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Formulation and characterization of sustainable bioadhesive films for wound treatment based on barley β-glucan extract obtained using the high power ultrasonic technique

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ABSTRACT

β-glucan is a well-known functional and bioactive food ingredient. Recently, some studies highlighted several interesting pharmacological activities, such as hypocholesterolemic, hypoglycemic, immunomodulatory, anti-tumor, antioxidant and anti-inflammatory. The aim of this study is to evaluate a novel application of β-glucan, obtained from barley, for the development of formulations for skin use. Several water suspensions were obtained from barley flour of different particle sizes treated by high power ultrasonic (HPU) technique. Barley flour fraction in the range of 400–500 μm allowed to obtain a stable suspension, represented both by a water soluble and water insoluble fraction of β-glucans, that showed excellent film forming ability. The plasticizer sorbitol as well as the bioadhesive biopolymer acacia gum were added to this suspension in order to obtain a gel suitable to prepare films by casting. The obtained films demonstrated suitable mechanical properties and ability to stimulate in vitro keratinocytes growth suggesting its possible application in dermatological field as for wound treatment. This study demonstrated the dual use of barley suspension: as excipient and as active ingredient.

1. Introduction

β-glucan is a polysaccharide composed by D-glucose units polymerized primarily via the β-1, 3 glycosidic bonds, in addition to the β-1, 4 and/or β-1, 6 bond (Nakashima et al., 2018; Du et al., 2019). It is widely present in cereals, yeast, some bacteria, mushrooms, algae.

β-glucan found in cereals (such as barley and oats) includes a mixture of β-1,3 and β-1,4 glycosidic bonds and shows no β-1, 6 branching. Barley (*Hordeum vulgare L.*) is an important cereal crop and ranks fourth among the total cereals production but very little of this grain is used as human food. It is currently gaining popularity as a "functional grain" because it contains higher amounts of bioactive compounds including β-glucan among others (Goudar et al., 2020). β-glucan is acknowledged as a functional and bioactive food ingredient owing to its biological activities (Goudar et al., 2020).

β-glucan can be divided in water soluble and insoluble fraction. The water-insoluble part of the β-glucan has been studied to treat digestive system pain and chronic constipation, while the water-soluble β-glucan is considered as a potent immunological booster and biological defense modifier. Many studies evidenced biological activities of β-glucan such as hypoglycemic, immunomodulatory, antitumor and antioxidant (Zhu et al., 2016). In addition, β-glucan from cereals reduces total serum cholesterol and low density lipoprotein (LDL) (Ho et al., 2016). The consumption of these cereals decreases the risk of chronic diseases (Maheshwari et al., 2017). One interesting application of β-glucan is in the dermatological field (Majtan and Jesenak, 2018). In fact, barley

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β-glucan shows anti-inflammatory activity as binds dectin-1 receptor and interleukins and modify the expression of inflammatory-associated genes and macrophage activation. Moreover, barley β- glucan has been described as cytokine production inducer (Fusté et al., 2019).

Thanks to these different activities, β-glucan has been used in many commercial marketed products for different applications, such as food supplements, medicines, cosmetics, feeds and other health products (Zhu et al., 2016).

About β-glucan application in dermatological field, it was used in many formulations (such as creams, ointments and suspensions) as a general skin protectant. Interestingly, β -glucan increases collagen production and reduces eczema, psoriasis, and other skin diseases, being of great interest in dermatological field. β -glucan was furthermore found to be a film forming, humectant and a promoter of wound healing. A study investigated the penetration of cereal β-glucan into human skin models and clinically evaluated its efficacy in reducing fine lines and wrinkles (Pillai et al., 2005). Other studies supports the use of β-glucan in the care and maintenance of healthy skin and in the cosmetic treatment of aging wrinkles (Baschong et al., 2009).

The search of natural and biosustainable materials to be used in health products in place of synthetic ones (Lang et al., 2022) in a necessity both for people and environment. In this context, we considered remarkable to evaluate the use of barley flour both as source of excipients and active ingredient (namely β- glucan). The extraction of barley from this source has been already performed by maceration by Tejinder (Tejinder, 2003). The novelty of this work is represented by the technique used to produce the extract. In fact, in the perspective of biosustainability, it was considered useful to treat barley by high power ultrasonic (HPU) technique using water as extraction solvent. HPU is more efficient than traditional extraction methods (e.g. maceration, infusion). In the field of molecule extraction from natural sources, HPU is an emerging technology because it can accelerate heat and mass transfer. As is well known, the phenomenon of acoustic cavitation, caused by the propagation of ultrasound in a liquid medium, results in the formation of cavitative bubbles that, after interaction with the natural matrix, alter its physical and chemical properties, facilitating both the release of extractable compounds and increasing mass transport by destroying the cell walls of the vegetal material. HPU is a green method because it avoids the use of large amounts of solvent and reduces work time because it can increase the extraction rates. There are many examples in literature about the use of ultrasound in the field of extraction of compounds from natural matrices that often require very long extraction times (hours or days) using traditional methods (Toma et al., 2001; Vinatoru, 2001; Luque-Garcìa and Luque de Castro, 2003; Riera et al., 2004). By HPU the extractions can be completed in a very short time with low solvent consumption, high reproducibility, increased purity of the final product, avoiding wastewater post-treatment. All these results in energy savings, compared to that consumed for conventional extraction methods such as Soxhlet extraction, maceration, or steam distillation.

This paper aims to propose an eco-friendly material obtained by a green method (HPU) for new applications in the health sector. With this idea, β- glucan extract was prepared by high power ultrasonic (HPU) technique, using different particle size of barley flour. The most suitable, able to give a stable suspension, was used to prepare bioadhesive film by casting method.

2. Materials and methods

Barley seeds (cultivation in Monteleone of Spoleto, Perugia, Italy) were purchased from Antica Spezieria Bavicchi (Perugia, Italy). Acacia gum, and ethanol 96% (EtOH) were purchased from Sigma Aldrich (Milan, Italy). Sorbitol was supplied by A.C.E.F. s.p.a. (Fiorenzuola d'Arda, Italy). Ultrapure water was obtained by reverse osmosis process in a MilliQ system Millipore (Rome, Italy). The simulated wound fluid (SWF) pH 6.5 was prepared by dissolving 8.30 g of NaCl and 0.28 g of $CaCl_2$ in 1000 mL of ultrapure water.

HaCat cells (Human immortalized keratinocyte cell line), was bought from I.Z.S.L.E.R. (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna).

2.1. Barley seeds grinding and seaving

Barley seeds were grinded by a knife mill GM 200 (Retsch, Predengo, Cremona, Italy) working at 4000 rpm, for 3.30 min. The obtained grinded powder was sieved by steel sieves (Endecotts Ltd, London, UK) using mesh sizes of 1 mm, 710 μm, 500 μm, and 400 μm.

2.2. Extract preparation

Before extraction, barley flour (20 g) was boiled in EtOH (80%, v/v) under reflux for 2 h, as reported in literature (Benito-Román et al., 2013), in order to inactivate the enzymes β-gluconases, responsible for β-glucan enzymatic hydrolysis. The suspension obtained after this treatment was centrifuged (10 min/4000 rpm/20 °C) the solvent removed and the solid submitted to extraction by high power ultrasonic (HPU) treatment. The HPU conditions were set according to a previous study (Benito-Román et al., 2013) properly modified: emitted power of 750 W and transmitted power of 200 W, frequency of 20 KHz, amplitude of 50% working at 55 °C, for 15 min, using Horn Type ultrasonic probe VCX750 (SONICS, Newtown, Connecticut, USA).

