applied sciences

MDPI

Article

Comparative Study of the Phytochemical Profile and Biological Activity of *Ajuga reptans* L. Leaf and Root Extracts

Anna Dziki [1], Magdalena Anna Malinowska [1], Agnieszka Szopa [2] and Elżbieta Sikora [1,*]

[1] Department of Organic Chemistry and Technology, Faculty of Chemical Engineering and Technology, Cracow University of Technology, Warszawska 24, 31-155 Kraków, Poland; anna.dziki@doktorant.pk.edu.pl (A.D.); magdalena.malinowska@pk.edu.pl (M.A.M.)
[2] Department of Medicinal Plant and Mushroom Biotechnology, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland; a.szopa@uj.edu.pl
* Correspondence: elzbieta.sikora@pk.edu.pl

Abstract: In this work, the phytochemical composition and the biological activity of the ethanolic extracts obtained from *Ajug reptans* L. (*Lamiaceae*) leaves and roots (growing in Lesser Poland Voivodeship, Poland) were compared. The phytochemical composition of the extracts were determined by the high-performance liquid chromatography with diode-array detection (HPLC–DAD) method. The dominant compounds in both extracts were verbacoside, isoverbacoside, 3,4-dihydroxyphenylacetic acid and rosmarinic acid. The antioxidant capacity of the extracts was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and 2,2′-azobis(3-ethylbenzothiazoline-6-sulfonate (ABTS) tests. The Folin–Ciocalteu assay was used to determine the total polyphenolic content. Additionally, enzyme (tyrosinase, collagenase) inhibition tests and metal chelating ability were studied to assess the anti-aging properties of the extracts. Moreover, the *A. reptans* extracts' capacity to absorb the whole range of ultraviolet radiation and high-energy visible (HEV) light was evaluated. The skin irritation test (SIT) EpiDerm was applied to evaluate the safety of the bugle extracts. The noteworthy point is that there is a lack of literature on the assessment of *A. reptans* root extract activity. Our study is the only one that compares the quantitative composition and biological activity of extracts from the root and leaves of *A. reptans*. The obtained results indicated that both of the extracts exhibit high antioxidant, chelating and photoprotective activity, but the extract from *A. reptans* roots showed a higher enzyme inhibition effect for mature skin. The *A. reptans* root extract, similarly to the leaf extract, could be applied as potentially multi-functional, safe and sensitive cosmetic raw materials, especially in anti-aging and anti-pollution cosmetics.

Keywords: *Ajuga reptans* leaf extracts; *Ajuga reptans* root extracts; antioxidant potential; chelating capacity; anti-collagenase; anti-tyrosinase; HEV protection; photoprotection; HPLC quantification

Citation: Dziki, A.; Malinowska, M.A.; Szopa, A.; Sikora, E. Comparative Study of the Phytochemical Profile and Biological Activity of *Ajuga reptans* L. Leaf and Root Extracts. *Appl. Sci.* **2024**, *14*, 5105. https://doi.org/10.3390/app14125105

Academic Editors: Przemysław Kowalczewski and Joanna Zembrzuska

Received: 3 May 2024
Revised: 5 June 2024
Accepted: 7 June 2024
Published: 12 June 2024

1. Introduction

Ajuga reptans L. (creeping bugleweed or bugle) is a plant of the *Lamiceae (Labiatae)* family [1]. More than 70 species of the genus *Ajuga* are found on almost every continent and, depending on the species, vary in occurrence, color and size of stems and leaves [2–4]. This is an herbal plant which reaches a height of up to 30 cm. Depending on the species and location, the plant's leaves exhibit colors ranging from green to red [5]. The plant has short main roots from which lateral roots branch [6]. *A. reptans* is a fast-growing variety of the plant with a creeping habit (the second part of its botanical name—'reptans'—means 'creeping'). In a short time, its excessive and rapid spread by producing runners becomes a problem, which is why crops are thinned, and any runners have to be cut back. Taking into account current global eco-trends, sustainability and factors associated with waste management, the use of all parts of

the plant as a raw material for obtaining active substances, for example for cosmetic applications, is justified.

Extracts of *Ajuga reptans* leaves as a source of many active ingredients [5] are used as raw materials in skincare products. There are only a few literature reports regarding *A. reptans* roots (Table 1). Therefore, the aim of this work was to compare the phytochemical composition and the biological activity of the ethanolic extracts obtained from leaves and roots of *A. reptans*, as potential multifunctional cosmetic raw materials.

Table 1. Active compounds occurring in different organs of *A. reptans*.

Part of a Plant	Name of the Compounds	References
Roots	Phytoecdysteroids: ajugasterone, 20-hydroxyecdysone, castasterone, norcyasterone, reptansterone, 28-epi-sengosterone, 2-dehydroajugalactone.	[5,16]
Above-ground parts	Iridoids: ajugoside, reptoiside, 8-acetylharpagide, harpagide, acubin, catalpol, harpagoside. Phytosterols: B-sitosterol, stigmasterol. Polyphenols: p-coumaric acid, ferulic acid, rutin, luteolin, apigenin, quercetin. Diterpenoids: ajugareptansin, ajugavensin A, ajugorientin,14,15-dehydroajugareptansin, 3α-hydroxyajugamarin F4, areptin A, areptin B, ajugatannin B1, ajugatannin D1. Phytoecdysteroids: 22-dehydro-12-hydroxycyasterone, 22-dehydro-12-hydroxy-29-norsengosterone.	[5,13,17,18]
Whole plant	Steroids: ecdysterone, ajugalactone, ajugasterone A, ajugasterone B, 29-northosterone, 29-norsengosterone, 25-hydroxyecdysone acetate, clerosterol, 22,23-didehydroclerosterol, reptanslactone A, reptanslactone B. Diterpenoids: ajugareptanzone A, ajugareptanzone B, ajugachin A. Iridoids: reposide, ajugol, ajureptazide A, ajureptazide B, ajureptazide C, ajureptazide D. Phenylpropanoid glycosides: teupolide, martinoside, verbascoside, isovascoside.	[5,7,13,17,19,20]
Leaves	Ecdysteroids: B-ecdysone, ajuga-lactone. Essential oils: 1-octen-3-ol, hexadecanoic acid, terpinolene and 6,10,14-trimethyl-2 pentadecanone.	[5,7,13,17,21]

Ajuga extracts are a known source of bioactive compounds and, among others, contain phytoecdysteroids, flavonoids, iridoids and diterpenoids (Table 1). The structure of selected *A. reptans* active substances are presented in Figure 1. The extracts pose many health benefits, characterized by antioxidant potential [7–11] and anti-inflammatory [7,12] and antibacterial [2,5,9,13,14], antifungal and anti-inflammatory effects [8–10,15], as well as photoprotective activity [11].

The biological activity assessed in our article encompassed various aspects of cosmetic action. The antioxidant potential tested by different methods aimed at precisely determining the mechanism of free radical neutralization. The enzymatic activity of tyrosinase and collagenase inhibition of the extract derived from the roots and leaves of *A. reptans* was investigated to elucidate their potential biological effects. Inhibition of collagenase activity allows for the improvement of the protein structure of the skin by preventing collagen breakdown. Conversely, tyrosinase inhibition prevents the formation of melanin, the excess of which manifests in the development of localized discolorations on the skin. Both phenomena occur with age and are common causes of premature aging of the skin.

Finally, the photoprotective effects of the examined extracts were compared, and their safety for use was assessed. The plant material was prepared using two drying methods: lyophilization and conventional drying at 40 °C. The activity profiles of these two plant organs will allow for the assessment and comparison of the potential of both parts of the plant, so that its cosmetic potential can be fully utilized.

