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Insights on the In Vitro Wound Healing Effects of *Sedum telephium* L. Leaf Juice

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Abstract: Chronic wounds remain a significant clinical challenge, necessitating the exploration of novel therapeutic agents. *Sedum telephium* L. (syn. *Hylotelephium telephium* (L.) H. Ohba) fresh leaf juice, known for its traditional medicinal uses, was evaluated to assess its efficacy and its mechanism in promoting wound healing in vitro. Fresh leaves were collected and processed to obtain the juice, which was analyzed for polyphenols, flavonoids, polysaccharides, and proteins content. Key bioactive compounds were identified, including complex polysaccharides (2.47%), proteins (0.16%) and kaempferol and quercetin tri- di- and mono-glycosides, 0.04%, expressed as quercetin equivalent. The juice and its polysaccharidic fraction were tested on human keratinocytes (HaCaT) and fibroblasts (HFF-1) to assess cell viability, wound closure, and the production of growth factors and pro-collagen I. Our results indicated that the whole juice significantly enhanced wound closure in both cell types: +33% in keratinocytes compared to control, at 24 h, with a marked increase in fibroblast growth factor (FGF) (+21% compared to control) and LAP(TGF-β1) (+60% compared to control) and +30% in fibroblasts compared to control at 24 h, without a significant upregulation of pro-collagen I expression. The polysaccharidic fraction alone showed limited efficacy (no efficacy in keratinocytes and +20% in wound closure compared to control at 24 h in fibroblast), emphasizing the importance of the complete phytocomplex. These findings suggest that *Sedum telephium* L. represents a promising candidate in the setting of skin regeneration and repair.

Keywords: phytomedicine; *Sedum telephium* L.; wound healing; skin

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1. Introduction

The healing process is a physiological complex of events comprising hemostasis, chemotaxis and inflammatory cascade, cell proliferation and tissue remodeling [1–3].

A multitude of strategies are currently available to address acute injuries and promote tissue regeneration [4–7]. Several safe and effective options to address minor and acute injuries include the use of topical antibiotics and/or disinfectants (including silver sulfadiazine, benzalkonium chloride, ethanol, and povidone iodine). On the other hand, chronic ulcers pose a persistent challenge, often being resistant to conventional treatments and prone to complications [8]. Chronic wounds are characterized by prolonged healing time and propensity for recurrence, with the subsequent diminished quality of life for patients and increased healthcare costs [6,9,10]. The social impact is profound, as chronic wounds can result in pain, disability, and reduced mobility, impairing patients' ability to perform daily activities and to engage in social interactions [11]. Depression, anxiety, and social isolation further exacerbate the burden on affected individuals [10,12]. Millions of

individuals are affected worldwide, giving a reason for the unbearable economic burden for healthcare systems [9,13].

Available strategies include traditional and advanced dressings, dermal scaffolds, growth factors, stem cells, systemic treatments [5,14]. The therapeutic choice varies upon the type of ulcer, patient comorbidities and the expertise of the healthcare team in treating cutaneous ulcers [2,15]. However, no definitive treatment for the cure of chronic wounds is available to date. As such, there is an urgent need for innovative therapies tailored specifically to these recalcitrant wounds. Current research is increasingly focusing on phytoderivatives, bioactive compounds derived from plants, as promising candidates for addressing this unmet medical need [16]. These natural compounds offer a multifaceted approach to wound healing, targeting inflammation, infection, and tissue repair processes [17,18]. With growing evidence supporting their efficacy and safety, phytoderivatives represent a compelling frontier in the quest for novel therapies to combat chronic ulcers and improve patient outcomes.

On the one hand, the interest towards medicinal plants in wound healing is supported by a vigorous push provided by growing literature in the setting of ethnopharmacology; on the other hand, another stimulus to the growing interest towards phytomedicine is provided by the official recognition of several herbal products, marketed as conventional drugs, enlisted in the Monographs on Selected Medicinal Plants of the World Health Organization (WHO) (who.int) and, in the European Union (UE), in those of the European Medicines Agency (EMA) (www.ema.europa.eu, accessed on 6 June 2024). The aim of this work is to provide an insight on the potential use of *Sedum telephium* L. (syn. *Hylotelephium telephium* (L.) H. Ohba) (Crassulaceae), in the setting of wound healing. *S. telephium* is a medicinal plant traditionally known in the Italian ethnobotany and initially studied by Balatri [19–21] who, first, introduced the use of fresh leaves juice in the modern medicine to heal wounds. Actually, this species has been only partially exploited but also not adequately explored, both for its phytochemistry and for experimental pharmacology: *S. telephium* has been shown to contain as main characteristic compounds polysaccharides and flavonols, consisting, respectively, in rhamnogalacturonans [21,22] and kaempferol and quercetin glycosides, especially tri- and di-glycosides, such as quercetin and kaempferol 7-*O*-rhamnoside with glucose or rhamnose or glucose-rhamnose *O*-bonded in position 3 [20]. Three different *S. telephium* lyophilized extracts were tested to compare the antioxidant (in vitro) and the photoprotective activity (in vivo) of the whole phytocomplex, one enriched in polysaccharidic and one enriched in flavonols fractions; from the study emerged that the whole phytocomplex and flavonols, but not polysaccharides demonstrated antioxidant and photoprotective effectiveness [23]. A methanolic extract of *S. telephium* sbsp. *maximum* showed anti-inflammatory activity in lipopolysaccharide-stimulated rat peritoneal macrophages [24], but also isolated *S. telephium* rhamnogalatturonans showed immunomodulatory and anti-inflammatory activity [22,24]. In the scarce number of papers on *S. telephium* is included one old study where the wound healing potentiality of this medicinal plant was found debatable, as fresh leaf juice and isolated polysaccharides, but not *S. telephium* flavonols demonstrated to inhibit MRC5 cell adhesion to laminin and fibronectin [21].

Products for topical application containing *S. telephium* are currently commercially available and possess interesting potential implications in the dermatological setting.

In the present study, we aimed at characterizing the juice obtained from *S. telephium* leaves from a chemical point of view and, even more importantly, to assess its trophic action though in vitro wound-healing assays. both on keratinocytes and fibroblasts.

We also compared the efficacy of whole fresh juice and its polysaccharidic fraction only. Lastly, we assessed the production of pro-collagen I by fibroblasts and the secretion of growth factors by keratinocytes induced by *S. telephium*, in order to better clarify its potential mechanism of action in the setting of wound healing.

2. Materials and Methods

2.1. Sampling

Fresh leaves of *S. telephium* were collected from domesticated plants at the end of July, classified and authenticated at the Botanical Garden of the University of Siena. At least 200 g of fresh leaves were taken. Plants were selected according to the following criteria: integrity, absence of parasite infestations, and provenience from areas not subject to phytosanitary treatments. Sedum juice (SED) was obtained by squeezing fresh leaves after having removed the leaf epidermis, using a kitchen centrifuge (Vitafruit, Moulinex, Écully, France).