The extraction was performed as reported in Scheme 1, briefly:

1. 20 g of barley flour (previously treated for enzyme inactivation) were suspended in 150 mL of bidistilled water ad treated by HPU;
2. the obtained suspension was centrifuged (10 min/ 4000 rpm/20 °C). The supernatant (liquid part) was recovered and punt in a flask (1 L capacity) and kept under magnetic stirring (600 rpm); the solid part was recovered suspended in 150 mL of fresh bidistilled water for a second treatment by HPU;
3. the obtained suspension was centrifuged (see point 2).

Three cycles of treatment by HPU were performed in order to obtain the highest extraction yield.

In order to evaluate the influence of HPU method on the final yield, the extraction was also performed by a traditional method (maceration):

1. 20 g of barley flour (previously treated for enzymatic inactivation by Et0H 80% v/v for 2 h) were suspended in 150 mL of bidistilled water and kept at 55 °C under magnetic stirring at 750 rpm, for 15 min,

2. the obtained suspension was centrifuged (10 min/ 4000 rpm/ 20 °C) and the supernatant recovered and poured into a flask (1 L capacity) and kept under magnetic stirring (600 rpm). The solid part was recovered suspended again in 150 mL of bidistilled water for a second treatment at 55 °C, under magnetic stirring at 750 rpm, for 15 min.

3 the obtained suspension was centrifuged (see point 2).

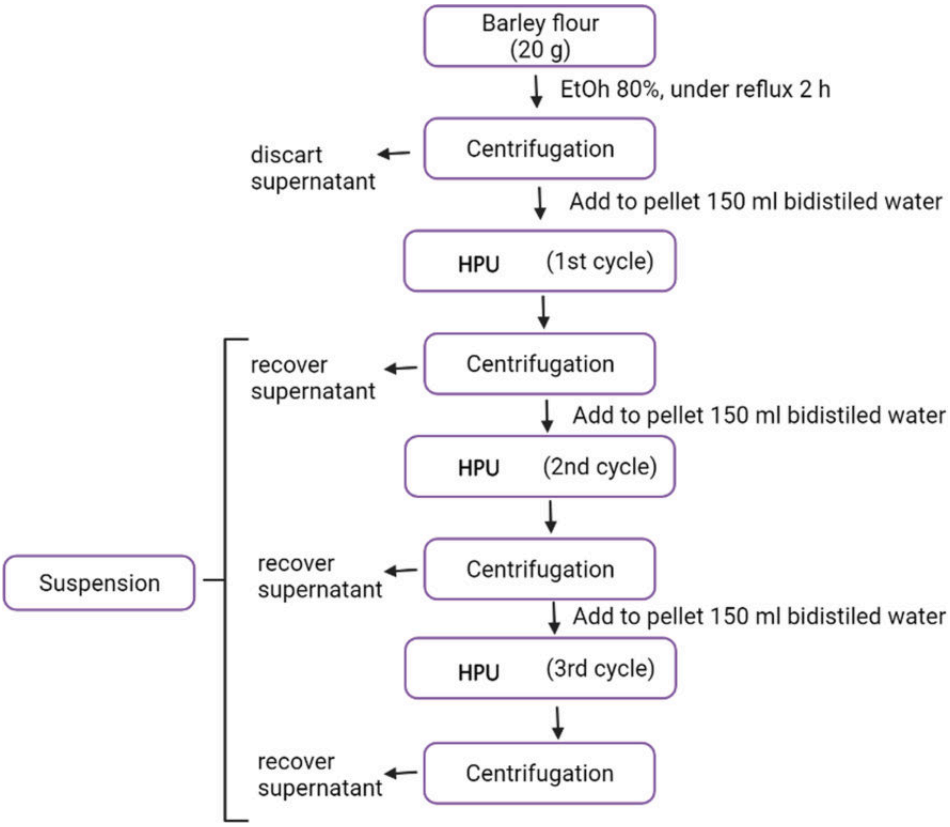
Three cycles were performed.

The extraction yield of β-glucan was calculated according to equation (1):

$$Yield(\%) = \frac{\%\,\beta\text{glucan in raw baley} - \%\,\beta\text{glucan in exhausted barley}}{\%\,\beta\text{ glucan in raw barley}} \times 100 \quad (1)$$

2.3. β-glucan content determination

β-glucan content was calculated using "Mixed-linkage β-glucan" from Megazyme International Ltd. The amount of β-glucan in the samples was determined by UV–vis spectrophotometry using an Agilent 8453 (Agilent, Germany) spectrophotometer. The analysis was performed at λ_{max} = 510 nm. The measurements were performed in triplicate.



Scheme 1. Extraction process of β- glucan from barley flour.

2.4. NMR analysis

Mono and bi-dimensional NMR spectra were recorded on a Bruker Avance NEO 600 spectrometer equipped with the ProdigyTM Bruker Cryoprobe (600 MHz for 1H) with a z gradient coil. Residual solvent resonances were used for referencing; reported chemical shifts are relative to external TMS. In all the cases, the samples were prepared by suspending 3–4 mg of the polysaccharides in 0.6 mL of D_2O.

2.5. Single particle optical sensing (SPOS) analysis

The dimensional analysis was performed by SPOS technique "Single Particle Optical Sensing" using an Accusizer C770 (PSS Inc., Santa Barbara, CA). The sizes were expressed as mean volume diameter (MVD) value (n = 5 ± SD).

2.6. Scanning electron microscopy analysis (SEM)

The morphology and thickness were investigated by Scanning Electron Microscopy using a FE-SEM LEO 1525 ZEISS (Carl Zeiss Microscopy, Jena, Germany). The sample was deposited on conductive carbon adhesive tape and metallized with chromium (8 nm) by sputtering. The diameters of particles were measured using ZEISS SmartSEM software.

2.7. Film preparation

Films were obtained by solvent casting method (Pagano et al., 2020) starting from β -glucan suspension (named β-GLU ULTX) prepared as described in par. 2.2. Sorbitol 7% w/w was added to β-GLU ULTX (92% w/w) under magnetic stirring (800 rpm) at room temperature (R.T.). AG (when used) was added to β-GLU ULTX - sorbitol mixture and vortexed for 5 min before casting. Afterwards, 71 g of the prepared hydrogel was casted into rectangular teflon moulds (10.5 cm × 15.5 cm) and stored at 4.0 °C for 12 h in order to remove the air bubbles. Thereafter, placed in ventilated oven at 40.0 °C ± 0.1 for 24 h. After preparation films were stored under $MgCl_2$ saturate solution (Wexler and Hasegawa, 1954).

2.8. Rheological properties

The viscosity was measured by a Stresstech HR (Rheological Instruments, AB Milan, Italy) rheometer, (cone-plate geometry, diameter 40 mm, angle 1°). The shear stress was set in the range 1–100 Pa working at 25.0 °C ± 0.1 (room temperature), n = 3 ± SD.

2.9. Mechanical properties

Tensile tests were performed by a digital microprocessor instrument (Llyod LR30K, Lloyd Instrument, Fareham, UK). Films were cut in portions 100 mm × 10 mm (UNI ISO 527) to have a useful length of 50 mm. The experiment was performed at 5 mm/min, cell load 50 N. The two ends of the film were fixed with clamps to the dynamometer. The sample was subjected to tensile stress until deformation and break. Values for maximum stress, deformation at maximum stress, and elastic modulus were registered. The obtained results are an average of five measurements (n = 5).

