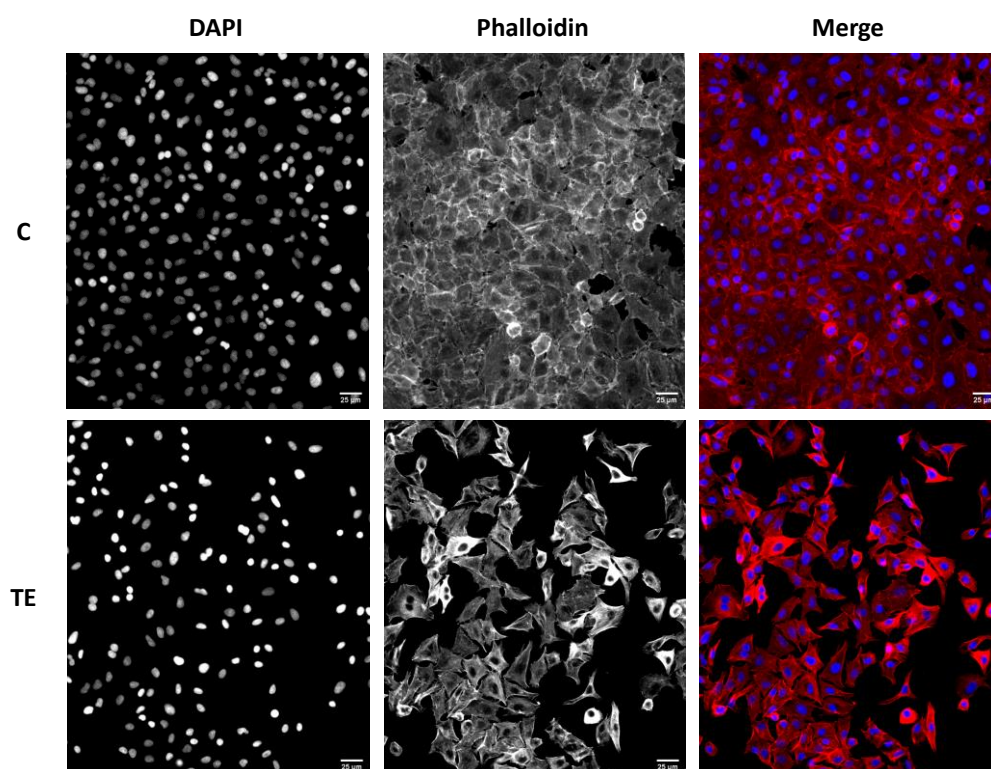


Figure 3. Morphology of A549 cells exposed to tuber extract (TE) of *A. italicum* species (250 µg/mL) and control (C) after 24 h of incubation (S Plan Fluor ELWD 20× DIC N1 objective). Overall cell morphology was evaluated using phalloidin (actin) and DAPI (chromatin status).

4. Discussion

4.1. Chemical Constituents

LC-MS analysis showed that *A. italicum* leaf and tuber extracts differ significantly in their phenolic composition; however, both extracts contain apigenin glycoside derivatives. This group of compounds was more abundant in the leaf extract. On the other hand, the tuber extract is rich in lignan derivatives, from which the most abundant are fraxiresinol derivatives (Table 1).

Besides apigenin derivatives, from which the apigenin 6-C-glucoside-7-O-glucoside (2) is the most abundant of all the quantified compounds and corresponds to 11.38 ± 1.63 µg/mg of the *A. italicum* leaf extract, kaempferol 3-O-glucoside (11), myricetin (13), chryseriol (14), and trimethyl myricetin (15) were also found. This group of compounds is essential because several pharmacological effects, including antiviral, antimicrobial, anticancer, anti-inflammatory, antidiabetic, antioxidant, and cardioprotective abilities, have been attributed to them [30]. It should be noted that this is the first report describing the chemical composition of *A. italicum* leaves and indicates the plant's potential to be a source of bioactive compounds.

Arum italicum tuber extract is extremely rich in lignans, and in addition to the fraxiresinol derivatives, from which fraxiresinol 1-O-glucoside (28) is the one with a higher area percentage, the medioresinol (19) and furofluranolignan derivatives (34) could also be detected (Figure 1; Table 1). This group of compounds is associated with some biological activities, namely antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [32]. Flavonoids were also present in the *A. italicum* tuber extract and were mainly represented by apigenin glycosides, although tricetin (33) was also detected (Table 1). It should be emphasized that some of these compounds were previously identified in the tuber extracts of *A. italicum* [12], and dicoumaroyl-spermidine was also detected in the tuber extract of *A. maculatum* [33], another species of the *Arum* genus.