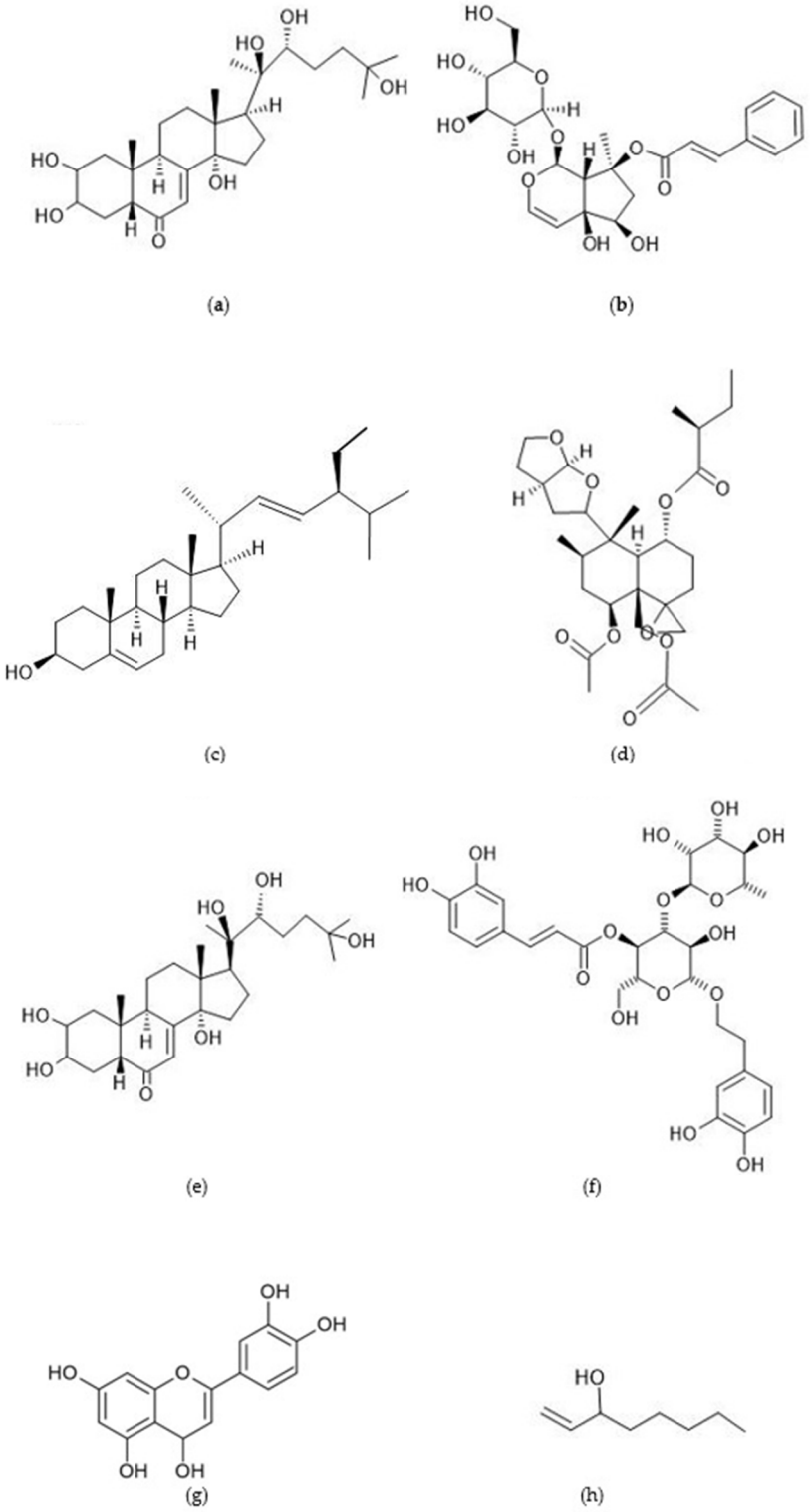

Figure 1. The selected active compounds present in *A. reptans* according to the literature: (**a**) 20-hydroxyecdysone (**b**) harpagoside (**c**) stigmasterol (**d**) ajugavensin A (**e**) ecdysterone (**f**) verbascoside (**g**) quercetin (**h**) 1-octen-3-ol.

2. Materials and Methods

2.1. Chemicals

Reagents used for the determination of antioxidant potential and phenolic content (DPPH, TPTZ, ABTS, Folin Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ethylenediaminetetraacetic acid (EDTA), ferrozine and $FeCl_2$) as well as enzymatic activity (collagenase, tricine buffer, collagenase substrate, tyrosine, L-DOPA, phosphate buffer) were of analytical grade and were purchased from Sigma Aldrich (St. Louis, MO, USA). Ethanol (anhydrous) was supplied by Avantor Performance Materials (Gliwice, Poland). Ultrapure water was obtained using Millipore Milli-Q system (Merck Millipore, Molsheim, France). Acetonitrile and methanol were purchased from Thermo Fisher Scientific (Illkirch-Graffenstaden, France). The standard compounds: chlorogenic, 3,4-dihydroxyphenylacetic, neochlorogenic, caffeic, rosmarinic, ferulic, kojic and vanillic acids, as well as apigenin, quercetin, rutin, verbacoside, isoverbacoside, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant Material

Plant material was supplied by the producer (Gospodarstwo Ogrodnicze Paweł Gardulski, Karpacz, Poland) in the springtime of 2023. Young plants were cleansed in deionized water, and roots separated from leaves. Fresh samples were then subjected to the drying procedure through either freeze-drying (24 h, Christ Alpha 1–4 LSC Basic, Donserv, Warszawa, Poland) or air-drying (24 h, 40 °C, Hartman, Cultura M, Almedica AG, Galmiz Sweden). All dried plant material (L_D—dried leaves, R_D—dried roots, L_L—freeze-dried leaves, R_L—freeze-dried roots) were ground in a mill to a particle size below 1 mm prior to extraction procedure.

2.3. Extraction Procedure

The process of bugle extraction was carried out by ultrasound assisted extraction using, as a solvent, 70% water ethanol solution (v/v). The extraction process, run in triplicates, proceeded during 30 min. In all the experiments, the ratio of the dry plant material mass to the solvent volume was constant (50 mg/mL). In order to obtain dry *A. reptans* extracts, the solvent was evaporated using Hei-VAP Advantage (Heidolph Instruments, Schwabach, Germany) and Rotavapor® R-215 (Büchi, Flawil, Switzerland) rotary evaporators. Before the extraction process, the *A. reptans* leaves were separated from the roots. The plant materials were cleaned of dust and debris from the soil by washing with tap water and subjected to drying processes at 40 °C (samples L_D—dried leaves and R_D—dried roots), and this was also done for the freeze-dried samples (L_L—freeze-dried leaves and R_L—freeze-dried roots). The extraction efficiency was determined for both drying methods by referring to the mass of material used for extraction and the obtained mass of dry extract after solvent removal. The results are shown as the average of three measurements along with the standard deviation.

2.4. HPLC–DAD Analyses

Each lyophilized plant's tissue sample (lyophilized leaves and roots), weighing 50 mg, underwent extraction using 70% ethanol (2 mL, HPLC-grade purity, Merck, Darmstadt, Germany) in falcon tubes through sonification (Sonic 2, POLSONIC Palczyński ultrasonic bath, Warsaw, Poland) for two cycles, each lasting 30 min at room temperature. Post-extraction, the resultant extracts were centrifuged at 4000 rpm for 8 min (MPW-223E, MPW, Warsaw, Poland), and the supernatant was filtered using syringe filters (pore diameter 0.2 µm Millex® GP, Filter Unit, Millipore, Bedford, MA, USA). Analysis of phenolic compounds was conducted via the HPLC–DAD method [22–24]. Employing a liquid chromatograph system (Merck—Hitachi, Japan) equipped with a Diode Array Detector (DAD) (L-245) operating within the ultraviolet (UV) light range (200–400 nm), an analytical column Purospher® RP—18e (4 × 250 mm, 5 µm; Merck, Darmstadt, Germany) was utilized with a flow rate of 1 mL/min at 25 °C, detection wavelength set at 254 nm, and an injection volume of 10 µL. The mobile phase comprised ethanol:0.5% acetic acid in a 1:4 (v/v) ratio for solvent A, and ethanol for solvent B. Gradient elution proceeded as follows: 100% A for 0–20 min; 100–80%

A for 20–35 min; 80–60% A for 35–55 min; 60–0% A for 55–70 min; 0% A for 70–75 min; 0–100% A for 75–80 min; and 100% A for 80–90 min, with a consistent flow rate of 1 mL/min. Triplicate injections were performed for all samples. Quantitative assessment of the active substances was expressed as mg/100 g DW (dry weight of the plant material). The results are shown as the average of three measurements along with the standard deviation.