2.10. Bioadhesion capacity

Pig skin samples, deriving from shoulder region, were obtained from Large White pigs weighing ~165–175 kg, supplied by Veterinary Service of ASL Umbria 1 Città di Castello (Perugia, Italy) and used within 12 h from pig death. The adhesion force was measured by a dynamometer. The experiment was performed as previously described (Pagano et al., 2022). The skin samples were cut in portions of 2 cm × 2 cm and fixed with cyanoacrylate glue on the surface of a glass support placed in a thermostatic bath at 32.0 °C ± 0.5. The film was fixed at the bottom of a

punch connected to the dynamometer. The skin sample was wetted with 200 μL of SWF and put in contact with the skin sample by applying a light force for 1 min. The force necessary for film detachment, expressed in N, from the skin was measured and expressed as an average of three measurements (n = 3 ± SD).

2.11. MTT and wound healing assays

The assays were performed in order to evaluate the effect of the water soluble fraction of β-glucan released from the film. The sample was prepared as follows: film (1 × 1 cm^2) was incubated overnight in 10 mL of DMEM complete medium, afterward centrifuged and the supernatant added to the cells. In order to know the amount of β-glucan released in the medium, the film (1 × 1 cm^2) was incubated in the same conditions described above in 10 mL of bidistilled water and then performed the quantification as described in par. 2.3.

Cells viability was evaluated on HaCat cells by MTT test (Pagano et al., 2021b). Cells were growth in monolayer cultures, with DMEM complete medium supplemented with 10% heat-inactivated FBS, 2 mM of L-glutamine, and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) and incubated at 37 °C under 5% CO_2 atmosphere. Cells were seeded onto 96-well plate with DMEM complete medium, as previously reported (Perioli et al., 2012) and treated for 24 h with different concentrations of β-glucan (from 12.9 to 0.21 μg/mL). In all experiments, untreated cells were used as negative control. The results were acquired by an automatic microplate reader (Eliza MAT 2000, DRG Instruments, GmbH). The absorbance values (OD) were measured spectrophotometrically at λ_{max} = 540 nm. Cell viability calculation by MTT assay was performed according to Eq. (2) (Denizot and Lang, 1986; Kumar et al., 2018):

$$\%viability = \frac{\text{Average of treated cells} - \text{background}}{\text{Average of control cells} - \text{background}} \text{ x } 100 \quad (2)$$

Three indipendent experiments were performed in triplicate. A one-way ANOVA test was performed using the Graphpad program (GraphPad Prism 9.2.0.332, GraphPad software, San Diego, CA, USA). The wound healing test was performed by CytoSelect™ Wound Healing Assay Kit (Cell Biolabs, Inc., San Diego, USA) allows to simulate in vitro a wound (Grada et al., 2017; Martinotti and Ranzato, 2020) and was used to study the water soluble fraction of β-glucan on keratinocytes growth at different dilutions.

This technique consists in the performing a linear thin scratch "wound" (creating a gap) in a confluent keratinocyte monolayer. The images of cells filling the gap are taken at regular time intervals. HaCat cell were seeded in a 24-well plate at the final concentration 5 × 10^4 cell/500 μL (1 × 10^5/mL) and incubated overnight. Inserts were removed and cells were washed with PBS 1X to remove dead cells and debris (Pagano et al., 2021b).

After 6, 12 and 24 h the treatments were removed and cells were stained as previoously described (Martinotti and Ranzato 2020). The total wound field surface area was calculated (Grada et al., 2017) considering the dimensions of the insert: Total Surface Area = 0.9 mm (length) × 1.8 mm = 1.62 mm^2. Wound area is calculated by manually tracing the cell-free area in captured images using the ImageJ public domain software (NIH, Bethesda, MD). The closure will increase as cells migrate over time. The % closure of wound field was calculated using Eq. (3):

$$\%closure = \frac{\text{Total Surface Area} - \text{Cells Free Area}}{totalsurfacearea} \text{ x } 100 \quad (3)$$

Where: Total Surface Area means the area immediately after removing the insert; Cells Free Area means the white area in the photograph.

Migration into the wound field was determined as previously described (Pagano et al., 2021a) and pictures of control cells (CTR) and treated cells after 6, 12 and 24 h were taken. Three indipendent experiments were performed in duplicate. Three fields for each condition were compared.

3. Results and discussions

3.1. Barley extract preparation and characterization

Before treatment, barley seeds were grinded by knife mill in order to obtain a flour as described in method section (par. 2.1.). Seeds grinding is an important step as allowing to obtain particles of small dimensions able to expose a high surface area, useful to improve the extraction efficiency. After grinding the flour was sieved by stainless steel sieves (par. 2.1.), in order to classify and collect the particles in different fractions. After a preliminary evaluation, three of them were selected: 400 – 500 μm, 500 – 700 μm and 710– 1000 μm (Table 1). Particles having size below 400 μm were discarded as make difficult the filtration process (necessary after treatment). Before to perform the extraction, the flour was boiled in EtOH (80%, v/v) following the procedure reported in literature (Benito-Román et al., 2013) (par. 2.2.) in order to inactivate β-glucanases, enzyme responsible for β-glucan enzymatic hydrolysis. Afterwards, the flour was centrifuged and submitted to extraction by High-Power Ultrasonic (HPU). This approach is more efficient in comparison to traditional ones such as maceration, percolation, reflux extraction, as it allows to work in short times, using low amounts of extraction solvent and by low temperatures with high yield values and low energy consumption. Following the procedure reported in literature (Benito-Román et al., 2013), barley flour was suspended in bidistilled water using a ratio of 20 g/150 mL (flour/bidistilled water). Three extraction cycles were performed, suspending 20 g of flour in 150 mL of fresh bidistilled water for three times as described in par. 2.3. Four different extracts, β-GLU A - β-GLU D, were prepared by combining the selected flour fractions in different ratios as reported in Table 1. All the extracts were suspensions consisting both of a soluble and an insoluble fraction of β-glucan (hereinafter called "extracts"). Each extract was characterized (Table 1) and the best results, in terms of β-glucan content and final yield, were obtained for the sample β-GLU D. This is attributable to the small fraction (400–500 μm) used to obtain this sample probably because it exposes a higher surface area to the solvent, than the other two fractions, with a consequent yield increase. This hypothesis is also confirmed comparing the yield values reported in Table 1. In fact, it increases from β-GLU A to β-GLU C samples proportionally to the increased percentage of 500–700 μm fraction and decreased percentages of 710–1000 μm fraction. Considering these results, β-GLU D was submitted to further characterization.

In order to evaluate the advantages in the use of HPU treatment in the improvement of the final yield, the extraction was also performed using the selected fraction (400–500 μm) in the same temperature and

Table 1
Characterization of β-glucan samples obtained HPU (n = 3 ± SD).

Extract	Barley flour fractions (%)			pH ± SD	β-glucan (g/100 g) ± SD	Yield (%) ± SD
	400–500 (μm)	500–700 (μm)	710–1000 (μm)			
β-GLU A	-	25	75	5.46 ± 0.05	4.49 ± 0.91	77.2 ± 1.5
β-GLU B	-	50	50	5.66 ± 0.15	4.71 ± 0.82	81.4 ± 1.3
β-GLU C	-	75	25	5.51 ± 0.05	5.39 ± 0.73	81.5 ± 2.3
β-GLU D	100	-	-	5.56 ± 0.04	6.30 ± 0.70	85.0 ± 1.4

time conditions but by a traditional maceration method (par. 2.2.). Comparing the yield value, expressed as β-glucan (g/100 g of barley flour), they were obtained 6.30 ± 0.1 and 3.80 ± 0.5 for HPU and maceration method respectively. An increase of the final yield of 40% was observed for HPU compared to maceration. This increase is due to the cavitating bubbles generated by HPU that are able to break the cell walls present in the solid starting material favoring the extraction as observed in literature for other matrices (Esclapez et al., 2011).

