European Polymer Journal
January 2023, Volume 183, Pages 111763 (15p.)
https://doi.org/10.1016/j.eurpolymj.2022.111763
https://archimer.ifremer.fr/doc/00811/92292/

A novel pectic polysaccharide-based hydrogel derived from okra (*Abelmoschus esculentusL. Moench*) for chronic diabetic wound healing

Maalej Hana [1, *], Maalej Amina [2], Bayach Asma [1], Zykwinska Agata [3], Colliec-Jouault Sylvia [3], Sinquin Corinne [3], Marchand Laetitia [3], Ktari Naourez [4], Bardaa Sana [5], Ben Salah Riadh [6], Chamkha Mohamed [2], Boufi Sami [7], Nasri Moncef [8]

[1] University of Gabes, Faculty of Sciences of Gabes, Laboratory of Biodiversity and Valorization of Arid Areas Bioresources (BVBAA), LR16ES36, Faculty of Sciences, Erriadh 6072, Gabes, Tunisia
[2] Environmental Bioprocesses Laboratory, Sfax Biotechnology Center, P.O. Box 1177, Sfax 3018, Tunisia
[3] Ifremer, MASAE, 44300 Nantes, France
[4] University of Gabes, Faculty of Sciences of Gabes, Department of Life Sciences, Gabes 6072, Tunisia
[5] Laboratory of Pharmacology, Faculty of Medicine of Sfax, University of Sfax, Tunisia
[6] Laboratory of Microorganisms and Biomolecules, Sfax Biotechnology Center, 3018 Sfax, Tunisia
[7] University of Sfax- LMSE-Faculty of Science-BP 802-3018, Sfax, Tunisia
[8] Laboratory of Enzyme Engineering and Microbiology, National School of Engineering of Sfax (ENIS), University of Sfax, Sfax 3038, Tunisia

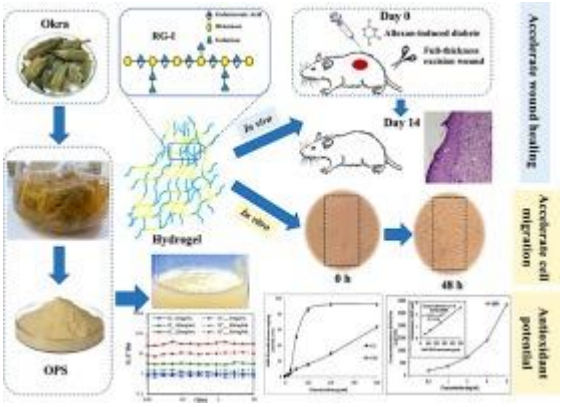
* Corresponding author : Hana Maalej, email address : hannou25@yahoo.fr

Abstract :

Hydrogels based on natural polysaccharides represent a growing group of suitable biomaterials for the elaboration of effective wound healing dressings, especially for the treatment of chronic wounds. This work was intended to prepare a polysaccharide-based hydrogel for diabetic wound healing which would help maintain the well-being of diabetes and improve their quality of life. For this purpose, a pectic polysaccharide (OPS) was extracted and purified, for the first time, from Tunisian okra pods and its physicochemical and rheological features, antioxidant and in vivo and in vitro wound healing activities were investigated. OPS, an acidic polysaccharide with a molecular weight of 3.28 × 106 Da and a polydispersity index of 1.03, was mainly composed of galactose (24.45%), galacturonic acid (24.6%) and rhamnose (18.25%). Combined with FT-IR and NMR analyses, it consisted of a pectic rhamnogalacturonan I (RG-I) structure with galactan side chains. The OPS demonstrated antioxidant potential, gelling ability, cytocompatibility properties, non-cytotoxicity and cell migration and proliferation promoting activities, which met the requirements for wound dressings. Then, the in vivo cutaneous wound healing effect of OPS-based hydrogel was investigated using an alloxan-induced diabetic rat model, and results showed that it significantly accelerated the wound healing process by acting in the acceleration of the recovery of the dermis and inducing more blood vessels formation and tissue granulation.

Overall, these results provide new insights into the development of a promising and effective okra pectin-based hydrogel for the treatment of chronic diabetic wounds.