Overall, data showed that the phenolic compounds detected in this study differ from those reported in the literature. For example, Ağalar et al. [12] state that ferulic, caffeic, and *p*-coumaric acids, as well as their derivatives, were the major components of the *A. italicum* Miller tubers growing in Turkey. On the other hand, Akar et al. [34] indicated that among the various compounds present, ferulic acid, luteolin, and rutin were the major ones in the edible and nonedible parts of the Turkish *A. italicum* tubers. None of the studies in the literature described the phytochemical composition of *A. italicum* leaves; however, studies focused on elucidating the chemical constituents of some phylogenetic species close to *A. italicum* have been found. *Arum dioscoridis* from Jordan was found to have apigenin, luteolin, quercetin, quercetin-3-O- β -glucoside, vitexin, isoorientin, esculin, and caffeic and ferulic acids [35]. Using the HPLC-MS technique, Afifi et al. [36] reported that an ethanol extract of *A. hygrophilum* leaves, a medicinal species indigenous to Jordan, revealed the presence of rosmarinic caffeic, ferulic, and gallic acids as well as quercetin-3-O-rhamnoside.

4.2. Biological Activities

4.2.1. Enzyme Inhibition Activities

Diabetes develops when the body's cells cannot absorb sugar (glucose) and utilize it to produce energy. This causes an accumulation of excess sugar in the bloodstream (hyperglycemia). Neglecting to treat diabetes may have disastrous effects, including damage to various organs and tissues, including eyes, heart, nerves, and kidneys.

α -Glucosidase and α -amylase are two key enzymes involved in the regulation of the absorption of glucose, which is generated from starch [37,38]. The inhibition of these enzymes is one of the main mechanisms currently used to control hyperglycemia [39–41]. The existing inhibitors, such as acarbose, voglibose, and miglitol, efficiently reduce intestinal glucose absorption and postprandial blood glucose. However, they possess various side effects, such as diarrhea, flatulence, and abdominal pain [42]. Therefore, further efforts should be undertaken to find novel anti-diabetics compounds from medicinal plants, particularly those renowned for their traditional usage in managing diabetes. Recently, the number of studies reporting α -glucosidase's and α -amylase's inhibitory effects of plant extracts has increased.

In the present study, the abilities of both the tuber and leaf ethanol extracts of the *A. italicum* species were evaluated in terms of their ability to inhibit α -glucosidase and α -amylase enzymes. The results showed that the tuber extract is more efficient in inhibiting α -glucosidase and α -amylase than the leaf morphological extract. It should be emphasized that the inhibitory potential of the tubers was significantly better than the acarbose used as a reference standard. However, a weak inhibitory effect against the α -amylase enzyme was recorded for both extracts.

Numerous studies have found that compounds that strongly inhibit the α -glucosidase enzyme but have a weak or medium inhibition potential against α -amylase are better for diabetes control than those inhibiting both enzymes. Indeed, excessive α -amylase inhibition causes abdominal discomfort due to anaerobic fermentation and the accumulation of undigested starches in the colon [43].

To the best of our knowledge, no studies on the inhibitory effect of the *A. italicum* species on the activity of both α -glucosidase and α -amylase enzymes have been reported

in the literature. However, the literature has already reported enzyme inhibitory activity for some species phylogenetically close to *A. italicum*. Studies with extracts from the leaves of *A. hygrophilum*, *A. dioscoridis*, and *A. palaestinum* species collected from Jordan showed a marked inhibition effect against the α -amylase and α -glucosidase enzymes attributed to the presence of polyphenols and flavonoids [35,36].

The higher activity of the *A. italicum* tuber extract against the α -glucosidase enzyme could be related to their richness in flavonoids, particularly flavone-C-glycosides, since these compounds have been previously described as strong inhibitors of this enzyme [44,45]. In a previous study, apigenin 8-C-glucopyranoside (vitexin), isolated from *Beta vulgaris* species, demonstrated strong inhibitory activity against α -glucosidase [44]. The same enzyme was also strongly inhibited by vicianin 2, another 6,8-di-C-glucoside of apigenin, in in vitro assays [46].

4.2.2. Cytotoxic Activity

Cancer is a complex disease characterized by an uncontrollable proliferation of cells, being one of the leading causes of premature death [47]. Scientists have been interested in searching for anticancer agents for a long time, and several drugs have since been discovered. However, the rising frequency of cancer treatment resistance suggests that further research is needed to find new anticancer drugs. Plant organisms are an inexhaustible source of bioactive substances for drug discovery, and many anticancer agents were directly isolated from plants.