2.2. Phytochemical Analyses

2.2.1. Polyphenol and Flavonoids Content

The total amount of polyphenols in SED was determined by the Folin–Ciocâlteu colorimetric method, optimized as described by Finetti et al. [25]. Gallic acid (Merck Sigma-Aldrich, Darmstadt, Germany) was used as reference standard and results were expressed as gallic acid equivalents (GAEs). Flavonoid content was quantified by means of direct absorbance [26]. Briefly, SED was diluted 100 mg/mL in bidistilled double-water, and the absorbance was detected at a wavelength of 366 nm. Quercetin (Merck Sigma-Aldrich) was used as a reference standard, with 366 nm being its peak of absorbance. Results were expressed as quercetin equivalents (QEs). A Shimadzu UV 1900 spectrophotometer (Kyoto, Japan) was used for absorbance quantification. All analyses were performed in triplicate.

2.2.2. Quantification and Isolation of Polysaccharides

SED polysaccharides (SEDPOL) were isolated through conventional ethanol precipitation. Basically, 1 g of SED was diluted 1:2 with ultrapure water and placed in a bain-marie at 100 °C for 15 min to promote sugar solubilization. The obtained solution was centrifuged at 2000 rpm for 5 min and precipitated material was discarded. Absolute ethanol (Merck Sigma-Aldrich) at −80 °C was added to the solution in a 1:1 ratio and determined the precipitation of the polysaccharides, in the form of a whitish gel. The solution was centrifuged and the supernatant was removed. The precipitate was recovered with 10 mL of ultrapure water and assayed through the colorimetric phenol-sulfuric method, already used and described in [18]. Briefly, 20 µL of the sample was diluted with 380 µL of ultrapure water and placed in a reaction tube; then, 200 µL of 6% m/v (mass/volume) aqueous phenol solution were added. Subsequently, 0.5 mL of 98% m/m sulfuric acid was added and the reaction tube was quickly closed. After 10 min at 80 °C, the absorbance was read at 490 nm with a Shimadzu UV 1900 spectrophotometer. The amount of polysaccharides was calculated interpolating data on the standard curve constructed with D(+)-glucose (Merck Sigma-Aldrich) and by multiplying ×0.9 to convert D(+)-glucose in polysaccharides. The test was conducted in experimental triplicate.

SEDPOL were further investigated for their qualitative structure by using a specific enzymatic kit able to identify the different monomeric components. More in detail, two available kits (D-fructose/D-glucose assay kit ref. K-FRUGL, and L-arabinose/D-galactose assay kit, ref. K-ARGA, Megazyme, Bray, Ireland) were used according to manufacturer's instructions. After enzymatic reactions, absorbance was read at 340 nm by using a Shimadzu UV 1900 spectrophotometer, and internal control (shipped with kits) was used.

2.2.3. Protein Dosage

The validated Bradford method [27] was used for the quantification of proteins in SED. Five µL of the SED sample were placed in 96-well plates; Ultrapure water was used as a control. An amount of 245 µL of Bradford's solution (B6916-500mL, Merck Sigma-Aldrich) was added to each well and plates were incubated for 15 min in the dark at room temperature. The plates were then read through Victor NIVO 3S (Perkin Elmer, Waltham, MA, USA) at 595 nm. The calibration curve was obtained using bovine serum

albumin (BSA) (A3059-100G, Merck Sigma-Aldrich). All the measurements were conducted in triplicate.

2.2.4. Flavonol Profile

HPLC-DAD (High-Performance Liquid Chromatography coupled with a Diode Array Detector) analysis was performed by using a Shimadzu Prominence LC 2030 3D instrument (Shimadzu, Kyoto, Japan), equipped with a Bondapak® C18 column, 10 mm, 125 Å, 3.9 mm × 300 mm column (Waters Corporation, Milford, MA, USA).

Water solutions containing 0.1% (*v*/*v*) formic acid (A) and acetonitrile with 0.1% (*v*/*v*) of formic acid (B) were used as mobile phases. The following program was applied: B from 10% at 0 min to 25% at min, then B 50% at 26 min to 10% at 35 min. Flow rate was set at 0.9 mL/min. Chromatograms were recorded at 366 nm. Analyses were performed using 20 µL of SED diluted 1:5 in ultrapure water; quercetin, kaempferol, isoquercitrin, hyperoside and kaempferitrin (Merck Sigma-Aldrich) were used as external standards. Three independent runs were performed.

2.3. Cell Biology Analysis

2.3.1. Cell Cultures

Aneuploid immortal keratinocytes from adult human skin (HaCaT) and Human Foreskin Fibroblasts (HFF-1) (kindly provided by the Dept. of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy) were cultured in polystyrene flasks (Sarstedt AG & Co. KG, Nümbrecht, Germany) using Dulbecco's Modified Eagle's Medium—High glucose (DMEM-Hi glucose—D5796, Merck Sigma-Aldrich) supplemented with 10% *v*/*v* Fetal Bovine Serum (FBS Merck Sigma-Aldrich) and 1% *v*/*v* 1:100 diluted Antibiotic-Antimycotic solution (Merck Sigma-Aldrich) at 37 °C in 5% CO_2 atmosphere.

Buffer Saline (PBS) solution and 1× Trypsin-EDTA solution (Merck Sigma-Aldrich) were used to detach cells from flasks when reaching 70% confluence; by means of centrifugation at 800 rpm for 5 min, the cell pellet was obtained and then resuspended into fresh medium. Therefore, cells underwent 2 passages in culture. Cells count was performed using a hemocytometer after staining with Trypan Blue dye (Merck Sigma-Aldrich).

2.3.2. Cell Viability

The Cell Counting Kit—8 (CCK-8, Merck Sigma-Aldrich) was used to evaluate cell viability of HaCaT and HFF in response to treatments with SED, SEDPOL and the reference drug (REFDRUG). SED was tested at different dilutions (0.1, 1 and 5 mg/mL, respectively), SEDPOL was tested at a concentration of 0.03 mg/mL, and REFDRUG, a cream containing 15% m/m *Triticum vulgare* L. standardized water extract + 1% m/m phenoxyethanol, was tested at 0.1 mg/mL.

Cells were seeded at a density of 5000 cells/well in a 96-well plate and incubated for 24 h at 37 °C in 5% CO_2 atmosphere. Supernatants were then removed and wells were filled with 90 µL of culture medium and 10 µL of treatment samples and again incubated for 24 h, as described above. Then, fresh DMEM and CCK-8 (9:1) was added into each well and incubated for 30–60 min. Absorbance was measured at 450 nm using a Perkin Elmer Victor Nivo 3S Microplate Reader.