β-GLU D sample was firstly characterized by SEM analysis in order to study the morphology of the insoluble fraction. β-GLU D shows particles with spherical shape, smooth surface and low dimensions, around ~1 μm detected as reported in par. 2.6. (Fig. 1A and 1B). Comparing these micrographs with those obtained from the starting flour (Fig. 1C and 1D), showing the coexistence of irregular and regular (with round edges) particles, it is clearly detectable the influence of HPU treatment. In fact, the working frequency used (20 kHz) is able to improve the uniformity of particle size distribution as well as to modify the particles surface without chemical structure impairment as observed from other authors (Yang et al., 2019).

The dimensional characterization of β-GLU D performed by SPOS analysis, showed an MVD of 428.42 ± 34.60 μm, attributable to the presence of aggregates formed during HPU treatment. The presence of aggregates improves the suspension instability and thus increases the possibility of sedimentation. In order to reduce the aggregates and thus to obtain a homogeneous and stable suspension, β-GLU D was treated by ultraturrax (ULTX). The most suitable conditions were obtained working at 24,000 rpm for 2 min. The dimensional analysis of the obtained suspension (hereinafter called β-GLU D-ULTX) showed a decrease of the MVD to 102.12 ± 26.97 μm suggesting the benefits of this treatment in the particle size reduction. Nevertheless, the measured diameter could be attributable to aggregates suggesting that the dimensions of the single particle could be lower than that obtained by SPOS analysis. In fact, observing β-GLU D-ULTX micrographs (Fig. 1E), separate particles having low dimensions (~300 nm) can be detected (Fig. 1F). It is important to underline that, compared to the sample β-GLU D not treated by ULTX (Fig. 1A and B), the single particles are not distinguishable. Moreover, after deposition of a drop of such suspension on a glass surface (Fig. 2A) and left to dry, it was possible to observe the

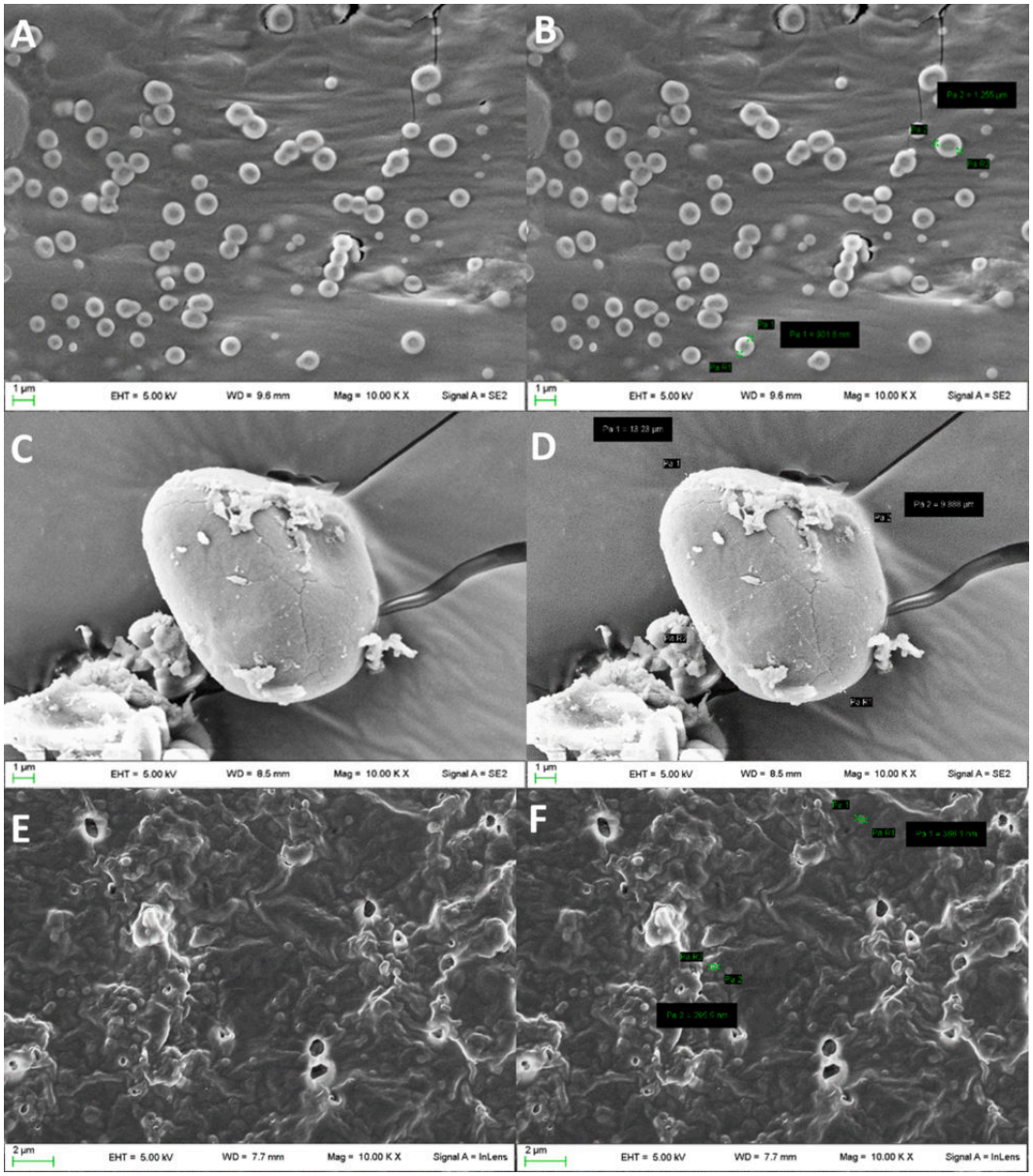


Fig. 1. Micrographs of β-GLU D (**A**, **B**), barley flour (**C**, **D**) and β-GLU D-ULTX (**E**, **F**). Magnification 10.00 KX.