2.5. Antioxidant Property Assays

The antioxidant capacity of the extracts was evaluated by DPPH, FRAP and ABTS methods, using a Nanocolor UV/VIS spectrophotometer (Macherey-Nagel, Düren, Germany). The dry plant extracts were used to prepare the water solutions by weighing 50 mg of each of the extracts and dissolving them in 1 mL of deionized water.

2.5.1. DPPH Assay

The ability of *A. reptans* extracts to scavenge free radicals was carried out according to our previous studies [25]. A total of 1.5 mL of 2,2-diphenyl-1-picrylhydrazyl 0.1 mM ethanolic solution was mixed with 0.25 mL of the *A. reptans* water extracts. As a blank sample, 0.25 mL of ethanol (70%) and 1.5 mL of DPPH solution were used. Three samples were prepared for each of the extracts. The change in optical density was measured at room temperature, 20 min after pre-mixing DPPH solution and the extract samples, at a wavelength of 517 nm. The average absorbance value was calculated and the DPPH radical reduction capacity, expressed as an inhibition percentage, was calculated according to Formula (1). The obtained values are presented as an average from three determinations along with the standard deviation.

$$\%\text{Inhibition} = \left(\frac{\text{Ao} - \text{At}}{\text{Ao}}\right) \times 100\%, \quad (1)$$

where:

Ao—average absorbance value for the blank sample
At—average absorbance value for the test sample

2.5.2. FRAP Assay

The methodology used was previously described by Ferrier et al. [26]. The FRAP reagent was obtained by mixing a 10 mM solution of ferro-2,4,6-tripyridyl-S-thiazine (TPTZ), 20 mM solution of $FeCl_3 \times 6H_2O$ and 300 mM solution of acetate buffer—pH 3.6 in a ratio of 1:1:10 ($v/v/v$). Trolox solutions were also prepared at concentrations of 800 µM/mL, 400 µM/mL, 200 µM/mL, 100 µM/mL, 50 µM/mL, 25 µM/mL. The test sample contained 10 µL of extract from individual parts of *Ajuga* mixed with 190 µL of FRAP solution, which was then left for 15 min, at 25 °C. After this time, the absorbance of the iron tripyridyl–triazine complex, was examined at a wavelength of 630 nm. In the measurement of the reference sample, the extract was replaced with prepared TROLOX solutions of different concentrations. Buffer was used in the blank sample. Each sample solution was prepared in triplicate. The results are presented as the mean of three measurements with the standard deviation value.

The iron ion reduction ability, measured by the FRAP reagent method, was calculated based on the standard curve (2) obtained by measuring of the absorbance of Trolox solutions of different concentrations.

$$y = 0.0003x + 0.1474, \quad (2)$$

2.5.3. ABTS Assay

Another method for testing the antioxidant properties [27] of extracts used the 7.4 mM solution of $ABTS^+$ and 2.6 mM solution of potassium persulfate mixed in equal amounts and left in the dark for 12 h at room temperature. The test solution was created by mixing 3 mL of the prepared $ABTS^+$ solution with 180 mL of ethanol. Then, 190 µL of the resulting solution was mixed with 10 µL of the plant extract to be tested and left in

the dark for 2 h. After this time, the absorbance of the samples was measured at 734 nm on a multiplate reader (Tecan Infinite 200 Pro, TK Biotech, Warszawa, Poland). As the reference samples, TROLOX solutions were also tested at concentrations of 200 µM/mL, 100 µM/mL, 50 µM/mL, 25 µM/mL. Ethanol solution (70%, *v*/*v*) was measured as a blank. Three determinations were made for each sample. The results are presented as the mean of all three measurements along with the standard deviation.

The antioxidant activity converted to Trolox against the ABTS reagent was calculated from the curve obtained by measuring the absorbance of prepared Trolox solutions of different concentrations (Equation (3)).

$$y = -0.0015x + 0.3029, \quad (3)$$

2.5.4. Metal Chelating Ability

The iron (Fe^{2+}) chelating ability of the *A. reptans* extracts was determined according to the methods of Kubica et al. [25]. Before the measurements, 1 mL of the tested material solutions with 0.5 mL of ethanol (99.85%), and 0.05 mL of $FeCl_2$ (0.2 mM), and 0.1 mL of ferrozine (0.5 mM) were mixed. 10 min after the addition of ferrozine, the absorbance of the mixtures was measured at 562 nm. As a blank sample, 0.5 mL of ethanol (99.85%), 0.05 mL of $FeCl_2$ (0.2 mM) and 0.1 mL of ferrozine (0.5 mM) mixed with 1 mL of ethanol (70%) were used. As a reference sample, 0.5 mL of ethanol (99.85%), 0.05 mL of $FeCl_2$ (0.2 mM) and 0.1 mL of ferrozine (0.5 mM) mixed with 1 mL of a previously prepared EDTA solution were applied (0.1 mM). The averaged value of absorbance obtained for each of the samples was calculated and used to express the value of Fe^{2+} inhibition (Equation (4)). The results are shown as the average of three measurements along with the standard deviation.

$$\% \text{ chelating ability} = \left(\frac{Ao - At}{Ao}\right) \times 100\%, \quad (4)$$

2.6. Total Polyphenols Content

The Folin–Ciocalteu reagent was used to determine the total polyphenol content in the *A. reptans* extracts [25]. The preparation of the analysis sample involved combining 100 mL of the sample solution with 0.2 mL of Folin–Ciocalteu reagent, 2 mL of H_2O, and 1 mL of 15% Na_2CO_3. Following a 2-h incubation at room temperature, the absorbance of the samples was measured at 765 nm using a Nanocolor UV/VIS spectrophotometer (Macherey-Nagel, Düren, Germany). The total polyphenol content (samples L_D—dried leaves and R_D—dried root) and also that of the freeze-dried raw material (L_L—freeze-dried leaves and R_L—freeze-dried root) were calculated using a standard curve and presented in terms of gallic acid (Equation (5)).

$$y = 0.3064x + 0.0572, \quad (5)$$

The total phenolic content was expressed as mg GAE/g extract ± standard deviation (SD). Three determinations were made for each of the extracts. The results are presented as the mean of three measurements.

2.7. UV–VIS Characteristic of the Extracts

The UV-absorption capacity of the bugle herb extracts was tested using a Nanocolor UV/VIS spectrophotometer (Macherey-Nagel, Düren, Germany). The absorbance of the ultraviolet radiation was measured in the range of 200–900 nm.

2.8. Enzymatic Studies

2.8.1. Inhibition of Collagenase

For the Tricine Buffer preparation, 8.96 mg of Tricine Buffer and 23.4 mg of NaCl were dissolved in 1 L of H_2O. The pH was adjusted to 7.5, at 25 °C, with 1M NaOH water solution, and 1.47 mg of $CaCl_2$, with pH rechecked and adjusted if necessary. The

collagenase, prepared extemporaneously at 1.1 units/mL in the tricine buffer (611 μg/mL), amounted to 1.2 mL per 96-well plate. The collagenase substrate, consisting of 0.676 mg of collagenase substrate and 1.2 mL tricine buffer, was prepared per plate. The experimental protocol entailed introducing 30 μL of extract, 10 μL of collagenase, and 60 μL of tricine buffer into the well plate. Following a 20-min incubation at 37 °C, 20 μL of 1 mM collagenase substrate solution or 20 μL of H_2O was added. Absorbance readings were made at 340 nm, using a multiplate reader (Tecan Infinite 200 Pro, TK Biotech, Warszawa, Poland), after 20 min incubation at 37 °C, with 0.1 mM EDTA as the positive control for inhibition.

The results obtained by measuring absorbance values using a plate reader were averages for each extract, and the inhibition value was expressed according to the formula:

The percent inhibition of collagenase is calculated as follows (Equation (6)):

$$\%\text{inhibition} = \left(\frac{A_{slope} - A_{test}}{A_{slope}}\right) \times 100\%, \quad (6)$$

where:

A_{slope}—absorbance of control

A_{test}—absorbance of test sample

The results are shown as the average of three determinations along with the standard deviation values.

