Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.05.007

Wound healing activity of *Ullucus tuberosus*, an Andean tuber crop

Nathalie Heil[1], Karent Bravo[1], Andrés Montoya[2], Sara Robledo[2], Edison Osorio[1*]

[1]*Grupo de Investigación en Sustancias Bioactivas, Facultad de Ciencias Farmacéuticas y Alimentarias, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia*

[2]*PECET – Medical Research Institute, School of Medicine, University of Antioquia, Calle 70 No. 52-21, Medellín, Colombia*

ARTICLE INFO

Article history:
Received 11 Oct 2016
Received in revised form 17 Nov, 2nd revised form 21 Dec 2016
Accepted 19 Mar 2017
Available online 29 May 2017

Keywords:
Wound healing
Collagenase
Pro-collagen
MMP-1
Ullucus tuberosus

ABSTRACT

Objective: This study was designed to investigate the wound healing activity of aqueous extracts of *Ullucus tuberosus* (*U. tuberosus*) using *in vitro* models.

Methods: Lyophilized pulp and acetone extracts of *U. tuberosus* were produced using ultrasound extraction. The capacity for collagenase activation was evaluated using fluorescence detection of the enzymatic activity. Then, the influence of *U. tuberosus* extracts on cell proliferation, cell migration and synthesis of the extracellular matrix (ECM) proteins, metalloproteinase (MMP-1) and pro-collagen was analyzed using human dermal fibroblasts in culture.

Results: An increase in collagenase activity of 12% supports the utility of *U. tuberosus* as an agent for scar treatment. In addition, the extracts showed an increase in the proliferation and migration of human dermal fibroblasts and the production of pro-collagen and MMP-1 after treatment with *U. tuberosus* extracts. The increase in proliferation, migration and pro-collagen levels positively influenced the regeneration of scarless tissue during the proliferation phase, whereas the increase in MMP-1 may have favored the wound healing process during the remodeling and cellular differentiation phases.

Conclusion: The results of this study show for first time that *U. tuberosus* is a promising candidate to support scarless tissue regeneration.

1. Introduction

The South American Andes exhibit the greatest expression of tropical biodiversity on Earth. These territories are home to approximately 45 000 vascular plants (20 000 endemic) and 3400 vertebrate species (1.567 endemic) in only 1% of the Earth's land surface [1]. The richness of this diversity is also reflected in its use because the Andean region, with its significant indigenous settlements and its special climate, is host to many medicinal plants. Previous studies have reported the chemical and pharmacological properties of many Andean species [2,3]. However, it is known that most of the species growing in high-elevation environments in tropical mountains remain largely unexplored, particularly from the chemical and biological standpoint [4]. This is the case for the Andean tuber *Ullucus tuberosus* (*U. tuberosus*) Caldas (family Basellaceae), which was domesticated in the Andean region during the Pre-Hispanic era approximately 5500 years ago [5,6]. It is commonly known as papa lisa, olluco, or melloco in the central and southern Andes, but is most widely known as olluco in spanish. Traditionally the tuber is also known for its medicinal properties to treat burns and to prevent scars, but no information on its efficacy, bioactive compounds or mechanisms of action is known. The identification of safer and more effective tissue regeneration agents is an urgent need because the frequency of impaired wound healing is increasing, and no synthetic or natural substance is able to provide optimal treatment [7].

The wound healing process involves four phases: i) hemostasis (coagulation), ii) inflammation, iii) proliferation, and iv) remodeling [2]. During the proliferative phase, the fibroblast and endothelial cells facilitate angiogenesis, fibroblast proliferation with collagen deposition, granulation tissue formation, wound contraction and re-epithelialization, while during the remodeling/cellular differentiation phase, the remodeling and

*Corresponding author: Edison Osorio, Grupo de Investigación en Sustancias Bioactivas, Facultad de Ciencias Farmacéuticas y Alimentarias, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia.
Tel./fax: +574 2196590
E-mail: edison.osorio@udea.edu.co (E. Osorio).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

realignment of the collagen tissue occur to produce greater tensile strength comparable to that of normal skin; in addition, cell and capillary density decrease [8]. However, the complex mechanisms of impaired wound healing are not very well understood. Most information about normal and pathologic wound healing has been obtained by comparing the scarless wound healing of fetuses with the scar-forming healing of adult skin [9]. In addition, it is known that the key processes that lead to scarred skin are the over-production of structural extracellular matrix (ECM) proteins, the up-regulated synthesis of protease inhibitors (PAI) and the down-regulated synthesis of proteases (PA) such as collagenase and matrix metalloproteinases (MMPs). These misdirected processes lead to excessive matrix deposition and reduced remodeling. Therefore, it seems plausible that the normal equilibrium cannot be reached only in the remodeling phase of the wound healing process [10].