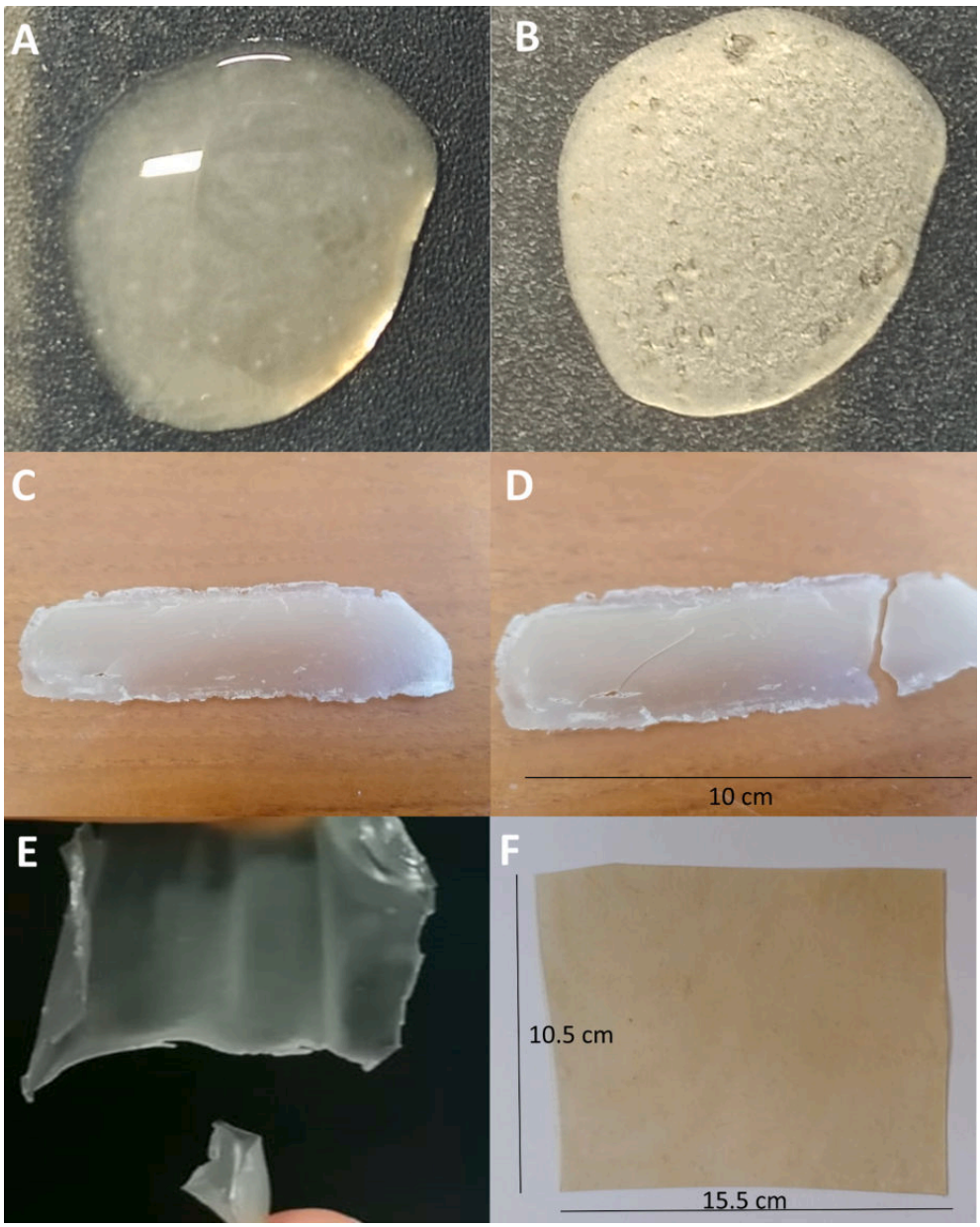


Fig. 2. Drop of β-GLU D treated by ULTX before (**A**) and after drying (**B**); Film obtained by casting β-GLU D-ULTX (**C**,**D**); film obtained using β-GLU D-ULTX and sorbitol as plasticizer (**E**); film obtained from composition G (**F**).

formation of a film (Fig. 2B). This observation suggested that the sample β-GLU D, treated by ULTX, could be a suitable material for films preparation.

3.2. Films preparation and characterization

In order to better investigate the film forming capacity of β-GLU D-ULTX, 12 g of this sample were casted in teflon moulds (2.0 cm × 10.0 cm) and the solvent removed in ventilated oven at 40.0 °C ± 0.1 for 24 h. After drying the formation of a continuous film (Fig. 2C) was observed however, characterized by brittleness (Fig. 2D).

The brittleness problem could be solved by the addition of a plasticizing agent and sorbitol was chosen for this purpose. Literature data (Harussani et al., 2021) reports that 30% w of sorbitol is able to improve mechanical properties of corn starch-based biopolymers. Thus, firstly this percentage of sorbitol was used and combined to *β*-GLU D-ULTX (70% w/w). However, after drying, the final product resulted very sticky and difficult to remove from the mould. For this reason, many other attempts were made reducing sorbitol %. The most suitable was in the range of 1–8% w/w providing not brittle and flexible films which nevertheless showed low tensile resistance (Fig. 2E). Another aspect to consider, is that the designed films must be applied on skin. Therefore, in addition to plasticizer it was necessary to include in the film composition a polymer capable to confer bioadhesion capacity without the need of synthetic adhesives.

In order to formulate a biocompatible and biodegradable product, the composition was modified by adding the biopolymer acacia gum (AG) recently successfully used in the development of bioadhesive films (Pagano et al., 2022). After many attempts, the AG amount was fixed to 1% w/w as the use of higher percentages produced sticky films. Thus, eight different films (A-H) were prepared starting from the compositions reported in Table 2.

The obtained films showed different appearances as reported in Table 2. The most interesting was that obtained from composition G (Fig. 2F), resulting uniform, flexible, easily removable from the mould and for this reasons considered for further characterization.

Table 2
Compositions of the starting suspensions used for film preparation.

Sample	β-GLUC D-ULTX (% w)	Sorbitol (% w)	AG (% w)	Appearance of the final film
A	98	1	1	rigid
B	97	2	1	rigid
C	96	3	1	rigid
D	95	4	1	rigid
E	94	5	1	flexible but fragile
F	93	6	1	flexible but fragile
G	92	7	1	flexible
H	91	8	1	sticky

It was interesting to evaluate the rheological properties of β-GLU D before and after treatment by ULTX and combined to sorbitol and AG in order to evaluate their effect on the flow properties. The rheological analysis (Fig. 3) performed on β-GLU D before and after treatment by ULTX showed a modification of the flow properties. In both cases, a pseudoplastic behavior is detectable.

The treatment by ULTX produces an increase of flow properties as testified by the shear rate range measured (28–4795 1/s *vs* 5 – 1332 1/s of β-GLU D and β-GLU D-ULTX respectively) this can be explained considering that ULTX treatment, coupled to the particle size reduction, probably is responsible for β-glucan chains breaking (even if this does not affect the filming capacity) and thus to the decrease of suspension viscosity (Bacha et al., 2017; Mcclear and Glennie-Holmes, 1985). The addition of sorbitol and sorbitol + AG produced a further increase of the flow properties (Fig. 3) low shear stress value to flow. Tensile characterization of the produced film G was then performed. It was observed that at low moisture content (films conditioned under $CaCl_2$ for 1 week, room temperature) the film shows high tensile modulus (mean value 0.14 GPa), high tensile strength (mean value 2.8 MPa) and low elongation values (mean value 22%) typical of materials in their glassy state. The same behaviour was described for similar systems by Razzaq et al. (Razzaq et al., 2016). When the samples were exposed to room humidity, the parameters changed in terms of decrease of both modulus and tensile strength and increase in elongation (Table 3) as observed also by other authors for similar systems (Skendi et al., 2003).

The morphology as well as the thickness of the prepared film were measured by SEM analysis. The surface appears wrinkled (Fig. 4A), useful for a better adhesion to skin and the thickness (Fig. 4B) is 384.13 ± 11.81 μm making the formulation, once applied on skin surface, not detectable.