2.8.2. Inhibition of Tyrosinase

Tyrosinase in 67 mM phosphate buffer (pH 6.8) was prepared at a concentration of 242 units/mL. L-DOPA (L-3,4-dihydroxyphenylalanine) was prepared as a working solution at 5 mM. The experimental protocol involved adding 50 μL of the tested extract, 80 μL of phosphate buffer, and 40 μL of tyrosinase (final concentration of 46 units/mL) to a 96-well plate. After a 5-min incubation at 25 °C, 40 μL of L-DOPA (final concentration of 0.95 mM) or 40 μL of phosphate buffer (for the blank) was added. Absorbance measurements were taken on a multiplate reader (Tecan Infinite 200 Pro, TK Biotech, Warsaw, Poland) at 475 nm after a 30-min incubation period, with kojic acid (50–250 μg/mL) serving as the positive control for inhibition. The results are presented as the average of three independent measurements along with the standard deviation.

The percentage inhibition of tyrosinase is calculated as follows (Equation (7)):

$$\%\ \text{inhibition} = \left(\frac{A_{slope} - A_{test}}{A_{slope}}\right) \times 100\% \quad (7)$$

2.9. Cytotoxicity Tests

Cytotoxicity studies of the extracts were prepared using the EpiDerm model. The Skin Irritation Test (SIT) EpiDerm was purchased from Mattek (Bratislava, Slovakia). Tissues were incubated for 60 min at 37 °C, 5% CO_2, and 95% relative humidity (RH). After this, they were returned for an 18-h overnight pre-incubation. Following overnight pre-incubation, extracts were applied to tissues. Tissues underwent pre-incubation for 60 min, then dosing occurred at 1-min intervals. After 35 min of incubation, plates were removed, and a 60-min waiting period followed for the first dosed tissue. A one-minute interval wash procedure with DPBS (Dulbecco's Phosphate Buffered Saline) was initiated. Tissues were rinsed, submerged, and dried. Post-incubation lasted 24 h at 37 °C, 5% CO_2, and 95% RH and, after media changing, an additional 18 h. A 1 mg/mL MTT solution was prepared and added to each sample. After a 3-h incubation, MTT medium was aspirated, and rinsing with DPBS followed. Inserts were transferred, and 2 mL isopropanol was added for MTT extraction over 2 h at room temperature. Extracts were transferred to a new plate, Optical Density (OD) was read at 570 nm. Mean values for each test substance, negative control (DPBS), and positive control (5% sodium dodecyl sulphate) were computed by averaging the relative viability from three tissues. A test chemical was classified as 'irritant' if MTT viability was

<50% relative to the negative control. Calculations for each tissue involve determining the Individual Relative Tissue Viability. All experiments were performed three times, and the presented values represent the average result supplemented with the standard deviation.

3. Results and Discussion

3.1. Extraction Procedure

The extraction process of *A. reptans* roots and leaves was conducted using ultrasound assisted extraction. To obtain information regarding the efficiency of the extraction method applied, the obtained dry extract was weighed after solvent evaporation, and the extraction yield was calculated (Table 2).

Table 2. Extraction yield of *A. reptans* (L_D—dried leaves, R_D—dried roots, L_L—freeze-dried leaves, R_L—freeze-dried roots).

Sample Name	Extraction Yield [%]
R_D	18.10 ± 2.16
L_D	19.04 ± 1.37
R_L	30.13 ± 2.07
L_L	27.13 ± 1.19

The extraction efficiency of the lyophilized raw material (27.13–30.13%) is significantly higher than that of the raw material dried in the traditional way (18.10–19.04%). This may be due to better drying process efficiency during lyophilization. Lyophilization, or freeze-drying, represents a methodical approach for water removal from a product by subjecting it to freezing and subsequent sublimation of ice, bypassing the intermediate liquid phase. This process offers the advantage of enhanced preservation of both nutritive and chemical constituents in contrast to conventional drying methods. Consequently, the heightened extraction yield observed in lyophilized raw material may be attributed to superior retention of biologically active and/or chemically significant constituents relative to conventionally dried counterparts. This, in turn, potentially leads to increased extractable substance content and consequent amplification of extraction efficiency.

3.2. HPLC–DAD Analyses

Due to its high extraction efficiency, the extract obtained from freeze-dried raw material was selected for qualitative analysis. Furthermore, as evident from the results of antioxidant activity analysis and total polyphenol content, lyophilization significantly influences the stability of metabolites present in the investigated plant material. Freeze-drying is the method for plant material drying allowing dried material of a much higher quality than in the case of other drying methods [28,29]. Convection air drying is the most commonly used method but it usually takes a long time. The other disadvantage of this method apart from the long time is that the technique does not allow for the adjustment of drying parameters, which can, in some cases, lead to the degradation of valuable nutrients [30] and does not allow for the adjustment of drying parameters [30–32]. Additionally, during air drying, plants can become contaminated. The phytochemical components of *A. reptans* extracts were determined using the HPLC–DAD method (Table 3). Among components presented in the leaf extracts, phenethyl glycosides (verbascoside, isoverbascoside), phenolic acids (chlorogenic acid, 3,4-dihydroxyphenylacetic acid, gallic acid, caffeic acid, neochlorogenic acid, rosmarinic acid, ferulic acid or vanillic acid) and flavonoids (apigenin, quercetin and rutin) were detected. In the root extracts, no presence of chlorogenic acid, neochlorogenic acid, ferulic acid, apigenin, quercetin or rutin was detected (Table 3).

Notable concentrations of chlorogenic acid were observed in leaves (49.75 mg/100 g DW), while its presence in roots was negligible. As for 3,4-dihydroxyphenylacetic acid, it displayed substantial variability across plant parts, with the highest concentration detected in leaves (227.65 mg/100 g DW), followed by roots (166.64 mg/100 g DW). Gallic acid, similar to chlorogenic acid, exhibited prominence in leaves (56.12 mg/100 g DW), with lower

concentrations observed in roots (14.17 mg/100 g DW). Minimal levels of caffeic acid were detected across both plant parts, with leaves showing higher concentration (7.26 mg/100 g DW). Neochlorogenic acid showed a relatively high level in leaves (44.76 mg/100 g DW), while its presence in roots was not detected. Among the phenolic acid compounds examined, rosmarinic acid exhibited notable concentrations in leaves (166.75 mg/100 g DW), followed by roots (163.65 mg/100 g DW). Ferulic acid was predominantly found in leaves (48.95 mg/100 g DW), with scant detection in roots. Vanillic acid had its highest concentration in leaves (19.83 mg/100 g DW), and slightly lower in roots (3.57 mg/100 g DW). Flavonoids (apigenin, quercetin, and rutin) were significantly present in leaves, while their presence in roots was minimal. Leaves exhibited the highest concentrations of both verbascoside and isoverbascoside, with notably lower levels detected in roots.

Table 3. Quantitative (mg/100 g DW ± SD) assessment of selected compounds in freeze-dried leaf and root extracts of *A. reptans*.

Target Compounds	LEAVES	ROOTS
Chlorogenic acid	49.75 ± 5.55	nd *
3,4-Dihydroxyphenylacetic acid	227.65 ± 2.93	166.64 ± 4.73
Gallic acid	56.12 ± 0.52	14.17 ± 0.55
Caffeic acid	7.26 ± 0.76	0.60 ± 0.04
Neochlorogenic acid	44.76 ± 6.04	nd
Rosmarinic acid	166.75 ± 1.13	163.65 ± 11.01
Ferulic acid	48.95 ± 3.71	nd
Vanillic acid	19.83 ± 2.91	3.57 ± 0.35
Apigenin	99.26 ± 1.86	nd
Quercetin	71.55 ± 7.25	nd
Rutin	51.18 ± 4.80	nd
Verbascoside	1114.64 ± 5.08	951.80 ± 17.14
Isoverbascoside	333.99 ± 12.08	277.79 ± 15.03

* nd—not detected.

Comparing the obtained results to the literature data, the extraction conditions applied allow for obtaining extracts richer in polyphenolic compounds. In the study by Toiu et al. (2019), ethanolic extracts derived from the aerial parts of *A. reptans* were assessed for total polyphenol content (19.81 mg GAE/g DW) and antioxidant activity using the DPPH method (42.75 µg/mL) and TEAC assay (60.98 mg/100 g DW). Our HPLC analysis revealed superior concentrations of rutin (51.18 mg/100 g DW), quercetin (71.55 mg/100 g DW), apigenin (99.26 mg/100 g DW), and ferulic acid (48.95 mg/100 g DW) in the leaf extracts [18].