Graphical abstract



Highlights

► RG-I pectic polysaccharide (OPS) was extracted and purified from okra pods. ► Physico-chemical features of OPS were elucidated by FTIR, GC, SEC-MALLS and NMR. ► OPS solution exhibited a gel-like behavior. ► OPS demonstrated notable antioxidant capacity and stimulated cell migration and proliferation. ► OPS hydrogel effectively accelerated wound healing in alloxan-induced diabetic rats.

Keywords : Okra polysaccharide, Pectin, Physicochemical features, rheological properties, Antioxidant potential, hydrogel, Wound healing, Scratch assay, Cell migration, Diabetic wound.

1. Introduction

Okra (*Abelmoschus esculentus L.*), also commonly known as gumbo or lady's finger, is an annual vegetable belonging to the *Malvaceae* family. Native of Africa, this flowering plant is mainly planted in tropical, subtropical and warm temperate regions of Southern Europe, Middle East, America and Asia [1]. Apart supplying common nutrient like vitamins and minerals, Okra fruit is also a rich source of natural food ingredients displaying promising nutritional, functional and biological characteristics, among which polysaccharides (PSs) (~10.38–16.89% by weight) have drawn a great deal of attentions [2-5]. Indeed, PSs derived from Okra have shown a broad spectrum of biological activities, including antioxidant, immunomodulating, analgesic, anti-hyperglycemic, anti-inflammatory, antihyperlipidemic, anti-fatigue and intestinal functions [6-10]. Among water-soluble polysaccharides, okra pods mainly contain pectin and, to a lower extent, xyloglucan and glucuronoxylan [11]. Its complex structure together with its location between cellulose microfilaments makes pectin extraction from the cell wall difficult [12]. Pectin is an acidic complex macromolecule which consists of linear and ramified regions. The linear homogalacturonan (HG) region consists of (1→4)-linked α-d-galacturonic acid (GalA*p*)-units, while the ramified region is represented by different hetero-polysaccharides, type I rhamnogalacturonan (RG-I) and type II rhamnogalacturonan (RG-II) [13]. Pectins have attracted a great attention as natural ingredients in the food industry for their thickening, gelling and stabilizing properties [14,15]. Indeed, the application of pectins in food is mainly based on their hydrocolloid properties. Their ability to form gels, the gelation mechanism and rheological properties of pectin gels depend on the structural features of these polysaccharides. Furthermore, some of them gain more and more interest as possible health promoting polysaccharides [16,17]. Recently, owing to its advantages of cytocompatibility, nontoxicity, moisturizing property and biodegradability, commercial pectin has been investigated for using in several wound healing applications, including composite wound dressing, scaffolding and

skin protection [18-20]. Natural and modified pectins demonstrated several advantages to be used for wound healing, including sufficient water uptake, acidic character of pectin macromolecules, and high affinity to cationic growth factors [18]. Such properties enhance removal of exudates, repulsion of bacteria and thus improve the healing process [21]. On the other hand, with the advances of technology, hydrogels dressings have shown great potentials for the treatment of both acute and chronic wounds [22]. Particularly, polysaccharide-based hydrogels which are made up with natural biomaterials that are biodegradable and biocompatible present unique features as wound dressings and are widely applicable in clinical practices. They share not only common characteristics of hydrogels such as excellent tissue adhesion, swelling, water absorption, etc., but also other properties, including antioxidant, antimicrobial and anti-inflammatory activities, to accelerate wound re-epithelialization, mimic skin structure and induce skin regeneration [23-26].

As far as we know, no studies were focused on pectic polysaccharide extracted from Tunisian okra and its healing activity on diabetic wounds. Indeed, diabetic ulcers which are a common type of chronic non-healing wound, are the major complication of trauma, thus affecting patients' quality of life and frequently causing the need of amputation [27]. The difficult-to-heal fact of diabetic wounds has been reported to be associated with some issues, including bacterial infection leading to uncontrollable inflammation, high oxidative stress mainly induced by excessive reactive oxygen species (ROS), impaired angiogenesis, vascular damage leading to nutrient and oxygen supply disorders to the wound site, etc. [28]. Therefore, besides strictly controlling blood glucose level, it is also very important to improve the diabetic wound local microenvironment and provide favorable conditions for wound healing. To achieve this goal, advanced research is carrying out to develop multifunctional wound dressing materials able to address simultaneously different aspects of the wound healing process. Novel wound dressings

are thus constantly being investigated. Their efficiency to achieve fast healing at reasonable cost together with minimal inconvenience to the patients is a common targeted feature [29-32]. Therefore, the development of novel wound dressing materials capable to decrease both risks of bacterial infections and oxidative stress, and accelerate the tissue regeneration process is utmost important to prevent long-term healing problems. For this purpose, in this study we purified a pectic polysaccharide from okra, assessed its physicochemical features and rheological properties, and evaluated, through *in vivo* and *in vitro* studies, its bioactivity and capacity, in form of hydrogel, for promoting wound healing in alloxan-induced diabetic rat model.