2.3.3. Scratch Test—Wound Healing Assay

We performed a revised version of the protocol published by Chen et al. [28], as also already described by Chiocchio et al. [29]. HaCaT and HFF cells were seeded into 6-well plates (Sarstedt AG & Co. KG) at a density of 50,000 cells/well with DMEM supplemented with 10% FBS and incubated at 37 °C in 5% CO_2 atmosphere until the achievement of approximately 80% confluence as a monolayer. Using a 1 mL pipette tip, the monolayer was scratched to create a cross at the center of each well. Supernatant (together with the detached cells) was then discarded, and DMEM supplemented with 3% FBSm, together with different treatment samples (SED 1 mg/mL, SEDPOL 0.03 mg/mL and REFDRUG

0.1 mg/mL), was added to the wells. Two independent experiments in technical triplicates (n = 6) were performed. Microscopic images of each well were taken at 0, 6 and 24 h after treatment by using a Leica DMIL microscope (Leica, Wetzlar, Germany). The wound size was evaluated using IC Measure software (The Imaging Source LLC, Version 2.0.0.286, https://www.theimagingsource.com/en-us/support/download/icmeasure-2.0.0.286/, accessed on 6 June 2024). Two different measurements were performed for each well. Untreated scratched cells represented the control. The percentage of wound closure was calculated using the following formula: [(Wound area t_0 − Wound area t)/Wound area t_0] × 100.

2.3.4. Growth Factors Dosages

The ELISA (Enzyme-Linked Immunosorbent Assay) test was used for the measurement of fibroblast growth factor (FGF), epidermal growth factor (EGF), precursor transforming growth factor latency-associated peptide (LAP(TGF-β1)) and procollagen I. While EGF, FGF and LAP(TGF-β1) were dosed on HaCaT-grown media, procollagen I measurement was performed on HFF-1 supernatant.

Cells were seeded into 24-well plates (10,000 cells/well) and treated with SED 1 mg/mL, SEDPOL 0.03 mg/mL and REFDRUG 0.1 mg/mL for 6 and 24 h. At the end of the treatments, 10 µL of protease inhibitor (Merck Sigma-Aldrich) 1× was added to each well and the plates were frozen. Following this, 3 cycles of freezing and thawing were performed in order to obtain complete cell lysis. Finally, the supernatants were collected in 1 mL tubes.

The human FGF-7 ELISA kit (RAB0188, Merck Sigma-Aldrich), human EGF ELISA kit (RAB0149, Merck Sigma-Aldrich), human LAP(TGF-β1) (88-50390-22, Thermo Fisher Scientific Inc., Waltham, MA, USA) and procollagen I α1 ELISA kit (ab210966, Abcam, Cambridge, UK) were used for ELISA assays. All ELISA dosages were performed following suppliers' data sheets. A Victor Nivo 3S Multimode Microplate Reader (Perkin Elmer) was used to read plates. Dosages were performed in two independent experiments with technical triplicates (n = 6).

2.4. Statistical Analysis

The one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to compare datasets and to assess the statistical analysis; $p < 0.05$ was set as the significance level. Data are presented as mean of replicates ± standard deviation (SD). Analyses were conducted using SPSS for Windows® v.25 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism (San Diego, CA, USA).

3. Results

3.1. Chemical Characterization of Sedum telephium L. Leaves Juice

In accordance with the previously published literature on *S. telephium*, chemical analyses showed that polysaccharides were the main constituents of SED: 2.47% m/m in fresh leaves. D-fructose, L-arabinose and D-galactose were not found in detectable amounts, whereas D-glucose and reactive glucose-derived monomers were clearly identified. The undetectable amount of D-fructose isolated in SEDPOL suggested that this fraction was not composed of sucrose, the main disaccharide of plants [30], but most likely by high molecular weight polysaccharides derived from cell wall pectins and hemicelluloses, as already reported by Sendl et al. who reported the major presence of pectin-derived rhamnogalatturonans in *S. telephium* [22]. In this work, we also highlighted the presence of proteins in SED, very plausibly as part of mucilage, also composed by glycoproteins [31]. Moreover, as previously reported, SED was enriched in polyphenols (793 mg GAE/Kg), especially flavonols, quantified by means of the UV method (420 mg QE/Kg), and clearly individuated through an HPLC-DAD analysis. As for the UV spectrum, the main peaks with retention time (RT) between 11.2 and 18.1 min were attributed to quercetin (RT = 11.2 min) and kaempferol glycosides (others) (Figure 1): considering the method used, we considered the main peaks as quercetin and kaempferol tri- and di-glycosides, as already reported by Mulinacci et al. [20] for *S. sedum* subsp. *maximum*. Unfortunately, quercetin and kaempferol

3-*O*-substituted 7-*O*-rhamnoside (already reported in the literature) are not available on the market as standards, with the exception of kaempferitrin, taken into account here and identified at RT = 18.1 min (0.007%). The HPLC-DAD quantification of the whole complex of quercetin and kaempferol tri- and diglycosides, expressed as kaempferitrin, confirmed this fraction to represent more than 90% (>400 mg/Kg) of total flavonoids. In accordance with the previous literature, quercetin mono-glycosides were also identified, including isoquercitrin and hyperoside, partially overlapping at RT = 19.3 and 19.8 min (0.002%) (Figure 1). Quercetin and kaempferol were identified at very low concentrations (<0.001%) as aglycones (Table 1).

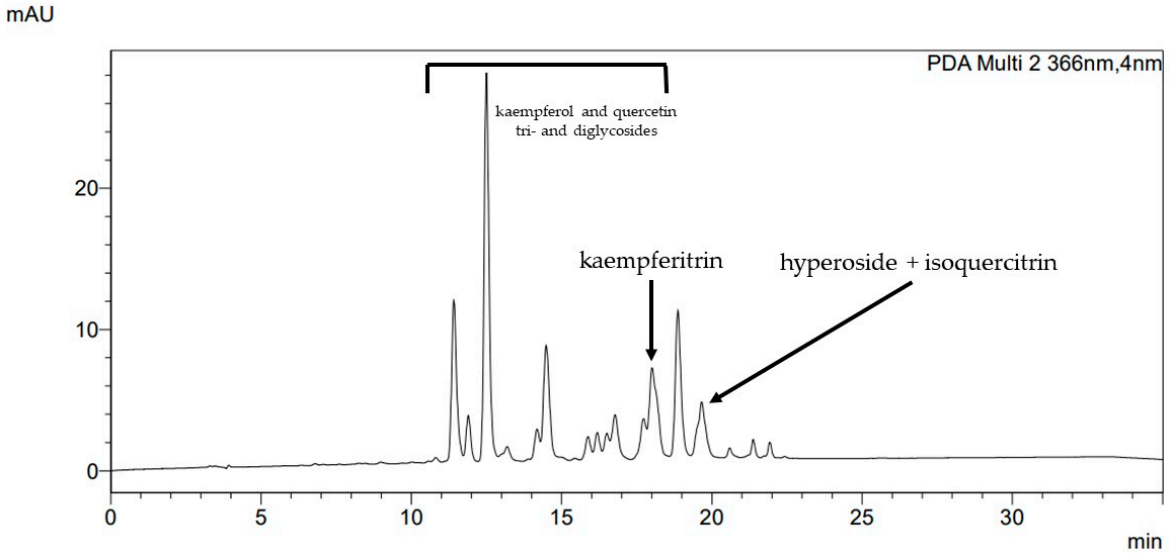

Figure 1. Chromatogram of the flavonoid fraction of *Sedum telephium* L. leaf juice. Main peaks could be attributed to kaempferol tri- (at lower retention time, RT) and di-glycosides, also including kaempferitrin (RT = 18.1 min); a quercetin tri-glycoside is recorded at RT = 11.2 min and mono-glycosides matching RT of isoquercitrin and hyperoside are recorded at RT = 19.3 and 19.8 min.