The HPLC analysis results, revealing diverse phenolic compound contents across different parts of *A. reptans*, provide insights into its rich phytochemical composition. The observed variations in the quantities of compounds, such as chlorogenic acid, gallic acid, and rosmarinic acid, suggest metabolic specialization within specific plant tissues. Furthermore, the detection of high concentrations of compounds, like verbascoside and isoverbascoside, particularly in leaves, may indicate their significance in plant defense against oxidative stress, and potential applications in the cosmetic industry.

3.3. Antioxidant Properties

3.3.1. DPPH Assay

For three samples of each of the extracts, the change in optical density, at 517 nm, according to previous described protocol [33], was measured (samples L_D—dried leaves

and R_D—dried roots) and also for the freeze-dried raw material (L_L—freeze-dried leaves and R_L—freeze-dried roots) (Table 4).

Table 4. DPPH inhibition efficiency for *A. reptans* extracts (L_D—dried leaves, R_D—dried roots, L_L—freeze-dried leaves, R_L—freeze-dried roots).

Inhibition Value of *A. reptans* Extracts (%)			
L_L	**R_L**	**L_D**	**R_D**
46.13 ± 0.02	16.60 ± 0.12	47.76 ± 0.02	16.47 ± 0.02

From the data values shown in the Table 4, we can observe that the strongest antioxidant properties are shown by the extract of the leaves of the bugle herb obtained from the plant dried at 40 °C (47.76%). Similar values were obtained by extracts of the leave lyophilized at 48 h (46.13%). The extracts from the plant's roots showed significantly lower antioxidant values.

3.3.2. FRAP and ABTS Assays

As for the FRAP (ferric reducing antioxidant power) and ABTS (2,2′-azobis(3-ethylbenzothiazoline-6-sulfonate)) assays, the obtained results for the antioxidant activity of the tested *A. reptans* extracts were measured by the FRAP method and expressed in mM TE/g (Table 5). The highest antioxidant value is shown by the extract obtained from the leaves of *A. reptans*, which was dried at 40 °C for a period of 24 h. The lowest values of antioxidant capacity were characterized by extracts extracted from the root of the plant. The results are expressed in mM TE/g (Table 5).

Table 5. Free radical scavenging capacity for extracts obtained from *A. reptans* when determined by the FRAP and ABTS method evaluated as Trolox equivalent (L_D—dried leaves, R_D—dried roots, L_L—freeze-dried leaves, R_L—freeze-dried roots).

Studied Extract	Fe^{3+} Ions Reducing Ability TROLOX eq. (μM/mL)	Fe^{3+} Ions Reducing Ability (mM TE/g)	Free Radical Scavenging Activity TROLOX eq. (μM/mL)	Free Radical Scavenging Activity (mM TE/g)
R_D	833.0 ± 0.01	2648.89 ± 0.02	69.27 ± 0.02	138.53 ± 0.02
L_D	1569.7 ± 0.03	4122.22 ± 0.04	97.47 ± 0.02	194.98 ± 0.02
R_L	1031.0 ± 0.02	3044.44 ± 0.02	73.47 ± 0.01	146.98 ± 0.02
L_L	1383.0 ± 0.03	3748.89 ± 0.03	129.6 ± 0.02	259.20 ± 0.04

The extract derived from the lyophilized leaves of *A. reptans* displays the highest antioxidant capacity, as determined through the extraction procedures. Conversely, the extracts originating from the root exhibit comparatively lower values. Metal chelating ability were presents as Trolox equivalent. The versenic acid (EDTA) solution was used as reference sample in measurements designed to demonstrate the iron (II) ions' chelating capacity for the *A. reptans* extracts (Table 6).

Table 6. Values of chelating capacity for iron (II) ions for extracts obtained from *A. reptans* (L_D—dried leaves, R_D—dried roots, L_L—freeze-dried leaves, R_L—freeze-dried root).

Iron (II) Ion Chelating Capacity of *A. reptans* Extracts (%)				
EDTA	**L_L**	**R_L**	**L_D**	**R_D**
55.48 ± 0.009	55.48 ± 0.01	84.68 ± 0.002	76.61 ± 0.002	57.74 ± 0.007

The lower value is shown by *A. reptans* leaf extract dried at 40 °C before extraction. The lowest values of chelating ability are shown by the extract of bugleweed leaves obtained

by previous freeze-drying. The obtained inhibition percents are higher than the obtained value for EDTA.

3.4. The Measurements of Total Polyphenol Content

The results of total polyphenol content measured by the Folin–Ciocalteu method in *A. reptans* ethanol extracts (samples L_D—dried leaves and R_D—dried roots) and also for the freeze-dried raw material (L_L—freeze-dried leaves and R_L—freeze-dried roots) are displayed in Table 7.

Table 7. Content of total polyphenols in *A. reptans* extracts (L_D—dried leaves, R_D—dried roots, L_L—freeze-dried leaves, R_L—freeze-dried roots).

Tested Extract	Total Polyphenol Content	
	(mg/mL)	(mg GAE/g)
R_D	0.10 ± 0.02	204.53 ± 0.03
L_D	0.16 ± 0.04	315.49 ± 0.04
R_L	0.12 ± 0.03	234.99 ± 0.03
L_L	0.14 ± 0.03	271.98 ± 0.02

The average content of polyphenols in the studied extracts are expressed as gallic acid equivalent (GAE) mg/g. These results show that the extracts obtained from the leaves of the plant have the highest polyphenol content. The lowest antioxidant values are characterized by the extract from the root dried at 40 °C for 24 h.

The obtained results proved that the extracts from the *A. reptans* leaves showed the highest polyphenol content (315.49 mg GAE/g) as well as antioxidant values: DPPH (inhibition% = 47.76), FRAP (4122.22 mM TE/g), and ABTS (259.20 mM TE/g). The results of ABTS measurements compared to other methodologies showed higher values of antioxidant capacity for the leaves dried by freeze-drying. This difference and the different ratio of results for individual extracts in the different methods are due to the fact that each of the test methods used is more sensitive to selected groups of compounds contained in the plant. Each of the methods used for the extracts confirmed that *A. reptans* root extracts dried at 40 °C showed the lowest antioxidant capacity. The chelating activity of the extracts from *A. reptans* has higher values (84.68%) than the comparative reference, which was 0.1 mM versenic acid (EDTA 55.48%). The results of these studies confirmed that *A. reptans* exhibits both antioxidant and chelating properties.

3.5. UV–VIS Characteristics of the Extracts

The results of testing the ultraviolet absorption capacity of the obtained extracts of *A. reptans* herb are depicted in Figures 2 and 3 through ultraviolet–visible (UV–Vis) spectra (samples L_D—dried leaves and R_D—dried root) and also for the freeze-dried raw material (L_L—freeze-dried leaves and R_L—freeze-dried root). Notably, for plants dried at 40 °C for 24 h and those dried using a freeze-dryer, extracts from the above-ground part of the plant exhibit superior UV–Vis absorption capabilities. When examining extracts in a 10 mm standard cuvette, particularly those dried using the freeze-drying process, the most characteristic peaks were observed.

In the case of the root extract, the peak I displayed a wavelength of 209.5 nm with an absorbance of 2.948, spanning a wavelength range of 201.8 nm to 213.8 nm. The absorbance value reached 2.948, and the integral value was 129.2. Additionally, peak II showed a wavelength of 216.5 nm and an absorbance of 2.876, covering a wavelength range from 213.75 nm to 277.0 nm. Absorbance measured 2.876, and the integral value was 322.2.

For the leaf extract, Peak I exhibited a wavelength of 326.5 nm with an absorbance of 1.324, encompassing a wavelength range from 308.0 nm to 632.0 nm. The half-width was 62.0, and the absorbance value was 1.324. The integral value was 341.6, and the Gauss integral measured 349.5. These detailed spectral characteristics provide comprehensive

insights into the distinct absorption peaks of both root and leaf extracts, enriching our understanding of their UV–Vis absorption capabilities.