2. Material and methods

2.1. Plant material, chemicals and animals

Mature okra (*Abelmoschus esculentus L.*) pods (5–10 cm in length) were collected during the month of October 2020 from a local market of Sfax city, Tunisia. Collected samples were carefully assorted, cleaned, washed and further air-dried for 2 days before being packaged and stored at − 20° C until use. The used standards and reagents are: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide), DPPH (2,2-phenyl-1-picrylhydrazyl), trichloroacetic acid (TCA), dimethyl sulfoxide (DMSO) and Gallic Acid (GA) were purchased from Sigma-Aldrich company (St. Louis, MO, USA). Neutrase P1236 (from *Bacillus amyloliquefaciens*) and Purafect 2000^{E} proteases were bought from Sigma-Aldrich (St. Louis, MO, USA) and Genencor International (Etats-Unis), respectively. Penicillin-streptomycin, fetal bovine serum (FBS), and Dulbecco's modified eagle medium (DMEM) were purchased from Life Technologies (Gibco, Paisley, UK). All other chemicals and solvent used were of analytical grade. B16 4A5 cells (RBRC-RCB0557) showing fibroblast-like characteristics, and Human embryonic kidney cells (HEK-293) (ATCC, CRL-1573, American Type Culture Collection, Manassan, VA) used in this study were prepared as described by Maalej et al. [33,34].

For the *in vivo* experiments, alloxan (CAS Number: 2244-11-3) was purchased from Sigma-Aldrich. « *Cytol Centella cream* ® » is a synthetic oil in water emulsion drug based on a natural titrated extract of *Centella asiatica* used as a therapeutic agent in wound healing. A total of 18 male Wistar rats, weighting 250 ± 40 g, were obtained from the Faculty of Sciences of Gabes-Tunisia (FSG). All animals were housed in an environmentally-controlled room, at constant temperature (22 ± 1 °C) with a 12/12 h light/dark cycle. All rats had free access to water and standard laboratory food. Procedures and animal comfort were controlled by the International Guidelines for Animal Care.

2.2. Purification of okra polysaccharide (OPS)

2.2.1. Extraction of the crude OPS

The okra pods (including seeds) were sliced and then mixed 10 times with distilled water, and were extracted at 70 °C, three times, for 2 h each time, under the same conditions. After centrifugation (6000 rpm, 15 min) to remove the debris of water insoluble materials, the recovered supernatants were pooled and concentrated to one third of the original liquid under a vacuum at 50 °C. The concentrate was precipitated twice with absolute ethanol (4 °C, overnight), followed by centrifugation at 6000 rpm for 15 min at 4 °C. Thereafter, the precipitate was dissolved in distilled water and then was lyophilized to obtain the crude okra polysaccharide.

2.2.2. Enzymatic deproteinization

The dried crude okra polysaccharide was dissolved in distilled water (0.2%, w/v) and the dispersion was treated for 2 h, successively, using two commercial proteases, Neutrase P1236 and Purafect 2000^{E} at the same enzyme to substrate ratio (E/S) of 5000 U/g of protein [35]. The pH was adjusted to the optimal level for each enzyme with 2.5 N NaOH prior to protease addition. The enzymatic hydrolyses were conducted at 50 °C for 2 h with pH values of 7.0 and 10.0, for Neutrase and Purafect, respectively. The optimal hydrolysis conditions were:

Neutrase, 50 °C, pH 7.0; Purafect, 50 °C, pH 10.0. Reactions were terminated by heating at 100 °C for 5 min. Then, the pH was adjusted to 7.0 and the mixture was precipitated with 95% ethanol and centrifuged (6000 rpm, 15 min). The obtained precipitate was finally lyophilized and regarded as enzyme-deproteinized okra polysaccharide.