Due to the high incidence of the malignancy from which they are derived, human gastric cancer (AGS) and human non-small cell lung cancer (A549) cell lines have been frequently used in cancer research. A549 cells, isolated from males with lung tumors, are often used in biological screenings in the study of lung cancer because they are among the most drug-resistant cell lines. AGS cell lines are derived from the stomach tissue of untreated human gastric adenocarcinoma, one of the leading causes of death with poor prognosis [48].

In the present study, the cytotoxic effects of both the tuber and leaf ethanol extracts of the *A. italicum* species were also evaluated against two cancer cell lines (AGS and A549). The results showed that the tuber extract was more efficient in inhibiting the growth of both cell lines than the leaf extract, which was nearly devoid of cytotoxicity. Its impact on the cell morphology of A549 cells, in which the greatest loss of viability was observed, was also assessed using fluorescence microscopy. The results showed a clear decrease in cell density (Figure 3) and a mild increase in chromatin condensation. This could point to a process of organized cell death. To assess the potential role of necroptosis, we co-incubated cells with necrostatin-1 (nec-1), a serine/threonine kinase RIP1 inhibitor. The fact that no changes in cell viability were detected shows there is no involvement of necroptotic pathways.

It is well-recognized that the cytotoxic effects of plant species are closely linked with their chemical constituents. Hence, the observed differences might be attributed to the distinctive profile of bioactive compounds recorded in Table 1.

In a previous report, the cytotoxicity of three subfractions from the tubers of *A. italicum* growing in Turkey was also tested in vitro against A549 and another tumor cell line, MCF-7 (human breast adenocarcinoma), using the MTT colorimetric method. The authors stated that methanol:water (2:8, v/v) subfraction exhibited promising cytotoxicity against A549 cell lines, while methanol:water (4:6, v/v) and methanol subfractions showed noticeable cytotoxic activities against both tested cancer cell lines [12]. We must highlight that our results agree with the study described by Ağalar et al. [12], in which they report that A549 cells were more sensitive against the tested subfractions.

The cytotoxic effect of the tuber extract toward AGS and A549 cell lines observed in the present study may be linked to its richness in lignans. Hence, previous research showed that the anticancer property of the lignan chemical class is its most notable bioactivity [12,49,50]. Relevantly, no impact on cell viability was detected when non-cancer cells were used,

namely the keratinocyte cell line HaCaT. This is a positive trait of the most active sample, TE, as it suggests that the effect detected could be selective for cancer cells.

5. Conclusions

The present study contributes to better knowledge of the chemistry of the Algerian *A. italicum* species, since this is the first report describing the phenolic profile of both leaf and tuber extracts. A wide variation in the occurrence of the phenolic compounds was observed. The leaf extract was rich in flavonoid glycosides, while the tuber extract was particularly rich in lignans. The tuber extract was more effective in inhibiting key enzymes linked to hyperglycemia than the leaf extract. The tuber extract also showed the most potent cytotoxic effect toward AGS and A549 cancer cell lines when assessing the cytotoxicity. Finally, we concluded that the tubers of *A. italicum* are considered, in this study, as the most promising part of this species, which will serve as a basis for more detailed future research. The current study suggests that the *A. italicum* species has the potential to be used as an antidiabetic treatment in folk medicine; in particular, the ingestion of tubers may reduce intestinal glucose absorption.

Author Contributions: H.R. contributed to the conceptualization, investigation, data curation, and writing—original draft preparation. A.H. contributed to the conceptualization, investigation, and data curation. D.M.P. contributed to the conceptualization, investigation, and data curation. R.B.P. contributed to the conceptualization, investigation, and data curation. D.C.G.A.P. contributed to the conceptualization, supervision, writing—reviewing the original draft, and writing—reviewing the final version. S.M.C. contributed to the conceptualization, data curation, and supervision. M.S.G.A.V.: software and methodology. A.M.S.S. contributed to the conceptualization, supervision, and writing—reviewing the final version. All authors have read and agreed to the published version of the manuscript.

Funding: This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e a Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects UIDB/50006/2020 e UIDP/50006/2020. Renato B. Pereira acknowledges FCT (PTDC/QUI-QFI/2870/2020) for the funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: Thanks for support are due to the University of Aveiro and PT national funds (FCT/MCTES, Fundação para a Ciência e a Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) for supporting the projects UIDB/50006/2020 e UIDP/50006/2020.

Conflicts of Interest: The authors declare no conflict of interest.

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