On the other hand, a key player in the wound healing process is collagen, a major protein of the ECM, which has a pivotal function in the maintenance of the skin's dermis structure. Initial wound healing is notable for the production of large quantities of randomly oriented collagen; however, during remodeling, the collagen becomes cross-linked and replaced with more organized collagen, which is better arranged to resist mechanical stress. In these processes, dermal fibroblasts synthesize pro-collagen, which is subsequently converted to collagen [11]. Nevertheless, the enzymes collagenase and MMP-1 strongly influence the final collagen concentration by protein degradation [12,13]. Interestingly, the *in vitro* analysis of collagen should focus on the formation of collagen in the ECM. As previously mentioned, the minimal deposition of collagen in aqueous cell cultures occurs because pro-collagen is not transformed to collagen [12]. Therefore, pro-collagen is directly measured in the fibroblast monoculture system. In an attempt to validate the traditional knowledge and to highlight potential therapeutic agents for wound healing, this study focused on determining the effect of the *U. tuberosus* extracts on collagen using human dermal fibroblasts (HDFa). Thus, lyophilized and acetone extracts of the peel and pulp of *U. tuberosus* were analyzed for their collagenase activation capacity. HDFa were used to evaluate the influence of extracts on the proliferation and wound closure capacity and the production of pro-collagen and MMP-1 in the cell supernatant to produce biologically active extracts and to yield a better understanding of the mode of action of this plant and for industrial usage.

2. Materials and methods

2.1. Plant material and extracts

For this study, *U. tuberosus* was purchased from regional cultivators from the west of Colombia, in the department of Valle de Cauca in the municipality of Sevilla. The voucher specimen were deposited in the HUA (Herbario de la Universidad de Antioquia) under no. Alzate 5275. The tuber crop was washed with 5% sodium hypochlorite, and the peel was removed. The pulp was homogenized with distilled water and lyophilized to obtain a dry powder with a small particle size distribution. The powder was stored at −20 °C for extraction or analysis. An acetone extract was prepared from the lyophilized plant material by solid–liquid extraction. Two gram of lyophilized plant material were extracted twice with 20 mL of acetone: water (75:25) using sonication for 45 min in 750 Wultrasonic bath (P60, Elma Schmidbauer GmbH). After extraction, solid–liquid-separation was carried out by centrifugation (Sorvall ST 16, Thermo Scientific; Waltham, USA) at 4000 rpm for 30 min. The supernatant was collected, concentrated in a roto-evaporator (R-215, Büchi) and dried in a centri-vaporizer (Labcono) at 45 °C. The dry samples were weighed and stored at −20 °C for the biochemical analysis.

2.2. Collagenase activity

The enzyme activity was measured according to the procedure described by Bravo *et al.* using the EnzCheck® Gelatinase/Collagenase assay kit (Molecular Probes Inc.) [2]. Aliquots of 20 µL sample solution, reaction buffer (control) or inhibitor (250 µM Oleanolic acid) and 80 µL DQ-collagen type IV substrate were added to each well of a 96-well plate. Directly before the fluorescence measurement, 100 µL of active enzyme (*Clostridium histolyticum*) was added. Collagenase activity was determined each minute for 20 min at 485 nm excitation and 515 nm emission using a Synergy HT Multi-Mode Microplate Reader (BioTek Instruments, Inc.; Winooski, USA). The increase in fluorescence was proportional to the proteolytic activity. Measurements were carried out in triplicate. The percent activation of the collagenase reaction was calculated as follows:

$$Collagenase\ activation\ (\%) = \left(1 - \frac{m_{control}}{m_{sample}}\right) \times 100$$

where, $m_{control}$ and m_{sample} were the slopes of the fluorescence vs. time graph of control and sample, respectively.

2.3. Cell culture

For all *in vitro* experiments, HDFa were obtained from Life Technologies. The cells were placed in a 75-cm^2 tissue culture flask, and they were cultured and maintained in a DMEM/F12, GLUTAMAX supplement medium (Gibco, Invitrogen) with penicillin (100 units/mL), streptomycin (100 µg/mL), and 10% fetal bovine serum (Gibco) in a humidified atmosphere containing 5% CO_2 at 37 °C until 80% confluence. Then, the cells were removed with a Trypsin-EDTA solution and transferred to and seeded in 96-well plates. The cells from passages 3–5 were used for the experiments.