Table 1. Chemical composition of *Sedum telephium* L. leaves juice. Polyphenols are expressed as gallic acid equivalents, flavonoids as quercetin equivalents, protein as bovine serum albumin equivalent and, finally, polysaccharides as D(+)-glucose.

Cf-Chemical Class	Content % (m/m)
Total polyphenols	0.08 ± 0.01
Total flavonoids	0.04 ± 0.01
Total proteins	0.16 ± 0.02
Total polysaccharides	2.47 ± 0.22

3.2. Wound Healing Activity of Sedum telephium L. Leaves Juicef in Human Keratinocytes

Cell viability assays in human keratinocytes showed that SED 5 mg/mL slightly decreased the cell viability at 6 h (−19% vs. ctrl, $p < 0.01$), even if it did not alter the cell viability at 24 h; on the other hand, SED 0.1 and 1 mg/mL and SEDPOL 0.03 mg/mL and REFDRUG 0.1 mg/mL did not impact cell viability either at 6 h or at 24 h. For this reason, we chose SED 1 mg/mL for the subsequent test, together with SEDPOL 0.03 mg/mL and REFDRUG 0.1 mg/mL. In human fibroblasts also, these samples did not impact cell viability.

Skin-healing tests were performed on both keratinocytes and fibroblasts: considering the preponderance of polysaccharide components within the SED phytocomplex, it was decided to also test SEDPOL alone at the same concentration as in the SED phytocomplex.

As shown in the representative images in Figure 2 and in the panel in Figure 3, there was a clear improvement in the healing rate in keratinocytes treated with SED, especially

at 24 h, with a mean of +33% of wound closure compared to the control ($p < 0.01$ vs. ctrl). A slight improvement (mean: +13%, $p > 0.05$ vs. ctrl) over the control was also observed at 6 h. In both cases, the activity of SED on keratinocytes was found to be higher than that of REFDRUG at 6 and 24 h (+3 and +8%, respectively, $p > 0.05$ vs. ctrl at 24 h). A null effect was recorded for SEDPOL in the wound closure, confirming the importance of the whole phytocomplex in exerting skin-healing activity.

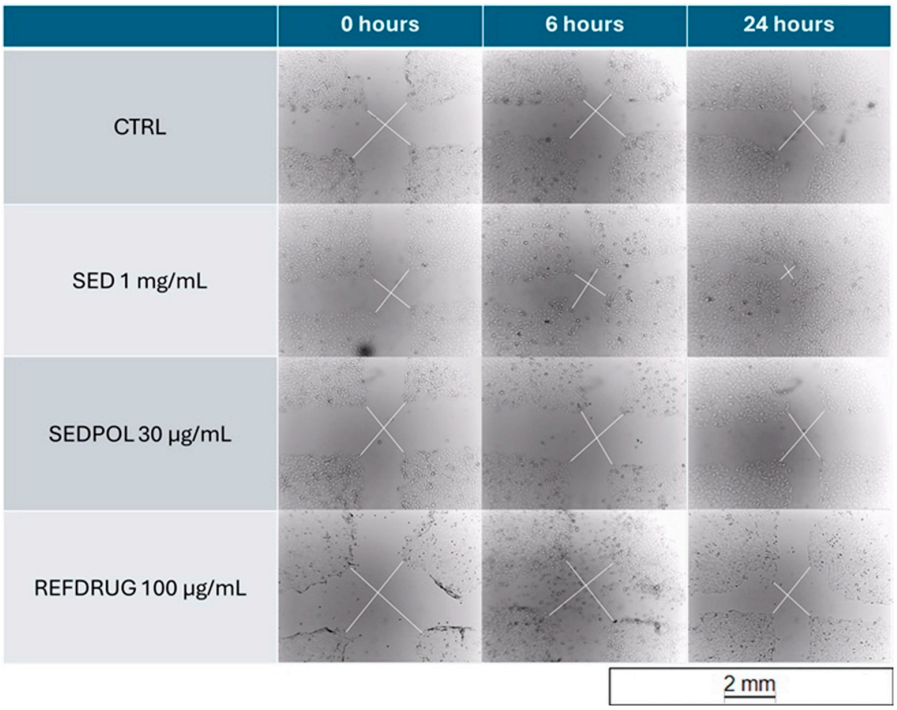

Figure 2. Microscopic images documenting the in vitro healing effect of *S. telephium* leaf juice (SED) 1 mg/mL, of the isolated polysaccharides (SEDPOL) at the same concentration in which they are present in SED (30 µg/mL) and of the reference drug (*T. vulgare* extract + phenoxy-ethanol, REFDRUG 100 µg/mL) in human keratinocytes, through the scratch test.

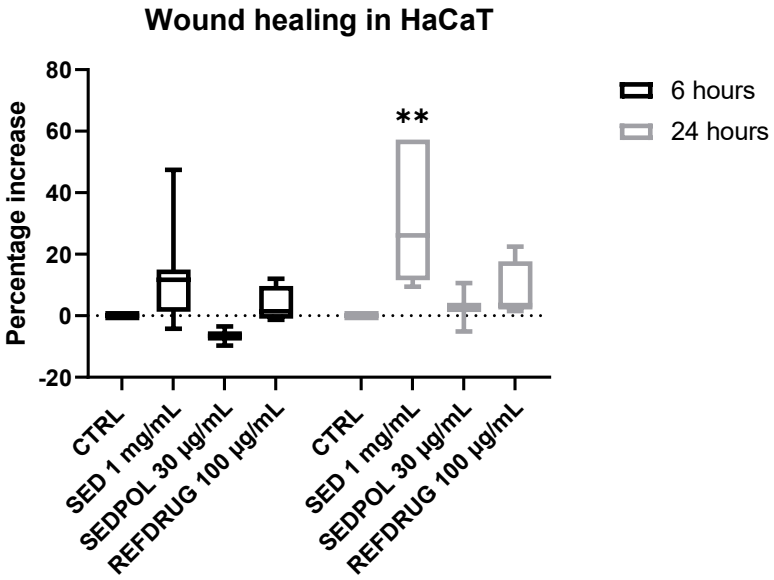

Figure 3. Box plot graphical representation of the in vitro wound-healing effect of *S. telephium* leaf juice (SED), of isolated polysaccharides (SEDPOL) and of the reference drug (REFDRUG) in human keratinocytes. **: $p < 0.01$ vs. ctrl (one-way ANOVA).