2.2.3. Cetyl trimethyl ammonium bromide (CTAB) precipitation

The deproteinized okra polysaccharide solution (0.5%) was precipitated by adding CTAB (100 mM) under continuous stirring. After incubation at room temperature for 1 h, the formed precipitate was collected by centrifugation at 6000 rpm for 15 min and then re-suspended in 100 mM NaCl solution. Then, the polysaccharide solution was precipitated by absolute ethanol (V/V), dialyzed against Milli-Q water and finally lyophilized. The obtained purified okra polysaccharide (OPS) was crushed in a pestle and mortar, weighted and stored at -20 °C until further use. The extraction yield was expressed as the % ratio of yield in grams of dried OPS to okra raw material.

2.3. Structural characterization of OPS

2.3.1. Chemical analyses

The total sugar content was determined by the phenol-sulfuric acid colorimetric method [36], using D-glucose as the standard. Total uronic acid content was estimated calorimetrically by the carbazole-sulfuric acid method [37], using D-glucuronic acid as standard. Sulfate content was determined according to the traditional method of barium chloride–gelatin [38]. The moisture and ash contents were determined according to the AOAC (Association of Official Analytical Chemists) standard procedures 930.15 and 942.05, respectively [39]. Soxhlet extraction with hexane was used to estimate the fat content (AOAC 920.85). Protein content (N × 6.25) was estimated using the Kjeldahl method [40] for the analysis of nitrogen. Elemental analyses were performed by the Central Micro-analysis Department of the CNRS at Gif/Yvette (France) for carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) contents.

125 **2.3.2. Monosaccharide components analysis by Gas Chromatography (GC)**

126 Monosaccharide content was determined by GC analysis of trimethylsilyl derivatives (TMS)
127 after acidic methanolysis [41]. GC analysis of the TMS methyl glycosides was performed on
128 an Agilent 6850 Series GC System, equipped with a HP-5MS column (30 m x 0.25 mm)
129 (Agilent Technologies, Palo Alto, CA, USA) and using He as carrier gas and a Flam Ionization
130 Detector.

131 **2.3.3. Molecular weight determination**

132 The average molecular weight of OPS was determined by high-performance size-exclusion
133 chromatography (HPSEC) coupled with a multiangle light scattering (MALLS, Dawn Heleos-
134 II, Wyatt Technology Sc) and a differential refractive index (RI) (Optilab Wyatt Technology
135 Sc) detectors. HPSEC system was composed of an HPLC system Prominence Shimadzu, a PL
136 aquagel-OH mixed, 8 μm (Agilent) guard column (U 7.5mm × L 50 mm), and a PL aquagel-
137 OH mixed (Agilent) separation column. Samples were eluted with 0.1 M ammonium acetate.
138 The molecular weight was calculated using dn/dc value of 0.145 ml/g. Elution was performed
139 at 1 ml/min with 0.1 M ammonium acetate containing 0.03% NaN_3, filtrated on 0.1 µm
140 membrane (Durapore Membrane, PVDF, Hydrophilic type VVLP, Millipore). Samples at 2
141 mg/ml were filtrated on 0.45 µm syringe filter prior to injection (100 µl). Data were computed
142 with Astra 6.1 software for absolute molar mass determinations.

143 **2.3.4. Infra-Red and NMR spectroscopic analyses**

144 The Fourier Transform Infra-Red (FTIR) spectrum of OPS was obtained using a Perkin Elmer
145 type FTIR 1000 spectrometer at room temperature and KBr pellets. The sample pellets were
146 prepared at a pressure of 5 tons for 2 min. Pellets were scanned at room temperature (25 °C) in
147 the 400-4000 cm^{-1} spectral range. Solid-state ^{13}C NMR spectroscopy was carried out using a
148 Bruker W300 spectrometer. 25 mg of sample was suspended in 1 ml D_2O at a high level of
149 deuteration (99.997 %) to avoid the presence of relatively high water. The spectrum was

registered at a temperature of 25 °C and with a frequency of 75.5 MHz, 50 ms acquisition time, 8 ms contact time and 5 s repetition time. ^{13}C chemical shifts (d, ppm) are quoted with respect to external sodium 4, 4-dimethyl-4-silapentane-1-sulfonate (0.0 ppm).

2.4. Rheological measurements

Viscosity and viscoelastic properties

OPS samples at different concentrations of 0.5, 1 and 1.5% were prepared in 0.9% NaCl and left overnight under continuous stirring to ensure complete solubilization.