2.4. Cytotoxicity and proliferation assay

The cell viability of HDFa was determined using WST-8 assay (Dojindo Molecules Technologies, Inc.), according to the manufacturer's instructions. Briefly, wells containing 1×10^4 cells/well were exposed to serial concentrations of extracts (2.5–200 µg/mL) for 24 h. After 24 h, the cells were washed twice with PBS, and 100 µL of a medium without fetal bovine serum and 10 µl of WST-8 were added to each well and incubated at 37 °C for 2 h. The absorbance was measured at 450 nm using a Synergy HT Multi-Mode Microplate Reader (BioTek Instruments, Inc.; Winooski, USA). For the cell proliferation assay, the cell viability was measured every 24 h for 72 h without changing the medium. The proliferation assay was also performed for 8 days (192 h) while changing the medium. Every 48 h, the cell number was determined and the medium with new samples was changed after washing the cells with PBS. The cell number was measured using the WST-8 kit

according the procedure described above, and cell viability was calculated.

2.5. Scratch assay

The scratch assay is used to evaluate the wound healing capacity of an extract or component. It is important because it reveals the capacity for cell proliferation and migration, which are essential for tissue repair. This assay was performed according to the procedure described previously [14]. The scratch was performed with "wound field" inserts from the Cell Biolabs CytoSelect™ Wound Healing Assay Kit that allowed for a regular reproducible gap (0.9 mm). The experiment was performed according to the supplier manual. Briefly, lyophilisate and organic extracts were prepared at 200 mg/mL. Wells of a 24-well plate were set up by adding wound healing inserts to each well. 200 µL of PBS were added in each well to close the insert side and prevent movement. Cells were detached from the culture flask, centrifuged, counted and adjusted at 5×10^5 cells/mL with supplemented DMEM/F-12. Five hundred µL/well were added into open insert side, and cells were incubated at 37 °C, 5% CO_2 for 24 h to form the monolayer. After incubation, the inserts were removed and the first images (t = 0) were captured with a digital camera (Nikon Digital Sight DS F.1) that was attached to a microscope (Nikon ELWD 03/0075). Then, the medium was removed, cells were washed with PBS, and 500 µL of fresh medium with lyophilisate and extracts were added. Gap closure was inspected after 12 (t = 12) and 24 h (t = 24) [15]. The speed of gap closure by the migration and the proliferation of cells exposed to *U. tuberosus* products was compared to a negative control without stimulating substances. The percent of gap closure was calculated according the following formulas:

$$gap\ closure_{0-12h}(\%) = \left(1 - \frac{L_{12h}}{\overline{L}_{0h}}\right) \times 100$$

$$gap\ closure_{0-24h}(\%) = \left(1 - \frac{L_{24h}}{\overline{L}_{0h}}\right) \times 100$$

$$gap\ closure_{12-24h}(\%) = \left(1 - \frac{L_{24h}}{\overline{L}_{12h}}\right) \times 100$$

where L_{0h}, L_{12h}, L_{24h} are the measured gap widths after the three different time points. Experiments were performed in duplicate, and data were analyzed in Corel Draw Graphic Suite ×6 software [16].

2.6. Pro-collagen production

Pro-collagen (Type I) was quantified using a commercially available Pro-Collagen Type I C-Peptide (PIP) assay kit (Takara Bio Inc.) according to the manufacturer's instructions. Previously, 1×10^4 cells/well were incubated in 96-well plates for 24 h in the presence of the samples. Then, samples were removed, and cells were washed twice with PBS and 100 µL of medium without fetal bovine serum were added again and incubated for 24 h. One hundred micro litre of antibody-POD conjugate solution was incubated with 20 µL of samples or standards for 3 h at 37 °C. The contents were removed, and wells were washed four times with 300 µL of washing buffer. Substrate solution (100 µL) was added to each well and incubated at room temperature for 15 min. Subsequently, 100 µL of stop solution was added to each well, and the absorbance was read at 450 nm. Pro-collagen concentration was calculated by the linear interpolation of the calibration curve with standards (Lyophilized Pro-collagen Type I, c = 0–640 ng/mL).