3.3. Wound Healing Mechanism of Sedum telephium L. Leaves Juice

To better understand the mechanism of action whereby SED acted in healing processes in HaCaT cells, the main growth factors produced by keratinocytes were dosed: FGF, EGF, and the precursor of TGF-β1, LAP(TGF-β1).

As shown in Figure 4, SED was able to increase FGF and LAP(TGF-β1), at 6 h and, to a higher extent, at 24 h: +21% ($p < 0.001$ vs. ctrl) and +60% ($p < 0.01$ vs. ctrl), respectively, compared to the control.

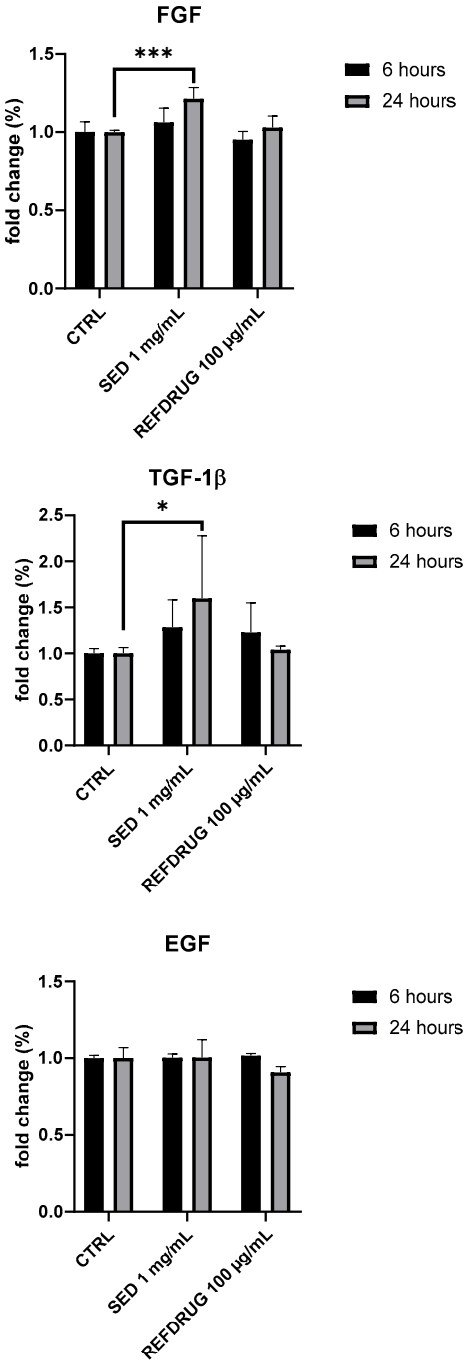

Figure 4. Histogram representation of growth-factor production induced by of *S. telephium* leaf juice (SED) 1 mg/mL, and of the reference drug (REFDRUG) 100 µg/mL. The upregulation of FGF and LAP(TGF-β1) produced by SED is correlated to the scratching wound-healing assay for SED at 24 h. *: $p < 0.05$ vs. ctrl; ***: $p < 0.001$ vs. ctrl (one-way ANOVA).

No upregulation of these growth factors was found for REFDRUG 0.1 mg/mL. On the other hand, the tested samples were devoid of any effect on EGF.

3.4. Wound Healing Activity of Sedum telephium L. Leaves Juicef and Pro-Collagen I Dosage in Human Fibroblasts

In order to further investigate the wound-healing potential of SED, we tested it also in human fibroblasts HFF-1. In the fibroblast line also, as shown in Figures 5 and S1, SED implemented cell migration and increased the rate of regeneration of the simulated wound maximally at 24 h, with an increase of 30% over the control ($p < 0.0001$). An increase in wound closure was also recorded at 6 h. In human fibroblasts, SEDPOL was also able to increase the rate of wound healing at 24 h of treatment (20%, $p < 0.01$ vs. ctrl), but confirming the above statement regarding the importance of the whole phytocomplex.

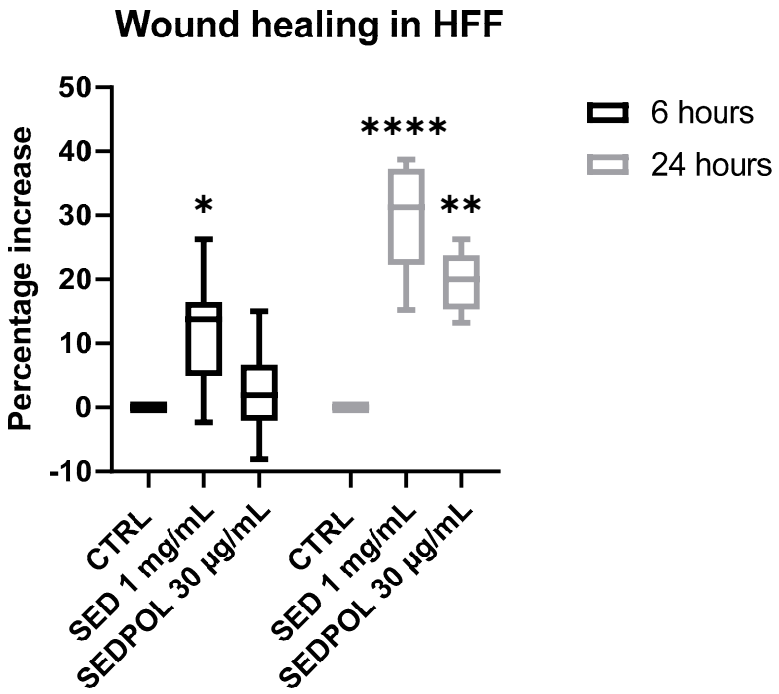

Figure 5. Box plot graphical representation of the in vitro wound-healing effect of *S. telephium* leaf juice (SED) and of the polysaccharidic fraction (SEDPOL) in human fibroblasts. * < 0.05 vs. ctrl. **: $p < 0.01$ vs. ctrl. ****: $p < 0.0001$ vs. ctrl (one-way ANOVA).

In fibroblasts, we also focused on the pro-collagen I expression as an indicator of SED trophic and stimulating activity, but we failed to observe any effect.

4. Discussion

Skin wounds have always represented a very important problem for human health, as they lead to a disruption of the skin barrier [32]. Maintaining skin integrity is fundamental not only for the protection against infectious agents, but also for regulation of body temperature, trans-epidermal water loss, and sensory perception [33–36]. Skin healing may be affected by several factors including age, metabolic conditions, drug consumption, inflammation, and oxidative stress. For these reasons, wound healing continues to be a challenging area for dermatological research [37]. This focus is particularly important for addressing minor wounds, which are frequently underestimated and often not managed adequately [38,39]. Despite their seemingly insignificant nature, even minor wounds can lead to complications if not properly treated, highlighting the necessity for improved therapeutic approaches and increased awareness of their potential impact on overall health [8,39].