Ex vivo bioadhesion capacity was evaluated following the procedure reported in par. 2.10. as the film is intended to be applied and to adhere

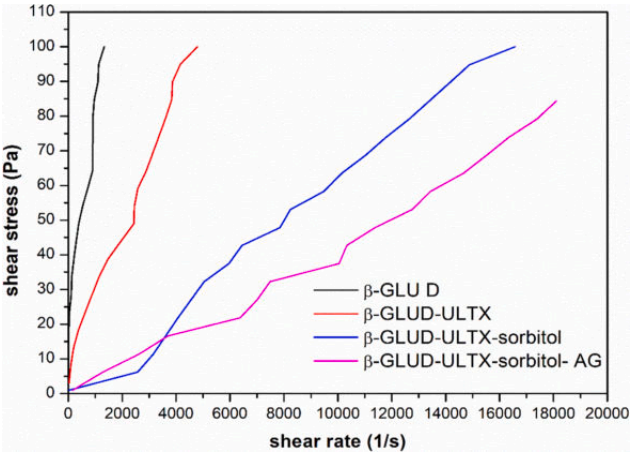


Fig. 3. Rheograms of β-GLU D; β-GLU D after treatment by ULTX; β-GLU D after treatment by ULTX combined to sorbitol; β-GLU D after treatment by ULTX combined to sorbitol and AG.

Table 3
Results of tensile test for film G.

Film G (storage conditions)	σ_{max} (MPa)	$\varepsilon_{@\ \sigma max}$ (%)	E(MPa)
under $CaCl_2$ for 1 week	2.80 ± 0.84	22 ± 6	140.00 ± 60.00
Room humidity	0.71 ± 0.03	29 ± 4	14.03 ± 1.55

on skin surface. The obtained results show an adhesion force of 0.100 ± 0.001 N. This value is interesting as, despite the measured value (mainly attributable to the biopolymer AG used) is low compared to films obtained using semi-synthetic polymers (Pagano et al., 2020) however, it allows a gentle adhesion to skin avoiding pain during the application time. This is an important aspect especially considering the possible use of the developed film for damaged skin (e.g. wounds) that is particularly sensitive to solicitations.

3.3. MTT e wound healing assays

MTT assay was performed in order to evaluate the possible citotoxic effect of the water soluble fraction of β-glucan released from film G. The sample, prepared as described in par. 3.11, produced a concentration of 12.9 μg/mL tested as is and diluted. At all the tested concentrations, from 0.21 to 12.9 μg/mL, allowed to obtain a viability up to 80% (Fig. 5A) resulting safe for the cells. Considering these results, the ability of β-glucan water soluble fraction released from film G to stimulate keratinocytes growth was studied in vitro as well. With this aim two concentrations were selected: 1.62 μg/mL and 3.23 μg/mL as both exhibited a cells viability very close to CTR cells (95.0 ± 2.8% and 93.6 ± 4.7%). The lowest concentration assayed, namely 1.62 μg/mL, demonstrated to stimulate cells growth in an excellent way (Fig. 5B). In detail, after 6 h the setup is approximately the same comparing untreated (CTR) and treated cells (Fig. 5B, on the left) as testified by the wound closure % measured: 26.4 ± 5.1% (for CTR), 32.5 ± 3.8% (for 1.62 μg/mL of β-glucan) and 33.2 ± 4.0% (for 3.23 μg/mL of β-glucan). Also after 12 h of treatment, no relevant differences were observed in the wound closure (around 45% both for CTR and treated cells). The most interesting results were observed after 24 h of treatment. In fact, a complete closure of the wound field is clearly visible for β-glucan concentration of 1.62 μg/mL (Fig. 5B), compared to the control cells (CTR) where the wound field was still open (82.1 ± 6.1%). The highest concentration of β-glucan assayed (3.23 μg/mL) was slightly better compared to CTR, however did not allow to achieve a complete closure after 24 h suggesting that the most promising results were obtained using the concentration of 1.62 μg/mL.

3.4. NMR analysis

The ^{1}H NMR spectrum of the water soluble fraction (Fig. 6Aa) shows the diagnostic resonances of the anomeric hydrogens (H1) of α- (5.33 ppm) and β-glucans (4.45 ppm) (Yao et al., 2021) suggesting that the sample is a mixture of two polysaccharides. This hypothesis is further confirmed by the ^{1}H, ^{13}C HSQC spectrum (Fig. 6Ba) in which the aforementioned peaks at 5.33 ppm and 4.45 ppm are scalarly coupled to two carbons resonating at 99.6 and 102.3 ppm (Yao et al., 2021) respectively. In addition, the ^{1}H, ^{13}C HSQC spectrum shows the presence of a third resonance belonging to an acetal carbon (C1) of a β-glucan resonating at 102.5 ppm and coupled to a hydrogen whose peak fall under the signal of HDO (around 4.7 ppm). Direct comparison of the spectra of the standards β-glucan (Fig. 6Ab and 6Bb) and corn starch (Fig. 6Ac and 6Bc) with the ones of the extract fully confirmed that the sample is a mixture of the two polysaccharides. Unfortunately, due to the presence of precipitate in the NMR tube, the quantitation of the composition of the sample was not possible.

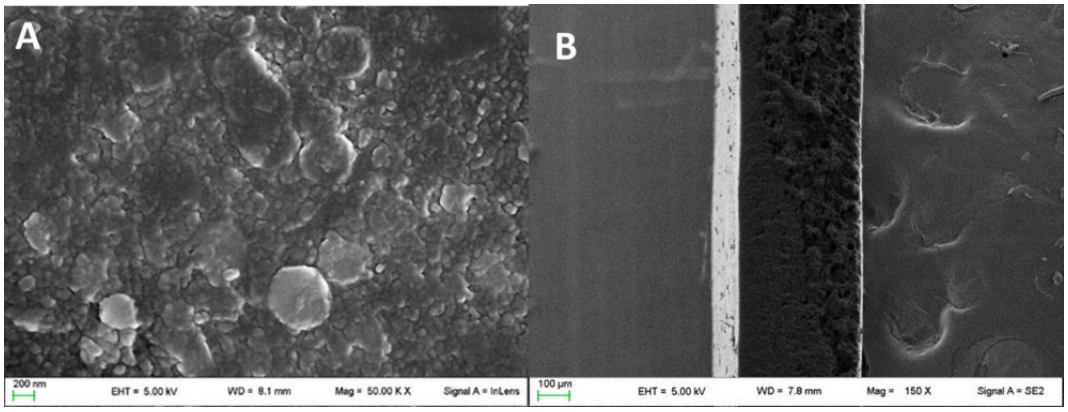
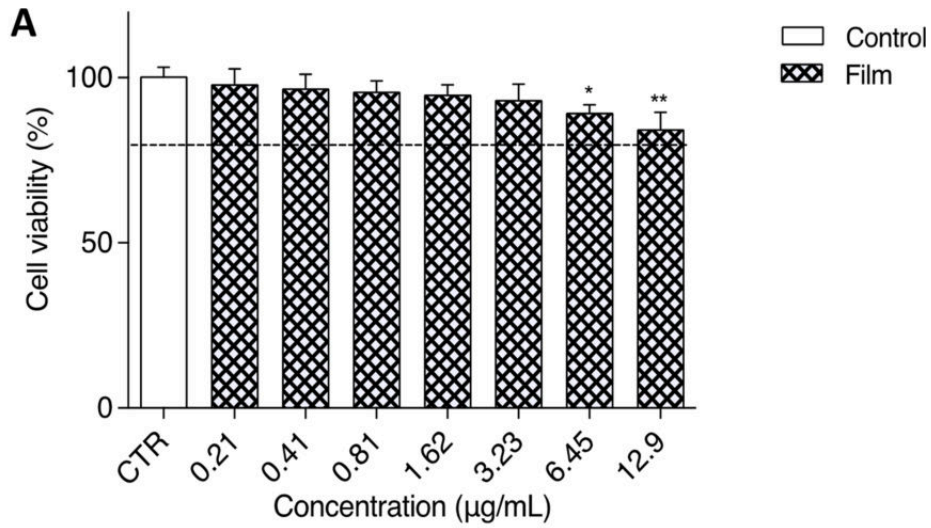


Fig. 4. Film G surface morphology (A) and thickness (B).