2.7. MMP-1 production

The MMP-1 production by fibroblasts was measured using Solid Phase Sandwich ELISA with the DuoSet® ELISA, Human Total MMP-1 (R&D Systems) according to the manufacturer's instructions. Previously, 1×10^4 cells/well were incubated in 96-well plates for 24 h in the presence of the samples. Then, the samples were removed, cells were washed twice with PBS and 120 µL/well of medium without fetal bovine serum were added. To stimulate MMP-1 production, the cells were irradiated with 100 mJ/cm^2 UVB using a radiation system UV Bio-Sun (VilbertLourmat, France), and cells were incubated for 72 h. Cells were washed twice as described previously, and 100 µL of the detection antibody diluted in reagent diluent were added to each well. Plates were incubated for 2 h at room temperature. After washing again, as described, 100 µL of the working dilution of Streptavidin-HRP was added to each well, and plates were incubated for 20 min at room temperature and protected from light. Plates were washed again, and 100 µL of substrate solution were added to each well. Plates were incubated for 20 min at room temperature and protected from light. Finally, 50 µL of a stop solution were added to each well, and after mixing, the optical density was read using a microplate reader set to 450 nm. The concentration of MMP-1 was also calculated by the linear interpolation of calibration curve with standards.

2.8. Statistical analysis

The results are expressed as the average ± standard deviation (SD). All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, EUA). Differences between controls and treatment were determined by analysis of variance (ANOVA) and were considered significant when the P value < 0.05.

3. Results

3.1. Collagenase activity

The lyophilisate and acetone extracts of *U. tuberosus* exerted an increased effect on collagenase with values near 5–12% (data not shown). The highest activity was obtained with the liophilisate pulp (111.5%). Significant differences respect control without samplers were observed. On the other hand, the *in vitro* assessment of hyaluronidase and elastase activity showed that any extracts from *U. tuberosus* had a significant activity on enzymes at 200 µg/mL (data not shown). Therefore, these findings suggest that *U. tuberosus* extracts could be effective through the moderate modulation of collagenase activity.

3.2. Cytotoxicity and cell proliferation assay

To determine the cytotoxicity of *U. tuberosus*, HDFa were incubated with lyophilized and acetone extracts of *U. tuberosus* at concentrations of 2.5–200 µg/mL. As shown in Figure 1A and B, no significant toxicity was observed in cells treated with