The use of herbal preparations, which leverage synergistic and multifaceted activities, is a hallmark of phytotherapy within pharmacology. EMA and WHO have all acknowledged that treating skin disorders and minor wounds is one of the most common applications for many medicinal plants globally. An in-depth review of medicinal plants officially recognized for their therapeutic role in wound healing reveals that their use is grounded in ethnobotanical knowledge and traditional medicine. This traditional knowledge is enhanced by contemporary clinical and experimental research aimed at elucidating their mechanisms of action. Recently, there has been a renewed interest in ethnobotanical knowledge and traditional medicine practices [40]. Promoting the traditional use of medicinal plants could represent a highly effective approach to pharmaceutical research, especially during challenging times for synthetic pharmaceutical chemistry [40].

In this work we investigated the in vitro wound healing effect of a medicinal plant with a consolidated traditional use in our country, *Sedum telephium* L. The pro-epithelizing properties of *S. telephium* are primarily attributed to its rich phytochemical composition. The plant contains various bioactive compounds such as flavonols and polysaccharides, which have demonstrated interesting properties potentially related to wound-healing. In fact, these compounds possess antioxidant, anti-inflammatory and immunomodulatory properties, all of which are crucial for promoting skin regeneration [22].

S. telephium exhibits strong anti-inflammatory activity, reducing the levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [24,40]. This reduction in inflammation is crucial for preventing chronic wounds and promoting a more favorable healing environment.

Notably, *S. telephium* extracts have been shown to scavenge free radicals and reduce oxidative stress, which is known to impair wound healing processes [23]. By neutralizing reactive oxygen species (ROS), the plant helps protect cells from oxidative damage and supports survival and proliferation of keratinocytes.

S. telephium has been described as possibly possessing anti-viral activity [41] and to possess anti-adhesive properties [21]; however evidence is scarce regarding a potential broad-spectrum antimicrobial activity, which, on the contrary, has already been documented for other *Sedum* species [42].

Our data confirm that *S. telephium* stimulates skin cells activity through the secretion of crucial to promote collagen and elastin neo-synthesis, which, in turn, provide structural support for.

Our findings reinforced the notion that *S. telephium* leaf juice (referred to in this work as SED) is effective in promoting cell migration and proliferation through the secretion of growth factors which contribute to tissue remodeling and wound contraction, therefore reducing wound size and promoting closure [43].

Indeed, in keratinocyte cultures SED induced the secretion of TGF-β1 and FGF, growth factors intrinsically connected [44] and dependent by the master TGF signalling [45]. Noteworthy, the upreguation of TGF-β1 and FGF is a pivotal mechanism also displayed by the well-known aloe gel (*Aloe vera* (L). Burm f. *gel ex folia sine cute*) [46].

SED showed to promote wound closure both in keratinocytes and in fibroblasts monolayers and, even more importantly, we clearly demonstrated the ameliorative effect of the whole phytocomplex compared to the polysaccharides fraction, the most enriched one in SED.

As expected, SED showed higher efficacy in wound closure compared to the reference drug; testing low concentrations of this sample was chosen after having preliminarily verified the negative effect at high concentrations, plausibly produced by phenoxyethanol, used in the formulation as a disinfectant and not as a trophic agent. However, the trophic effect of the patented water extract of *Triticum vulgare* L. water extract alone has been demonstrated in in vitro studies at very high, but not toxic, concentrations of 30 and 150 mg/mL [47].

Overall, the pro-epithelizing properties of *S. telephium* make it a promising candidate for the development of novel wound-healing therapies. This makes it suitable for various types of wounds, including surgical incisions, burns, and traumatic injuries.

Beyond its pro-epithelizing properties, *S. telephium* holds promise for various other applications in dermatology due to its multifaceted pharmacological profile. For example, certain components of *S. telephium*, such as polysaccharides, have a known moisturizing and hydrating properties. The antioxidant activity already reported for *S. telephium* phyotocomplex can help counteract oxidative stress-induced skin damage, including premature aging caused by environmental factors such as UV radiation and pollution [48–50]. By scavenging free radicals and protecting skin cells from oxidative damage, the *S. telephium* may hold promise as an anti-aging agent.

The anti-inflammatory effect of *S. telephium* leaf juice makes it beneficial for managing various dermatological conditions characterized by inflammation and infection [24]. These include, among others, acne, atopic dermatitis, psoriasis, and rosacea, where reducing inflammation and controlling microbial overgrowth are key therapeutic goals. However, we also acknowledge that larger clinical trials are needed to validate the efficacy and the safety of *S. telephium* in the dermatological setting, especially in the field of wound healing.

We are clearly aware that this work represents only a small step forward in the road of the research on *S. telephium*, but—undoubtedly—these insights into the phytocomplex's healing activity compared to its main fraction and that of the main trophic mechanism provide the basis for further studies. Coming back to the beginning, this work also allowed us to consider how the quality control of *S. telephium* leaf juice could be assessed from now on by monitoring the total polysaccharide content and the flavonoid profile, with kaempferitrin representing a good specific marker, available on the market as a standard.

Encouraged by the confirmation obtained in this work, the next steps of the research focused on this medicinal plant will take into consideration moisturizing and hydrating properties, potentially exerted by polysaccharides and glycoproteins, as well as antimicrobial activity.

5. Conclusions

Sedum telephium L. leaf juice represents a versatile botanical ingredient with a range of potential applications in dermatology. Its wound-healing properties, accompanied by additional features such as anti-inflammatory and antioxidant activities, make it a promising candidate for the development of novel skincare products targeting various skin conditions and concerns. However, further research, including larger clinical trials, is needed to validate its efficacy and safety for dermatological use.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics11040131/s1, Figure S1: Microscopic images of the scratch wound healing assay performed in human fibroblasts testing *S. telephium* leaf juice (SED) 1 mg/mL, and the isolated polysaccharides (SEDPOL) at the same concentration in which they are present in SED (30 μg/mL).