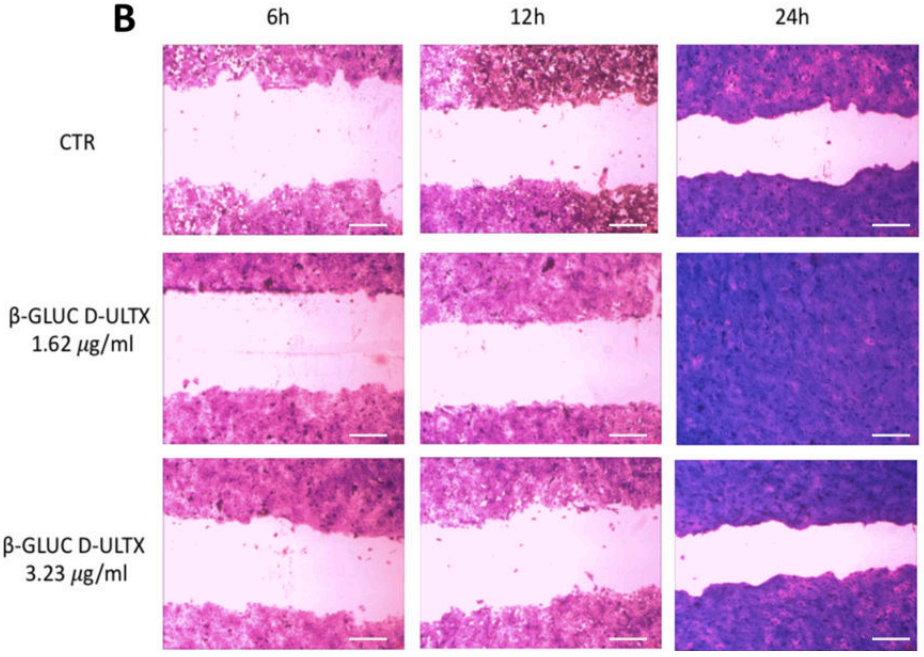


Fig. 5. (**A**) HaCaT cells viability incubated with different concentrations of the water soluble fraction of β-glucan released from film G. The control is represented by untreated cells (CTR) in DMEM and set at 100 %. The percentage of viable cells respect to the control was reported as the mean ± SD of three independent experiments. Dotted lines indicate 80 % cells viability respectively. * $p < 0.01$, ** $p < 0.001$, and *** $p < 0.0001$, treatments versus control (one-way ANOVA test). (**B**) Wound field observed at 6, 12 and 24 h for untreated cells (CTR) and for cells treated with two different concentrations the water-soluble fraction of β-glucan released from film G (1.62 µg/mL and 3.23 µg/mL), the scale bar represents 200 µm.

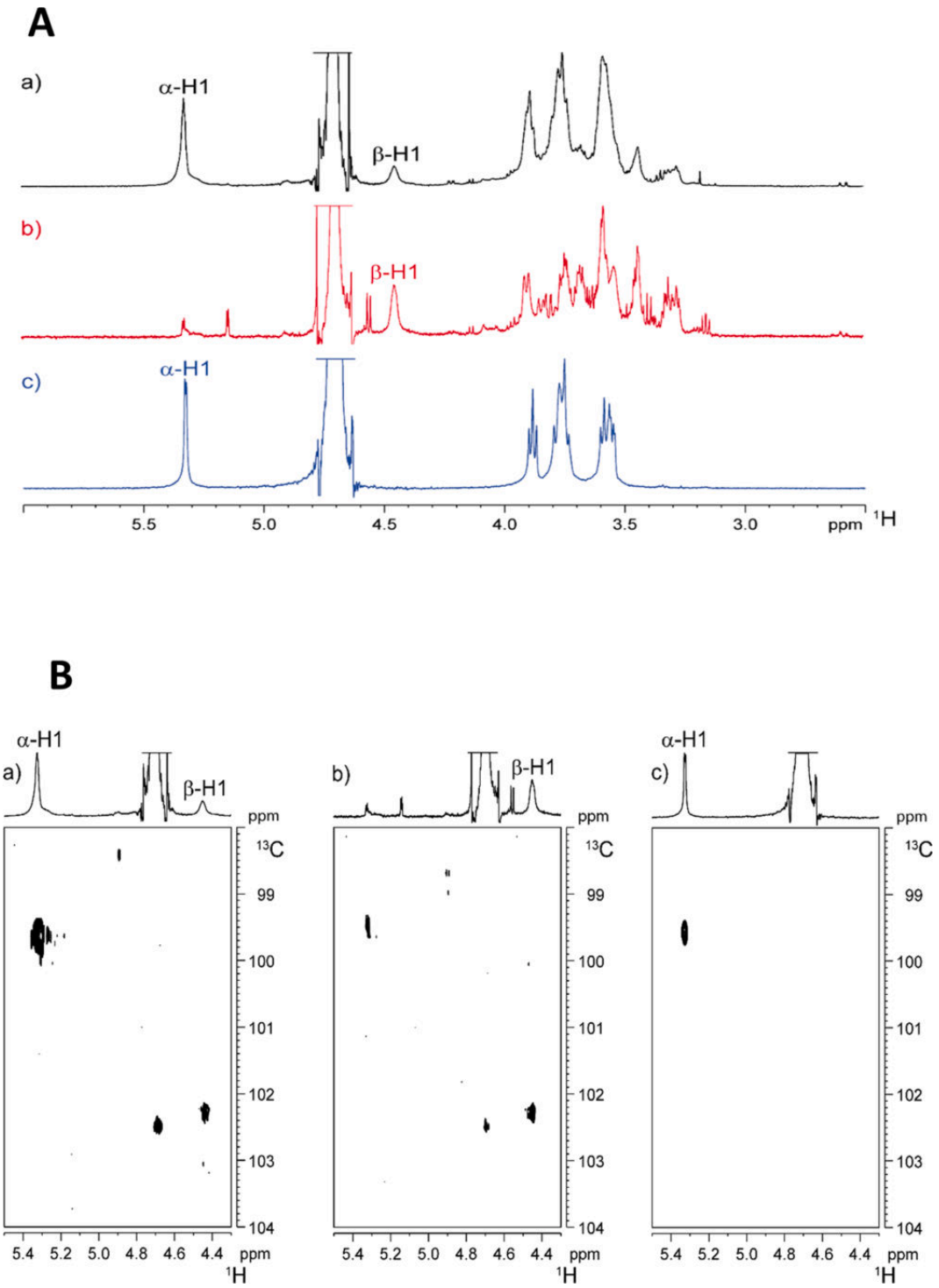


Fig. 6. (**A**) **a**: section of the ^{1}H NMR spectrum of the extract highlighting the α-H1 and β-H1 resonances (600 MHz, D_2O, 298 K); **b**: section of the ^{1}H NMR spectrum of the β-glucan reference standard highlighting the β-H1 resonance (600 MHz, D_2O, 298 K); **c**: section of the ^{1}H NMR spectrum of the corn starch highlighting the α-H1 resonance (600 MHz, D_2O, 298 K); (**B**) **a**: section of the ^{1}H, ^{13}C HSQC NMR spectrum of the extract highlighting the scalar correlation between α-H1/α-C1 and β-H1/β-C1 resonances (D_2O, 298 K); **b**: section of the ^{1}H, ^{13}C HSQC NMR spectrum of the β-glucan reference standard highlighting the scalar correlation between β-H1/β-C1 resonances (600 MHz, D_2O, 298 K); **c**: section of the ^{1}H, ^{13}C HSQC NMR spectrum of the corn starch highlighting the scalar correlation between α-H1/α-C1 resonances (600 MHz, D_2O, 298 K).