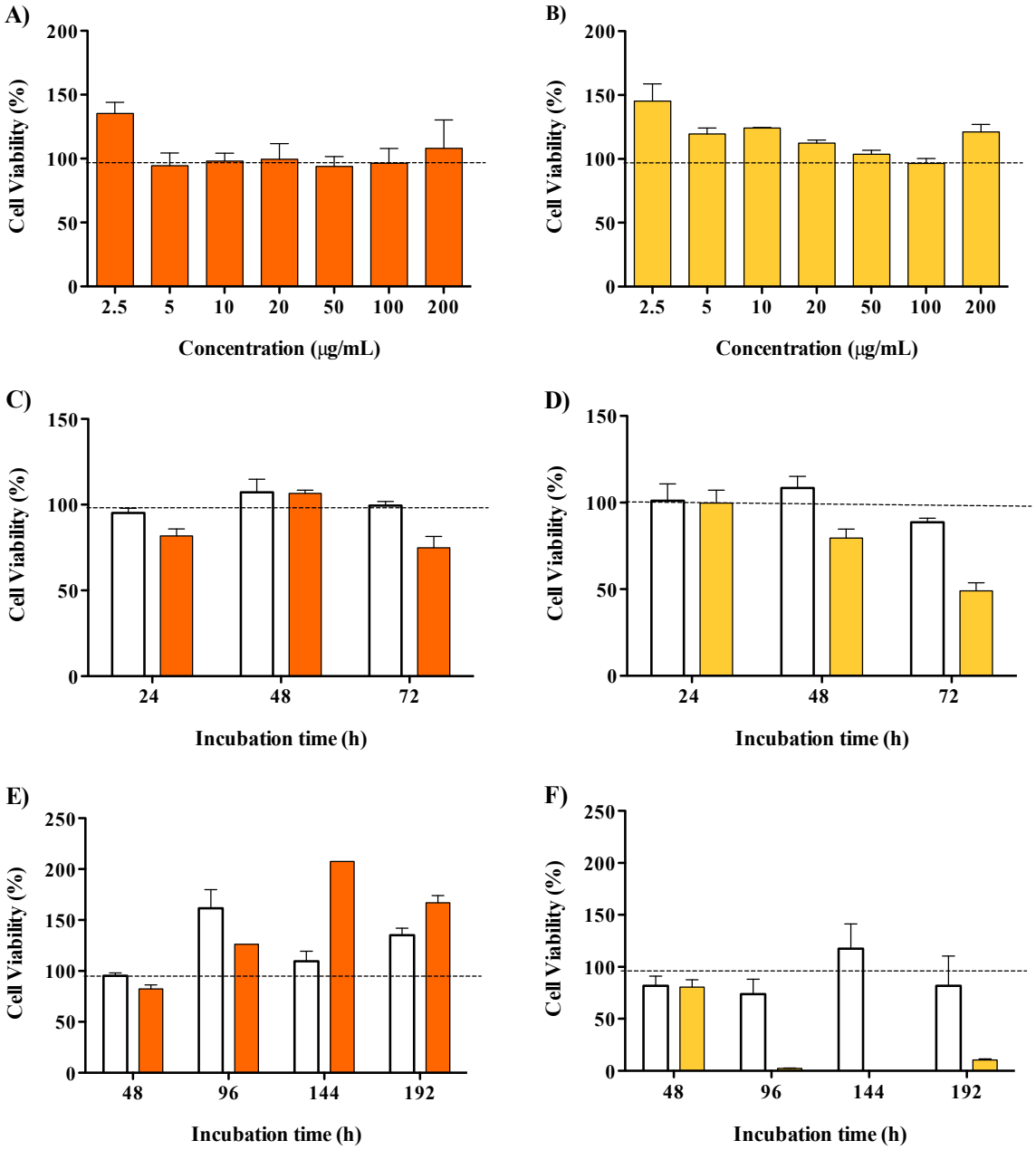

Figure 1. Cytotoxicity and cell proliferation assay on HDFa by *U. tuberosus* as measured by WST-8 assay.
(A) Cytotoxicity of lyophilized pulp; (B) Cytotoxicity of acetone extract; (C) Proliferation assay on lyophilized pulp for 72 h without medium change; (D) Proliferation assay on acetone extract for 72 h without medium change; (E) Proliferation assay on lyophilized pulp for 192 h with medium change; (F) Proliferation assay on acetone extract for 192 h with medium change. The assays were performed with two concentrations: 0.25 μg/mL is represented by white bars, and 2.5 μg/mL is represented by black bars.

U. tuberosus for 24 h. Conversely, an increase in cell viability was observed, mainly at low concentrations. The proliferative ability of the extracts, expressed as increased cell viability relative to untreated controls, which are 100% viable, was tested at low concentrations, and cell viability was increased compared to controls. The assays in which the medium was not changed, as shown in Figure 1C and D, did not exhibit cytotoxic or proliferative effects to 0.25 μg/mL concentrations. However, at 2.5 μg/mL, a decrease in the fibroblast viability was observed 72 h after treatment. The decreased viability observed 72 h after treatment in the assay without changed medium could be attributed to the lack of nutrients in the culture medium, which could induce cell death. When the medium was changed, after 8 days (192 h) of seeding, it was observed that the incubation of HDFa with lyophilisate (Figure 1E) displayed a significant increase in the viability, which was attributed to cell proliferation. The viability increased 100% after 144 h of treatment with 2.5 μg/mL of *U. tuberosus* and more than 50% at 96 h after treatment with lyophilized extract to 0.25 μg/mL. Treatment with acetone extract did not elicit proliferative effects, while a concentration of 2.5 μg/mL elicited cellular death after 48 h of treatment (Figure 1F).

3.3. Scratch assay

In the period from 0 to 12 h and 0–24 h, the pulp extract at 200 μg/mL exhibited a 20% and 10% increase in closure speed relative to the control, respectively. The acetone extract did not

increase the closure speed for those periods (Figure 2). In the period from 12 to 24 h, the closure speed was higher than the control for both extracts. A migration rate with a gap closure of 29% to pulp and 53% to acetone extract was found compared to control, which was considered to be statistically significant.