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References

1. Pazyar, N.; Yaghoobi, R.; Rafiee, E.; Mehrabian, A.; Feily, A. Skin Wound Healing and Phytomedicine: A Review. *Skin Pharmacol. Physiol.* **2014**, *27*, 303–310. [CrossRef] [PubMed]
2. Azevedo, M.; Lisboa, C.; Rodrigues, A. Chronic Wounds and Novel Therapeutic Approaches. *Br. J. Community Nurs.* **2020**, *25*, S26–S32. [CrossRef] [PubMed]
3. Wallace, H.A.; Basehore, B.M.; Zito, P.M. Wound Healing Phases. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2022.
4. Ireton, J.E.; Unger, J.G.; Rohrich, R.J. The Role of Wound Healing and Its Everyday Application in Plastic Surgery: A Practical Perspective and Systematic Review. *Plast. Reconstr. Surg. Glob. Open* **2013**, *1*, e10–e19. [CrossRef] [PubMed]
5. Nicholas, M.N.; Yeung, J. Current Status and Future of Skin Substitutes for Chronic Wound Healing. *J. Cutan. Med. Surg.* **2017**, *21*, 23–30. [CrossRef] [PubMed]
6. Powers, J.G.; Higham, C.; Broussard, K.; Phillips, T.J. Wound Healing and Treating Wounds: Chronic Wound Care and Management. *J. Am. Acad. Dermatol.* **2016**, *74*, 607–625; quiz 625–626. [CrossRef] [PubMed]
7. Kaisang, L.; Siyu, W.; Lijun, F.; Daoyan, P.; Xian, C.J.; Jie, S. Adipose-Derived Stem Cells Seeded in Pluronic F-127 Hydrogel Promotes Diabetic Wound Healing. *J. Surg. Res.* **2017**, *217*, 63–74. [CrossRef] [PubMed]
8. Han, G.; Ceilley, R. Chronic Wound Healing: A Review of Current Management and Treatments. *Adv. Ther.* **2017**, *34*, 599–610. [CrossRef] [PubMed]
9. Olsson, M.; Järbrink, K.; Divakar, U.; Bajpai, R.; Upton, Z.; Schmidtchen, A.; Car, J. The Humanistic and Economic Burden of Chronic Wounds: A Systematic Review. *Wound Repair Regen.* **2019**, *27*, 114–125. [CrossRef] [PubMed]
10. Klein, T.M.; Andrees, V.; Kirsten, N.; Protz, K.; Augustin, M.; Blome, C. Social Participation of People with Chronic Wounds: A Systematic Review. *Int. Wound J.* **2021**, *18*, 287–311. [CrossRef]
11. Gethin, G.; Probst, S.; Stryja, J.; Christiansen, N.; Price, P. Evidence for Person-Centred Care in Chronic Wound Care: A Systematic Review and Recommendations for Practice. *J. Wound Care* **2020**, *29*, S1–S22. [CrossRef]
12. Costa, D.; Ielapi, N.; Caprino, F.; Giannotta, N.; Sisinni, A.; Abramo, A.; Ssempijja, L.; Andreucci, M.; Bracale, U.M.; Serra, R. Social Aspects of Diabetic Foot: A Scoping Review. *Soc. Sci.* **2022**, *11*, 149. [CrossRef]
13. Nussbaum, S.R.; Carter, M.J.; Fife, C.E.; DaVanzo, J.; Haught, R.; Nusgart, M.; Cartwright, D. An Economic Evaluation of the Impact, Cost, and Medicare Policy Implications of Chronic Nonhealing Wounds. *Value Health* **2018**, *21*, 27–32. [CrossRef] [PubMed]
14. Paganelli, A.; Naselli, A.G.; Bertoni, L.; Rossi, E.; Azzoni, P.; Pisciotta, A.; Cesinaro, A.M.; Benassi, L.; Kaleci, S.; Garbarino, F.; et al. Wound Healing after Acellular Dermal Substitute Positioning in Dermato-Oncological Surgery: A Prospective Comparative Study. *Life* **2023**, *13*, 463. [CrossRef] [PubMed]
15. Lindholm, C.; Searle, R. Wound Management for the 21st Century: Combining Effectiveness and Efficiency. *Int. Wound J.* **2016**, *13* (Suppl. S2), 5–15. [CrossRef] [PubMed]
16. El-Sherbeni, S.A.; Negm, W.A. The Wound Healing Effect of Botanicals and Pure Natural Substances Used in in Vivo Models. *Inflammopharmacology* **2023**, *31*, 755–772. [CrossRef] [PubMed]
17. Pressi, G.; Rigillo, G.; Governa, P.; Borgonetti, V.; Baini, G.; Rizzi, R.; Guarnerio, C.; Bertaiola, O.; Frigo, M.; Merlin, M.; et al. A Novel *Perilla Frutescens* (L.) Britton Cell-Derived Phytocomplex Regulates Keratinocytes Inflammatory Cascade and Barrier Function and Preserves Vaginal Mucosal Integrity In Vivo. *Pharmaceutics* **2023**, *15*, 240. [CrossRef]
18. Paganelli, A.; Pisciotta, A.; Bertani, G.; Di Tinco, R.; Tagliaferri, N.; Orlandi, G.; Azzoni, P.; Bertoni, L. Food Supplements for Skin Health: In Vitro Efficacy of a Combination of *Rhodiola rosea*, *Tribulus terrestris*, *Moringa oleifera* and *Undaria pinnatifida* on UV-Induced Damage. *Cosmetics* **2023**, *10*, 83. [CrossRef]
19. Balatri, S. L'uso del *Sedum telephium* L. Nella Pratica Chirurgica Minore. *Boll. Soc. Tosco-Umbra Chir. XLII* **1981**, 3–8.
20. Mulinacci, N.; Vincieri, F.F.; Baldi, A.; Bambagiotti-Alberti, M.; Sendl, A.; Wagner, H. Flavonol Glycosides from *sedum telephium* Subspecies *Maximum* Leaves. *Phytochemistry* **1995**, *38*, 531–533. [CrossRef]
21. Raimondi, L.; Banchelli, G.; Dalmazzi, D.; Mulinacci, N.; Romani, A.; Vincieri, F.F.; Pirisino, R. *Sedum telephium* L. Polysaccharide Content Affects MRC5 Cell Adhesion to Laminin and Fibronectin. *J. Pharm. Pharmacol.* **2010**, *52*, 585–591. [CrossRef]
22. Sendl, A.; Mulinacci, N.; Vincieri, F.F.; Wagner, H. Anti-Inflammatory and Immunologically Active Polysaccharides of *Sedum telephium*. *Phytochemistry* **1993**, *34*, 1357–1362. [CrossRef]
23. Bonina, F.; Puglia, C.; Tomaino, A.; Saija, A.; Mulinacci, N.; Romani, A.; Vincieri, F.F. In-Vitro Antioxidant and in-Vivo Photoprotective Effect of Three Lyophilized Extracts of *Sedum telephium* L. Leaves. *J. Pharm. Pharmacol.* **2000**, *52*, 1279–1285. [CrossRef] [PubMed]
24. Altavilla, D.; Polito, F.; Bitto, A.; Minutoli, L.; Miraldi, E.; Fiumara, T.; Biagi, M.; Marini, H.; Giachetti, D.; Vaccaro, M.; et al. Anti-Inflammatory Effects of the Methanol Extract of *Sedum telephium* ssp. *Maximum* in Lipopolysaccharide-Stimulated Rat Peritoneal Macrophages. *Pharmacology* **2008**, *82*, 250–256. [CrossRef] [PubMed]