4. Conclusions

High Power Ultrasonic (HPU) is a suitable, low cost and eco-friendly technique to obtain a suspension rich in β-glucan from barley grains. By this study it was demonstrated that HPU allows to improve the extraction yield, compared to the traditional method maceration, in less time and with low energy consumption.

The suspension obtained by HPU, represented both by a water soluble and a water insoluble fraction of β-glucan, demonstrated to be a suitable natural filler, film forming agent as well as active ingredient. It was successfully employed, combined to the plasticizing agent sorbitol and to the bioadhesive polymer acacia gum, to prepare a biosustainable

film by casting method. The film, at room temperature and humidity (conditions of use) showed high elongation (29%) as well as suitable tensile (0.71 ± 0.03 MPa) and elastic (14.03 ± 1.55 MPa) properties mainly attributable to the insoluble fraction of the extract testifying a valuable mechanical resistance to solicitations. In vitro studies on keratinocytes demonstrated that the formulation is able to stimulate cells growth attributable to the water-soluble fraction working at very low concentrations (1.62 μg/mL). These results suggest the possible application for wound treatment.

The developed formulation was conceived to exploit barley extract both as excipient and as active ingredient. Future studies will be focused in the evaluation of formulation efficacy.

The film was developed in the total respect of the environment as it is obtained using only natural and sustainable materials. The processing method (HPU) require low energy consumption based on short work times and low temperatures.

It is important to highlight that the waste produced during barley HPU processing (exhausted barley) can be reused in many ways (e.g. animal feed, for bioplastic production, for energy production, ecc.). In the case of the purposed formulation, no special wastes are produced after its application. The film in fact, can be removed by simple washing with water producing a biodegradable residue. All this is a valuable contribution to pollution reduction and environment protection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Update

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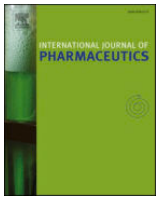

Corrigendum

Corrigendum to "Formulation and characterization of sustainable bioadhesive films for wound treatment based on barley β-glucan extract obtained using the high power ultrasonic technique" [Int. J. Pharm. 638 (2023) 122925]

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The authors regret that Fig. 5 is not correct. It should be replaced by the following:

The authors would like to apologise for any inconvenience caused.

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[1] These authors equally contributed to the study.

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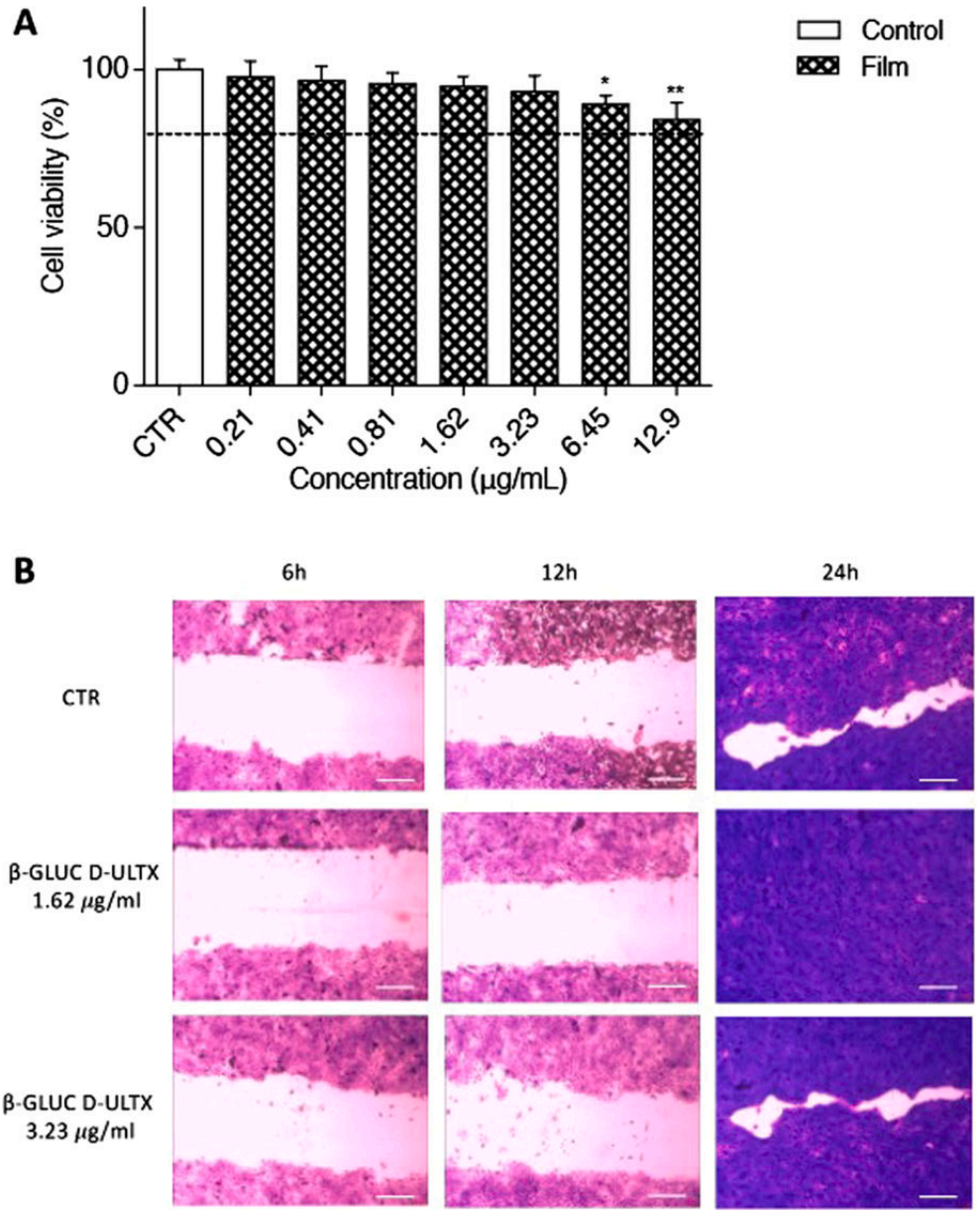

Fig. 5. (A) HaCaT cells viability incubated with different concentrations of the water soluble fraction of β-glucan released from film G. The control is represented by untreated cells (CTR) in DMEM and set at 100 %. The percentage of viable cells respect to the control was reported as the mean ± SD of three independent experiments. Dotted lines indicate 80 % cells viability respectively. *$p < 0.01$, **$p < 0.001$, and ***$p < 0.0001$, treatments versus control (one-way ANOVA test). (B) Wound field observed at 6, 12 and 24 h for untreated cells (CTR) and for cells treated with two different concentrations the water-soluble fraction of β-glucan released from film G (1.62 μg/mL and 3.23 μg/mL), the scale bar represents 200 μm.