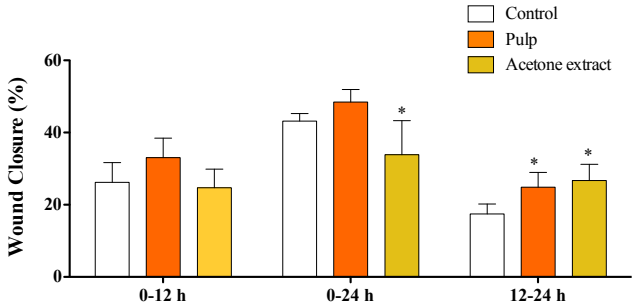

Figure 2. Scratch assay. Evaluation of wound closure capacity with "wound field" inserts on HDFa of different extracts to a final concentration of 200 µg/mL compared to control without sample. The results represent the means ± SD of at least five independent experiments.
*Indicates statistical significance with respect to control (one-way ANOVA followed by Tukey's test, $p < 0.05$).

3.4. Pro-collagen and MMP-1 production

The effect of 100 µg/mL lyophilized and acetone extracts of *U. tuberosus* on pro-collagen and MMP-1 production by HDFa are shown in Figure 3. The production of pro-collagen type I (Figure 3A) increased by 27% and 34% after treatment for 24 h with lyophilized pulp and acetone extracts, respectively, with respect to basal production. Similarly, the MMP-1 production in HDFa, stimulated by UBV radiation, increased 24% and 35% after treatment for 24 h with lyophilized pulp and acetone extracts, respectively (Figure 3B).

effective through the moderate modulation of collagenase activity. The wound healing process begins with hemostasis and inflammation and proceeds with proliferation and reconstitution of the ECM components [8]. Although the uncontrolled activation of metalloproteinases is harmful and the effects of these enzymes are strictly controlled by several mechanisms due to their highly destructive activity, several studies have shown that MMPs are essential for cutaneous wound repair [17,18]. MMPs are responsible for the destruction of foreign substances and dead tissues as well as the components of the extracellular matrix for the reconstitution process of the remodeling phase [18]. Additionally, it has been reported that MMPs contribute to healing by acting as proteinases that degrade the extracellular matrix and that regulate the activity of signaling molecules and growth factors [19]. Thus, at a low level and during specific steps of the process, the activation of MMPs is desirable.

Further, compounds with wound healing properties can act through different mechanisms, including the proliferation of skin cells [20]. In this sense, fibroblasts, the most common connective tissue cells, play a critical role. After an injury to the skin occurs, fibroblasts are stimulated by local growth factors and cytokines, which are produced by platelets, macrophages and fibroblasts themselves, to proliferate and migrate to the wound. Fibroblasts in the wound edges begin to grow and migrate into the provisional matrix of the wound to form granulation tissue [21]. Proliferative effects have been reported in other species. Xing *et al.* studied the fibroblast cell proliferation rate of some well-investigated medicinal plants [7]. For example, in *Aloe vera*, the oligosaccharide acemannan was identified as the component responsible for wound healing. In fact, *A. vera* gel is an extremely complicated mixture of natural products, but its biological activity is principally attributed to polysaccharides and glycoproteins that are present in the leaf pulp. It is well known that *U. tuberosus* contains a large quantity of polysaccharides [5], which are present in the

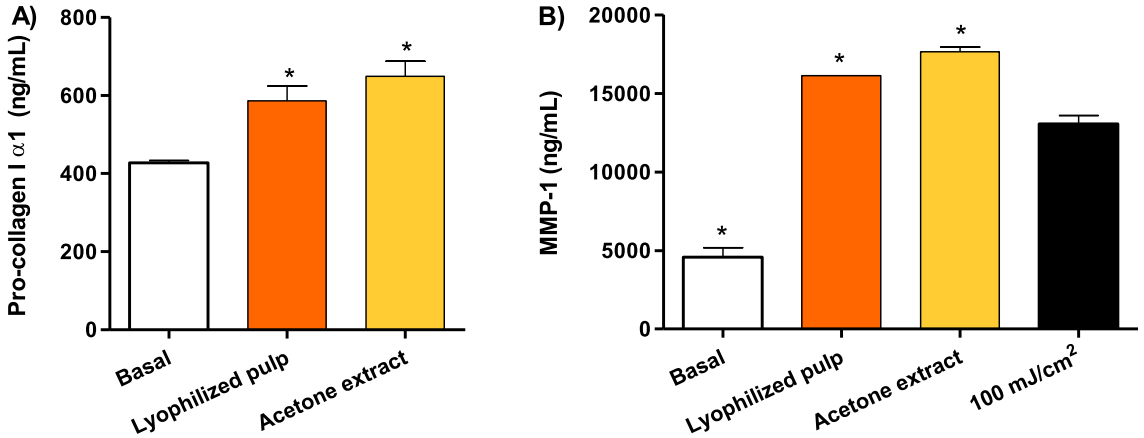

Figure 3. Effects of *U. tuberosus* on type I pro-collagen and MMP-1 production in HDFa measurement by ELISA after 24 h of pre-treatment.
(A) Type-I procollagen production; (B) MMP-1 production stimulated by UVB irradiation (100 mJ/cm^2). The results are mean values ± SD (n = 3).
* Indicates statistical significance when treated groups were compared to control (one-way ANOVA followed by Tukey's test, $p < 0.05$).