25. Finetti, F.; Biagi, M.; Ercoli, J.; Macrì, G.; Miraldi, E.; Trabalzini, L. *Phaseolus vulgaris* L. Var. Venanzio Grown in Tuscany: Chemical Composition and In Vitro Investigation of Potential Effects on Colorectal Cancer. *Antioxidants* **2020**, *9*, 1181. [CrossRef] [PubMed]
26. Governa, P.; Biagi, M. *Copaifera langsdorffii* Desf.: In Vitro Investigation on Anti-*Helicobacter pylori* and Anti-Inflammatory Activities of Oleoresin and Fruit Methanolic Extract. *Plant Biosyst. Int. J. Deal. All Asp. Plant Biol.* **2020**, *154*, 117–124. [CrossRef]
27. Zhang, Y.; Cheng, W.; Di, H.; Yang, S.; Tian, Y.; Tong, Y.; Huang, H.; Escalona, V.H.; Tang, Y.; Li, H.; et al. Variation in Nutritional Components and Antioxidant Capacity of Different Cultivars and Organs of *Basella alba*. *Plants* **2024**, *13*, 892. [CrossRef] [PubMed]
28. Chen, X.; Peng, L.-H.; Li, N.; Li, Q.-M.; Li, P.; Fung, K.-P.; Leung, P.-C.; Gao, J.-Q. The Healing and Anti-Scar Effects of Astragaloside IV on the Wound Repair in Vitro and in Vivo. *J. Ethnopharmacol.* **2012**, *139*, 721–727. [CrossRef] [PubMed]
29. Chiocchio, I.; Poli, F.; Governa, P.; Biagi, M.; Lianza, M. Wound Healing and in Vitro Antiradical Activity of Five *Sedum* Species Grown within Two Sites of Community Importance in Emilia-Romagna (Italy). *Plant Biosyst. Int. J. Deal. all Asp. Plant Biol.* **2019**, *153*, 610–615. [CrossRef]
30. Everard, J.D.; Loescher, W.H. Primary Products of Photosynthesis, Sucrose and Other Soluble Carbohydrates. In *Encyclopedia of Applied Plant Sciences*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 96–104, ISBN 978-0-12-394808-3.
31. Cakmak, I.; Lambers, H.; Grant, C.A.; Zhao, F.-J. Arbuscular Mycorrhizal Fungi: Key Players in Avoiding Cadmium Accumulation in Food Crops. *Plant Soil.* **2023**, *484*, 13–32. [CrossRef]
32. Jensen, J.M.; Proksch, E. The Skin's Barrier. *G. Ital. Dermatol. Venereol.* **2009**, *144*, 689–700.
33. Wong, R.; Geyer, S.; Weninger, W.; Guimberteau, J.-C.; Wong, J.K. The Dynamic Anatomy and Patterning of Skin. *Exp. Dermatol.* **2016**, *25*, 92–98. [CrossRef]
34. Nguyen, A.V.; Soulika, A.M. The Dynamics of the Skin's Immune System. *Int. J. Mol. Sci.* **2019**, *20*, 1811. [CrossRef]
35. Harris-Tryon, T.A.; Grice, E.A. Microbiota and Maintenance of Skin Barrier Function. *Science* **2022**, *376*, 940–945. [CrossRef] [PubMed]
36. Tomic-Canic, M.; Burgess, J.L.; O'Neill, K.E.; Strbo, N.; Pastar, I. Skin Microbiota and Its Interplay with Wound Healing. *Am. J. Clin. Dermatol.* **2020**, *21*, 36–43. [CrossRef] [PubMed]
37. Baron, J.M.; Glatz, M.; Proksch, E. Optimal Support of Wound Healing: New Insights. *Dermatology* **2020**, *236*, 593–600. [CrossRef] [PubMed]
38. Davis, E. Minor Traumatic Wounds. *Nurs. Stand.* **2016**, *31*, 64–65. [PubMed]
39. Langford, J.H.; Artemi, P.; Benrimoj, S.I. Topical Antimicrobial Prophylaxis in Minor Wounds. *Ann. Pharmacother.* **1997**, *31*, 559–563. [CrossRef]
40. Dalla Costa, V.; Piovan, A.; Filippini, R.; Brun, P. From Ethnobotany to Biotechnology: Wound Healing and Anti-Inflammatory Properties of *Sedum telephium* L. In Vitro Cultures. *Molecules* **2024**, *29*, 2472. [CrossRef]
41. Shirobokov, V.P.; Evtushenko, A.I.; Lapchik, V.F.; Shirobokova, D.N.; Suptel', E.A. Antiviral activity of representatives of the family Crassulaceae. *Antibiotiki* **1981**, *26*, 897–900.
42. Boriollo, M.F.G.; Marques, M.B.; da Silva, T.A.; da Silva, J.J.; Dias, R.A.; Silva Filho, T.H.N.; Melo, I.L.R.; Dos Santos Dias, C.T.; Bernardo, W.L.d.C.; de Mello Silva Oliveira, N.; et al. Antimicrobial Potential, Phytochemical Profile, Cytotoxic and Genotoxic Screening of *Sedum praealtum* A. DC. (Balsam). *BMC Complement. Med. Ther.* **2020**, *20*, 133. [CrossRef]
43. Biagi, M.; Miraldi, E.; Giachetti, D.; Tsioutsiou, E.E.; Governa, P.; Giordani, P.; Cornara, L. *Sedum telephium* L.: From Traditional Use to Pharmacological Research. In Proceedings of the III International Plant Science Conference (IPSC), Rome, Italy, 21–23 September 2016.
44. Song, Q.H. TGF-Beta1 Regulates TGF-Beta1 and FGF-2 mRNA Expression during Fibroblast Wound Healing. *Mol. Pathol.* **2002**, *55*, 164–176. [CrossRef]
45. Li, X.; Xie, R.; Luo, Y.; Shi, R.; Ling, Y.; Zhao, X.; Xu, X.; Chu, W.; Wang, X. Cooperation of TGF-β and FGF Signalling Pathways in Skin Development. *Cell Prolif.* **2023**, *56*, e13489. [CrossRef] [PubMed]
46. Hormozi, M.; Assaei, R.; Boroujeni, M.B. The Effect of Aloe Vera on the Expression of Wound Healing Factors (TGFβ1 and bFGF) in Mouse Embryonic Fibroblast Cell: In Vitro Study. *Biomed. Pharmacother.* **2017**, *88*, 610–616. [CrossRef] [PubMed]
47. Tito, A.; Minale, M.; Riccio, S.; Grieco, F.; Colucci, M.G.; Apone, F. A *Triticum vulgare* Extract Exhibits Regenerating Activity During the Wound Healing Process. *CCID* **2020**, *13*, 21–30. [CrossRef] [PubMed]
48. Ngoc, L.T.N.; Moon, J.-Y.; Lee, Y.-C. Antioxidants for Improved Skin Appearance: Intracellular Mechanism, Challenges, and Future Strategies. *Int. J. Cosmet. Sci.* **2023**, *45*, 299–314. [CrossRef]
49. Eftekhari, A.; Dizaj, S.M.; Chodari, L.; Sunar, S.; Hasanzadeh, A.; Ahmadian, E.; Hasanzadeh, M. The Promising Future of Nano-Antioxidant Therapy against Environmental Pollutants Induced-Toxicities. *Biomed. Pharmacother.* **2018**, *103*, 1018–1027. [CrossRef]
50. Fernandes, A.; Rodrigues, P.M.; Pintado, M.; Tavaria, F.K. A Systematic Review of Natural Products for Skin Applications: Targeting Inflammation, Wound Healing, and Photo-Aging. *Phytomedicine* **2023**, *115*, 154824. [CrossRef]