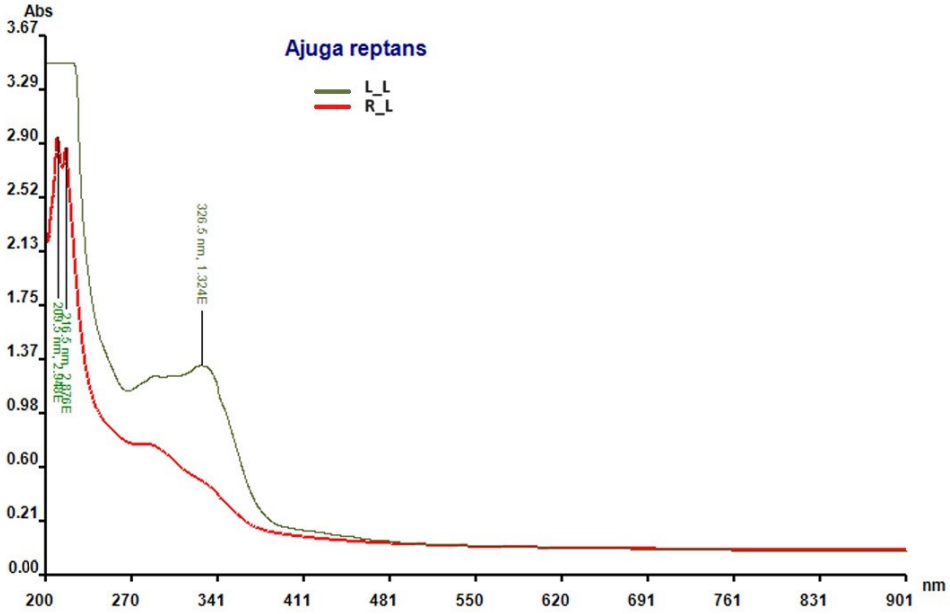

Figure 2. UV–Vis spectrum of freeze—dried plants (L_L—freeze-dried leaves and R_L—freeze-dried root).

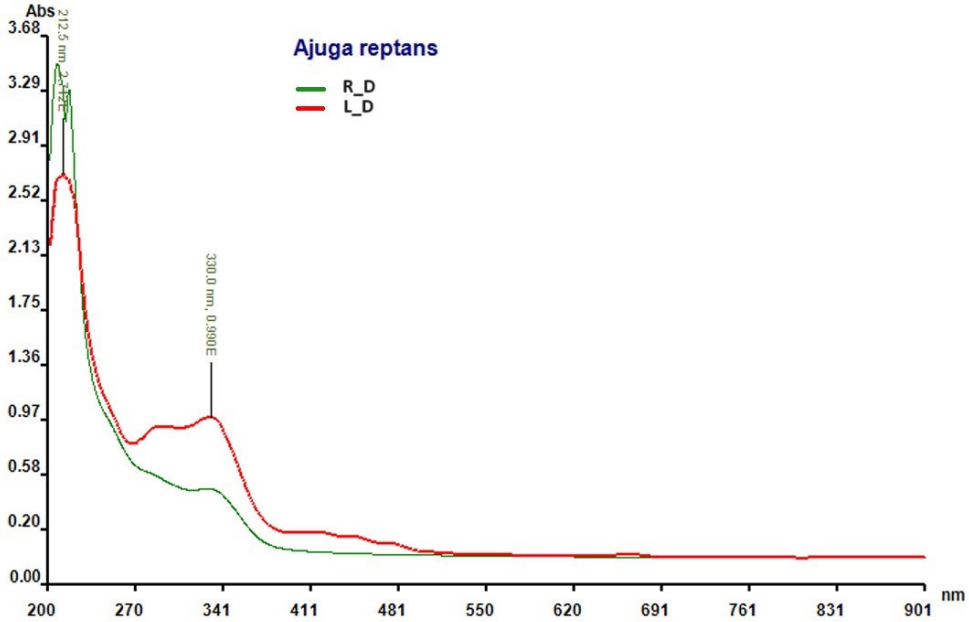

Figure 3. UV–Vis spectrum of plants dried at 40 °C (L_D—dried leaves and R_D—dried roots).

The examination of extracts in a 10 mm standard cuvette, dried at room temperature, revealed distinctive peaks, particularly evident in the leaf extract. For the leaves, Peak I displayed a wavelength of 212.5 nm with an absorbance of 2.712, covering a wavelength range from 210.5 nm to 214.8 nm. Absorbance reached 2.712, and the integral value was 48.5.

Furthermore, Peak II in the leaves extract exhibited a wavelength of 330.0 nm with an absorbance of 0.990, spanning a wavelength range from 307.8 nm to 392.0 nm. The half-width measured 57.0, and the absorbance value was 0.990. The integral value was 211.9, and the Gauss integral amounted to 240.3.

These detailed findings provide a nuanced understanding of the leaf extract's UV absorption characteristics, showcasing specific peaks and their associated parameters. The observed peaks contribute valuable insights into the UV absorption capacity of the leaves, offering a comprehensive perspective on potential applications.

The analysis of the obtained spectra of *A. reptans* extracts confirmed their UV-absorption ability in the ranges of 280–380 nm wavelengths. The higher absorbance values for extracts obtained from lyophilized material are worth noting.

3.6. Enzymatic Studies

Inhibition of Collagenase and Tyrosinase

Measurements aimed to demonstrate the collagenase and tyrosinase inhibition capacity of the investigated extracts lyophilized from leaves and roots of *A. reptans* (samples L_L—freeze-dried leaves and R_L—freeze-dried roots).

The extract derived from the lyophilized root of *A. reptans* displays the highest inhibition of collagenase capacity. Extracts originating from the root of exhibit comparatively lower values. From the values (Table 8), we can observe that the strongest inhibition of tyrosinase properties is shown by the extract of the root of the bugle herb obtained from the plant dried by lyophilization (47.52%). Similar values were obtained by extracts of the leaf (41.33%).

Table 8. Inhibition activity of collagenase and tyrosinase for extracts obtained from *A. reptans* (L_D—dried leaves, R_D—dried roots, L_L—freeze-dried leaves, R_L—freeze-dried roots).

Inhibition collagenase value of *A. reptans* extracts (%)	
L_L	R_L
49.37 ± 0.03	66.96 ± 0.02
Inhibition tyrosinase value of *A. reptans* extracts (%)	
L_L	R_L
41.33 ± 0.04	47.52 ± 0.01

The investigations into cytotoxicity, tyrosinase activity, and collagenase activity have revealed the multifaceted potential of *A. reptans* extracts. The findings suggest that these extracts exhibit versatility, holding promise not only as antioxidants and chelating agents but also showcasing attributes with broad applications in skincare. These attributes include the potential to impact skin pigmentation and collagen metabolism. It is worth noting that, in terms of enzymatic activity, the root extract of ajuga demonstrates higher catalytic efficiency than leaf extract (17.59% of collagenase inhibition and 6.19% of tyrosinase inhibition, respectively), prompting further investigation of this raw material for its rejuvenating properties. This component induces natural skin renewal processes by promoting the synthesis of structural protein, such as collagen. Additionally, the root extract inhibits melanin synthesis, which may result in skin whitening effects.

3.7. Cytotoxicity Tests

The EpiDerm model faithfully replicates the three-dimensional structure of human skin. In vitro studies provide not only precise information regarding the impact of substances on skin cells but also obviate the necessity of animal testing. Models like EpiDerm become crucial tools in refining safety standards within the cosmetic, pharmaceutical, and other industries related to skincare, facilitating more ethical and precise in vitro research [34]. The results shown in a graph (Figure 4) present the percentage of relative viability with standard deviations (±SD).