4. Discussion

In the present study, the *in vitro* inhibitory effect of extracts of *U. tuberosus* on collagenase was investigated to clarify the wound healing mechanism. Therefore, an increased effect on collagenase was observed, which suggest that *U. tuberosus* extracts could be

lyophilized extract but not in the acetone extract. It would be interesting to determine whether these compounds are responsible for the observed proliferative activity. In this study, fibroblast proliferation was doubled and evaluated as a significant result to aid in wound healing. Generally, the assay revealed a higher proliferation rate at lower concentrations.

In addition to cell proliferation, cell migration plays an essential role in the proper evaluation of the wound healing capacity of an extract or component [22]. Otherwise, increased cell proliferation may be misinterpreted because it is possible that the cells just accumulate in a disordered manner at one side. Some authors describe this as one of the pathologic mechanisms for the formation of scars [9,23]. Regarding the extracts of *U. tuberosus*, the increased wound closure speed observed by the monolayer scratch assay was significant when compared to literature data [16,24].

From the protein level determination, decreased pro-collagen synthesis and increased MMP-1 synthesis were found, similar to the results obtained in previously studies [25]. In this work, the increased pro-collagen synthesis functions in the opposite direction of the increased collagenase activity determined by biochemical screens. However, the obtained results need to be considered carefully because the influence of the ECM-cell-interaction is very limited in monoculture cell culture systems. The monolayer cell culture system has its limitations. The most crucial limitation is the fact that no mature collagen is synthesized in the aqueous environment. This is very problematic because on the one hand, collagen plays a vital role during wound healing and tissue regeneration and on the other hand, ECM-cell interactions are nearly completely neglected. Therefore, to assess and confirm the effect identified in the monolayer model, 3D test models, such as fibroblast populated collagen lattices or co-cultures of different skin cells, can be used. In this way, a test system physiologically closer to *in vivo* conditions can be established, although it cannot in any way functionally substitute *in vivo* tests with volunteers.

For the first time, *U. tuberosus* was analyzed for its wound healing activity. Biochemical and *in vitro* assays were used to show its medicinal potential and obtain active and safe extracts. The collagenase activity assay confirms the assumption of its activity against scar formation. The cytotoxicity assay demonstrates that *U. tuberosus* and its extracts are not toxic to fibroblasts, even at high concentrations. Furthermore, the increased proliferation rate and wound closure capacity supports its activity as a wound agent aid. However, not all the results are significant; therefore, further studies are necessary.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work was supported by the Universidad de Antioquia CODI-Estrategia de Sostenibilidad 2016-2017 GISB.

References

[1] Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J. Biodiversity hotspots for conservation priorities. *Nature* 2000; **403**(6772): 853-8.
[2] Bravo K, Alzate F, Osorio E. Fruits of selected wild and cultivated Andean plants as sources of potential compounds with antioxidant and anti-aging activity. *Ind Crops Prod* 2016; **85**: 341-52.
[3] Bravo K, Sepulveda-Ortega S, Lara-Guzman O, Navas-Arboleda AA, Osorio E. Influence of cultivar and ripening time on bioactive compounds and antioxidant properties in Cape gooseberry (*Physalis peruviana* L.). *J Sci Food Agric* 2015; **95**(7): 1562-9.
[4] Carrillo-Hormaza L, Mora C, Alvarez R, Alzate F, Osorio E. Chemical composition and antibacterial activity against *Enterobacter cloacae* of essential oils from Asteraceae species growing in the Páramos of Colombia. *Ind Crops Prod* 2015; **77**: 108-15.
[5] Valcárcel-Yamani B, Rondán-Sanabria GG, Finardi-Filho F. The physical, chemical and functional characterization of starches from andean tubers: Oca (Oxalis tuberosa molina), olluco (Ullucus tuberosus caldas) and mashua (Tropaeolum tuberosum ruiz & pavón). *Braz J Pharm Sci* 2013; **49**(3): 453-64.
[6] McMoran D, Gauthier J. Experimenting with growing ulluco as a niche crop for fun and profit. *J Hortic* 2014; **2**(1): 119-20.
[7] Xing W, Guo W, Zou CH, Fu TT, Li XY, Zhu M, et al. Acemannan accelerates cell proliferation and skin wound healing through AKT/mTOR signaling pathway. *J Dermatol Sci* 2015; **79**(2): 101-9.
[8] Agyare C, Boakye YD, Bekoe EO, Hensel A, Dapaah SO, Appiah T. Review: African medicinal plants with wound healing properties. *J Ethnopharmacol* 2016; **177**: 85-100.
[9] Rolfe KJ, Grobbelaar AO. A review of fetal scarless healing. *ISRN Dermatol* 2012; **2012**: 698034.
[10] Diegelmann B. Wound healing: an overview of acute, fibrotic and delayed healing. *Front Biosci* 2004; **4**: 1-8.
[11] Ramata-Stunda A, Boroduskis M, Vorobjeva V, Ancans J. Cell and tissue culture-based *in vitro* test systems for evaluation of natural skin care product ingredients. *Environ Exp Biol* 2013; **11**: 159-77.
[12] Chen CZ, Raghunath M. Focus on collagen: *in vitro* systems to study fibrogenesis and antifibrosis state of the art. *Fibrogenes Tissue Repair* 2009; **2**: 7.
[13] Gomez I, Benyahia C, Louedec L, Leséche G, Jacob MP, Longrois D, et al. Decreased PGE content reduces MMP-1 activity and consequently increases collagen density in human varicose vein. *PLoS One* 2014; **9**(2): e88021.
[14] Montoya A, Daza A, Muñoz D, Ríos K, Taylor V, Cedeño D, et al. Development of a novel formulation with hypericin to treat cutaneous leishmaniasis based on photodynamic therapy in *in vitro* and *in vivo* studies. *Antimicrob Agents Chemother* 2015; **59**(9): 5804-13.
[15] Demirci S, Doğan A, Demirci Y, Şahin F. *In vitro* wound healing activity of methanol extract of Verbascum speciosum. *Int J Appl Res Nat Prod* 2014; **7**(3): 37-44.
[16] Muhammad AA, Pauzi NAS, Arulselvan P, Abas F, Fakurazi S. *In vitro* wound healing potential and identification of bioactive compounds from *Moringa oleifera Lam*. *Biomed Res Int* 2013: 2013.
[17] Hayden DM, Forsyth C, Keshavarzian A. The role of matrix metalloproteinases in intestinal epithelial wound healing during normal and inflammatory states. *J Surg Res* 2011; **168**(2): 315-24.
[18] Küpeli Akkol E, Süntar I, Ilhan M, Aras E. *In vitro* enzyme inhibitory effects of Rubus sanctus Schreber and its active metabolite as a function of wound healing activity. *J Herb Med* 2015; **5**(4): 207-10.
[19] Nagase H, Woessner J. Matrix metalloproteinases. *J Biol Chem* 1999; **274**(31): 21491-4.
[20] Alerico GC, Beckenkamp A, Vignoli-Silva M, Buffon A, Von Poser GL. Proliferative effect of plants used for wound healing in Rio Grande do Sul state, Brazil. *J Ethnopharmacol* 2015; **176**: 305-10.
[21] Zhang Q, Fong CC, Yu WK, Chen Y, Wei F, Koon CM, et al. Herbal formula Astragali Radix and Rehmanniae Radix exerted wound healing effect on human skin fibroblast cell line Hs27 via the activation of transformation growth factor (TGF-β) pathway and promoting extracellular matrix (ECM) deposition. *Phytomedicine* 2012; **20**(1): 9-16.
[22] Liang C-CC, Park AY, Guan J-LL. *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nat Protoc* 2007; **2**(2): 329-33.
[23] Tredget EE. Pathophysiology and treatment of fibroproliferative disorders following thermal injury. *Ann N. Y Acad Sci* 1999; **888**: 165-82.
[24] Fronza M, Heinzmann B, Hamburger M, Laufer S, Merfort I. Determination of the wound healing effect of Calendula extracts using the scratch assay with 3T3 fibroblasts. *J Ethnopharmacol* 2009; **126**(3): 463-7.
[25] Poormasjedi-Meibod MS, Hartwell R, Kilani RT, Ghahary A. Anti-scarring properties of different tryptophan derivatives. *PLoS One* 2014; **9**(3): e91955.