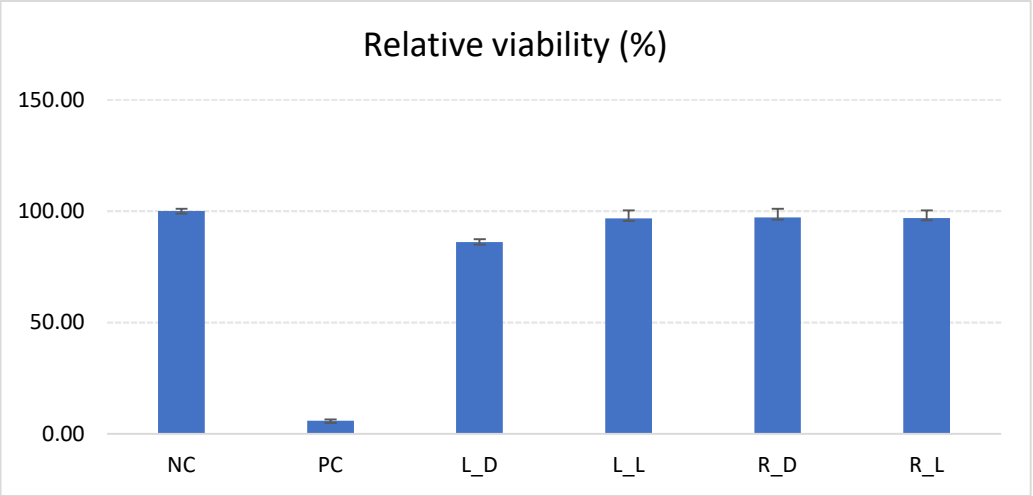

Figure 4. Relative tissue viability with the application of A. reptans (L_D—dried leaves, R_D—dried roots, L_L—freeze-dried leaves, R_L—freeze-dried roots, NC—negative control, DPBS, PC—positive control, 5% sodium dodecyl sulphate).

Summarizing the results of the cytotoxicity analysis, the obtained percentages of relative cell viability are as follows: negative control (NC) achieved 100% cell viability, indicating the absence of toxicity for this substance. Positive control (PC) led to a decrease in cell viability to 5.93%, confirming its toxicity. The extract from dried leaves showed a moderate impact with 86.05% cell viability, while freeze-dried leaves achieved 96.70% viability. The dried roots extract presented 97.26% viability, and freeze-dried roots obtained a result of 96.95%. Conclusions drawn from the analysis suggest that the tested substances from two samples of leaves (L_D, L_L) and two samples of roots (R_D, R_L) seem to exhibit a low level of cytotoxicity compared to the positive control, indicating potential safety for cells. The reliability of the conducted test was confirmed by the negative control, which showed the absence of toxicity for the control substance. These findings contribute valuable insights into the safety profile of the investigated substances, supporting their potential application with minimal adverse effects on cell viability.

4. Conclusions

Considering the conducted comparative studies of the activity of extracts from *A. reptans*, it can be concluded that the root extract is as valuable an active substance as that already used in cosmetics extracted from the leaves of ground plant. What is worth emphasizing is that the article compared the cosmetic potential of extracts from both the herb of the bugleweed and the root extract of this plant, which has not been previously described in the literature as an active ingredient in cosmetics. This allowed for the assessment of the potential use of both parts of the plant and the evaluation of their activity in terms of specific skin care. For instance, the roots extract demonstrates significantly higher effectiveness in inhibiting collagenase activity, thereby slowing down the degradation of the skin's key structural protein. The bugleweed root extract also proved to be more effective in the process of tyrosinase inhibition, indicating a higher potential for skin brightening. At the same time, extracts from the herb of the bugleweed exhibited a higher capacity for chemically reducing free radicals and chelating metal ions, which supports protection against the effects of reactive oxygen species on the skin. The multifunctional activity of both extracts shown in this article proves that both ingredients exhibit action on multiple levels of skin care, while remaining safe for use. We have also demonstrated how the drying method of the raw material affects the antioxidant and enzymatic activity of the obtained extracts. The use of freeze-drying method has shown that the resulting extract retains significantly higher ability to chelate metal ions in the case of the root (with 26.83% higher activity than in the case of conventionally dried root). It is also worth emphasizing that

the antioxidant activity in both ABTS, DPPH, and FRAP methods was higher for extracts obtained from material dried by lyophilization.

The high ability to neutralize free radicals, confirmed by various methods, the ability to induce collagen biosynthesis in the skin, as well as the brightening effect, represent invaluable actions in multiple aspects of skin protection against aging. Simultaneously, the investigated extracts protect the skin from UV radiation, further enhancing its natural defense against structural degeneration caused by sunlight. A comparison of the qualitative composition of the examined root and leaf extracts reveals differences in the content of major metabolites, which consistently correlate with slightly different antioxidant, enzymatic, and photoprotective activities. It is worth emphasizing that the root extract exhibits a stronger influence on collagen synthesis in the skin. This ingredient has not been described in the literature regarding its cosmetic action before, thus the presented research serves as valuable material for further studies on this raw material. However, it is undoubtedly a safe active ingredient, as confirmed by tests on a reconstructed skin model.

To sum up, the results of our study indicated that the *A. reptans* root extracts, similarly to the plant leaf extracts, could be applied as potentially multi-functional, safe and sensitive raw materials in anti-aging and anti-pollution cosmetics.

Author Contributions: Conceptualization, M.A.M. and E.S.; methodology, M.A.M., A.S. and A.D.; validation, A.S. formal analysis, A.D., A.S. and M.A.M.; investigation, A.D.; resources, M.A.M. and A.S.; data curation, M.A.M., A.D. and A.S.; writing—original draft preparation, A.D.; writing—review and editing, M.A.M., E.S. and A.S.; visualization, M.A.M.; supervision, E.S. and M.A.M.; project administration, M.A.M.; funding acquisition, M.A.M. and E.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the non-competition project TRL 4.0 "Support for management of research and commercialization of R and D results in scientific units and enterprises" within the action 4.4 "Enhancing human resources of the R and D sector" of the Operational Program Intelligent Development co-financed by the European Regional Development Fund.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

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

References

1. Israili, Z.H.; Lyoussi, B. Ethnopharmacology of the Plants of Genus Ajuga. *Pak. J. Pharm. Sci.* **2009**, *22*, 425–462. [PubMed]
2. Kalwij, J.M. Review of "The Plant List, a Working List of All Plant Species". *J. Veg. Sci.* **2012**, *23*, 998–1002. [CrossRef]
3. Penev, L.; Paton, A.; Nicolson, N.; Kirk, P.; Pyle, R.L.; Whitton, R.; Georgiev, T.; Barker, C.; Hopkins, C.; Robert, V.; et al. A common registration-to-publication automated pipeline for nomenclatural acts for higher plants (International Plant Names Index, IPNI), fungi (Index Fungorum, MycoBank) and animals (ZooBank). *Zookeys* **2016**, *2016*, 233–246. [CrossRef] [PubMed]
4. Szafer, W.; Kulczyński, S.; Pawłowski, B. *Rośliny Polskie*; PWN: Warsaw, Poland, 1953.
5. Rana, S.; Kaur, N.; Devi, P. A Review on Pharmacognostic Profile of Ajuga Reptans. Research & Reviews. *J. Pharmacogn.* **2020**, *7*, 9–18.
6. Činčura, F.; Feráková, V.; Májovský, J.; Ladislav Šomšák, J.Z. *Pospolite Rośliny Środkowej Europy*; Państwowe Wydawnictwo Rolnicze i Leśne: Warsaw, Poland, 1990.
7. Esposito, T.; Sansone, F.; Auriemma, G.; Franceschelli, S.; Pecoraro, M.; Picerno, P.; Aquino, R.P.; Mencherini, T. Study on Ajuga Reptans Extract: A Natural Antioxidant in Microencapsulated Powder Form as an Active Ingredient for Nutraceutical or Pharmaceutical Purposes. *Pharmaceutics* **2020**, *12*, 671. [CrossRef] [PubMed]
8. Toiu, A.; Vlase, L.; Gheldiu, A.M.; Vodnar, D.; Oniga, I. Evaluation of the Antioxidant and Antibacterial Potential of Bioactive Compounds from Ajuga Reptans Extracts. *Farmacia* **2017**, *65*, 353–354.
9. Toiu, A.; Mocan, A.; Vlase, L.; Pârvu, A.E.; Vodnar, D.C.; Gheldiu, A.; Moldovan, C.; Oniga, I. Phytochemical Composition, Antioxidant, Antimicrobial and in Vivo Anti-Inflammatory Activity of Traditionally Used Romanian Ajuga Laxmannii (Murray) Benth. ("Nobleman's Beard"—Barba Împăratului). *Front. Pharmacol.* **2018**, *9*, 7. [CrossRef] [PubMed]
10. Popova, E.; Titova, M.; Tynykulov, M.; Zakirova, R.P.; Kulichenko, I.; Prudnikova, O.; Nosov, A. Sustainable Production of Ajuga Bioactive Metabolites Using Cell Culture Technologies: A Review. *Nutrients* **2023**, *15*, 1246. [CrossRef]
11. Vertuani, S.; Ziosi, P.; Toso, R.D.; Vicentini, C.B.; Manfredini, S. Dualistic Properties of Cosmetic Formulations Based on Phenylpropanoids from Ajuga Reptans. *J. Cosmet. Dermatol. Sci. Appl.* **2013**, *3*, 64–72. [CrossRef]

12. Frezza, C.; Venditti, A.; Serafini, M.; Bianco, A. Phytochemistry, Chemotaxonomy, Ethnopharmacology, and Nutraceutics of Lamiaceae. *Stud. Nat. Prod. Chem.* **2019**, *62*, 125–178. [CrossRef]
13. Qing, X.; Yan, H.M.; Ni, Z.Y.; Vavricka, C.J.; Zhang, M.L.; Shi, Q.W.; Gu, Y.C.; Kiyota, H. Chemical and Pharmacological Research on the Plants from Genus Ajuga. *Heterocycl. Commun.* **2017**, *23*, 245–268. [CrossRef]
14. Aslan Bayhan, S.; Bayhan, H.A.; Çölgeçen, E.; Gürdal, C. Effects of Topical Acne Treatment on the Ocular Surface in Patients with Acne Vulgaris. *Contact Lens Anterior Eye* **2016**, *39*, 431–434. [CrossRef] [PubMed]
15. Karłowicz-Bodalska, K.; Han, S.; Bodalska, A.; Freier, J.; Smoleński, M. Przeciwzapalne Właściwości Wybranych Roślin Zawierających Związki Irydoidowe. *Postępy Fitoter.* **2017**, *18*, 229–234. [CrossRef]
16. Tanaka, N.; Uozumi, N.; Kobayashi, T. Genetic Transformation of *Ajuga Replans*. In *Transgenic Medicinal Plants*, 45th ed.; Bajaj, Y.P.S., Ed.; Springer: Berlin/Heidelberg, Germany, 1999; ISBN 364258439X.
17. Göger, G.; Köse, Y.B.; Demirci, F.; Göger, F. Phytochemical Characterization of Phenolic Compounds by LCMS/MS and Biological Activities of *Ajuga reptans* L., *Ajuga salicifolia* (L.) Schreber and *Ajuga genevensis* L. from Turkey. *Turk. J. Pharm. Sci.* **2021**, *10*, 616–627. [CrossRef] [PubMed]
18. Toiu, A.; Mocan, A.; Vlase, L.; Pârvu, A.E.; Vodnar, D.C.; Gheldiu, A.; Moldovan, C.; Oniga, I. Comparative Phytochemical Profile, Antioxidant, Antimicrobial and In Vivo Anti-Inflammatory Activity of Different Extracts of Traditionally Used Romanian *Ajuga genevensis* L. and *A reptans* L. (Lamiaceae). *Molecules* **2019**, *24*, 1597. [CrossRef] [PubMed]
19. Camps, F.; Coll, J.; Cortel, A. 29-Norsengosterone and 29-Norcyasterone, New C-28 Phytoecdysteroids from *Ajuga reptans* (Labiatae). *Chem. Lett.* **1982**, *11*, 1313–1316. [CrossRef]
20. Alekseeva, L.I.; Volodin, V.V.; Luksha, V.G.; Lafont, R. Ecdysteroid Acetates from *Ajuga reptans*. *Chem. Nat. Compd.* **1999**, *35*, 532–534. [CrossRef]
21. Giuliani, G.; Benedusi, A.; Bellinvia, S. Composition Based on Vegetal Extracts of Ajuga Reptans for Preventing Hair Loss, Stimulating the Growth of Hair, Regulating the Production of. Sebum. Patent WO2006100101, 28 September 2006.
22. Ellnain-Wojtaszek, M.; Zgórka, G. High-Performance Liquid Chromatography and Thin-Layer Chromatography of Phenolic Acids from Ginkgo Biloba L. Leaves Collected within Vegetative Period. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22*, 1457–1471. [CrossRef]
23. Szopa, A.; Starzec, A.; Ekiert, H. The Importance of Monochromatic Lights in the Production of Phenolic Acids and Flavonoids in Shoot Cultures of *Aronia melanocarpa*, *Aronia arbutifolia* and *Aronia* × *Prunifolia*. *J. Photochem. Photobiol. B Biol.* **2018**, *179*, 91–97. [CrossRef] [PubMed]
24. Szopa, A.; Kokotkiewicz, A.; Bednarz, M.; Jafernik, K.; Luczkiewicz, M.; Ekiert, H. Bioreactor Type Affects the Accumulation of Phenolic Acids and Flavonoids in Microshoot Cultures of *Schisandra chinensis* (Turcz.) Baill. *Plant Cell. Tissue Organ Cult.* **2019**, *139*, 199–206. [CrossRef]
25. Kubica, P.; Szopa, A.; Kokotkiewicz, A.; Miceli, N.; Gniewosz, M.; Elansary, H.O.; Mahmoud, E.A.; El-ansary, D.O. Production of Verbascoside, Isoverbascoside and Phenolic Acids in Callus, Suspension, and Bioreactor Cultures of Verbena Officinalis and Biological Properties of Biomass Extracts. *Molecules* **2020**, *25*, 5609. [CrossRef] [PubMed]
26. Ferrier, M.; Billet, K.; Drouet, S.; Tungmunnithum, D.; Malinowska, M.A.; Marchal, C.; Dedet, S.; Giglioli-Guivarc'h, N.; Hano, C.; Lanoue, A. Identifying Major Drivers of Antioxidant Activities in Complex Polyphenol Mixtures from Grape Canes. *Molecules* **2022**, *27*, 4029. [CrossRef] [PubMed]
27. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Hawkins Byrne, D. Comparison of ABTS, DPPH, FRAP, and ORAC Assays for Estimating Antioxidant Activity from Guava Fruit Extracts. *J. Food Compos. Anal.* **2006**, *19*, 671. [CrossRef]
28. Gaidhani, K.A.; Harwalkar, M.; Bhambere, D.; Nirgude, P.S. Lyophilization/Freeze Drying—A Review. *World J. Pharm. Res.* **2015**, *4*, 516–543.
29. Bisht, D.; Iqbal, Z. Lyophilization—Process and Optimization for Pharmaceuticals. *Int. J. Drug Regul. Aff.* **2018**, *3*, 30–40. [CrossRef]
30. Calín-Sánchez, Á.; Lipan, L.; Cano-Lamadrid, M.; Kharaghani, A.; Masztalerz, K.; Carbonell-Barrachina, Á.A.; Figiel, A. Comparison of Traditional and Novel Drying Techniques and Its Effect on Quality of Fruits, Vegetables and Aromatic Herbs. *Foods* **2020**, *9*, 1261. [CrossRef] [PubMed]
31. Lewicki, P.P. Design of Hot Air Drying for Better Foods. *Trends Food Sci. Technol.* **2006**, *17*, 153–163. [CrossRef]
32. Thamkaew, G.; Sjöholm, I.; Galindo, F.G. A Review of Drying Methods for Improving the Quality of Dried Herbs. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 1763–1786. [CrossRef] [PubMed]
33. Florczak, J.; Karmańska, A.; Karwowski, B. Badanie Zawartości Związków Polifenolowych Oraz Aktywności Przeciwutelniającej Niektórych Jadanych Grzybów Wieloowocnikowych. *Bromatol. Chemiia Toksykol.* **2016**, *49*, 719–724.
34. Olejnik, A.; Gościańska, J.; Nowak, I. Zastosowanie Modeli Sztucznej Skóry Do Badań Kosmetyków Innowacyjnych. *Chemik* **2011**, *65*, 76–81.