The shear rheological measurements were made on DHR3 rheometer (TA Instruments). A plate-plate cell with a diameter of 25 mm was used. Its surface was rough to prevent slippage to the wall. The temperature was 25 °C maintained by Peltier heating system. Dynamic mode tests under small strains were performed by measuring firstly storage modulus G' and loss modulus G" *vs.* strain to determine the linear behavior domain. Then, frequency sweeps at a fixed strain within the linear viscoelastic domain were performed. For viscosity measurements, the effect of shear rate (0.01–10 s^{-1}) on OPS solutions viscosity was also examined.

All rheological measurements were carried out on freshly prepared samples and data were analyzed with the software that supported the rheometer.

Zeta Potential measurement

The Zeta potential was measured at 25 °C using a laser Doppler electrophoresis apparatus (Malvern Nano-Zetasizer ZS, UK). The concentration of OPS was around 0.5 mg/ml and the pH was adjusted by NaOH or HCl solution (0.001 M). The measurements were performed three times for each sample.

2.5. Bioactivities of OPS

2.5.1. Determination of antioxidant activities *in vitro*

2.5.1.1. DPPH free radical scavenging activity assay

The DPPH radical scavenging activity was carried out based on the method described by Brand-Williams et al. [42] with slight modifications. Briefly, in a 96 well plate, 10 µl of OPS solution at different concentrations (12.5-400 µg/ml) were mixed with 10 µl of DPPH ethanolic solution (124 µg/ml). Gallic acid (GA), the control (containing ethanol and DPPH solution) and the blank of each sample were prepared with the same method and under the same conditions.

All solutions obtained were then incubated for 30 min at room temperature in the dark and the absorbance (A) was recorded at 517 nm. The percentage of inhibition of samples was calculated from obtained absorbance by the equation:

$$\% \text{ Inhibition} = [(A_{control} - A_{blank\ of\ control}) - (A_{sample} - A_{blank\ of\ sample})]/(A_{control} - A_{blank\ of\ control}) \times 100$$

The equation of the obtained curve was allowed to calculate the IC_{50} corresponding to the sample concentration that reduced the initial $DPPH^{\bullet}$ absorbance of 50%.

2.5.1.2. Ferric reducing antioxidant power (FRAP) assay

The reducing power of OPS was evaluated using the ferric reducing ability of plasma (FRAP) assay according to the method described by Benzie & Strain [43] with slight modifications. Briefly, in a 96 well plate, the FRAP reagent (Fe^{3+}- TPTZ (2, 4, 6- tripyridyl triazine) complex) was mixed with OPS sample (0.5 to 8 mg/ml) or standard solutions.

Standard solutions consisted of $FeSO_4.7H_2O$ in different concentrations ranging from 0 to 0.5 mg/ml. The blank of each sample and the control were also prepared under same conditions. The standard calibration curve was then plotted using ferrous sulfate ($FeSO_4.7H_2O$) ranged from 0.062 to 0.5 mg/ml. The absorbance was read at 593 nm after 10 min incubation at 37 °C. The reducing potential of iron, expressed as mmol $EFeSO_4$/g of OPS, was calculated using the linear regression curve of ferrous sulfate standard.

2.5.2. Cytocompatibility (MTT test)

The MTT assay was performed to evaluate the cytotoxicity effect of the purified OPS on HEK-293 cells [33]. Different sample concentrations (0–1000 µg/ml) were applied to the cells (1 x

199 10^5 live cells/ml) for 48 h. The absorbance (A) was measured at 570 nm using a microplate
200 reader (Thermo Scientific Varioskan Flash). The cells treated with medium alone were
201 considered 100% viable (control). The blank of each sample and the control was also prepared
202 under same conditions. The cell viability (%) was measured as follows:

203 $$\% \text{ Cell viability} = (A_{sample} - A_{blank\ of\ sample} / A_{control} - A_{blank\ of\ control}) \times 100$$

204 **2.5.3. Wound healing potential of OPS**

205 **2.5.3.1. Scratch wound assay *in vitro***

206 B16 cells, showing fibroblast-like characteristics, were seeded in a 24-well plate at a cell
207 density of 10^5 cells/ml until completely confluent cell monolayer was obtained. Then, the cell
208 monolayer was scratched in a straight line using a sterile micropipette tip, which could mimic
209 an incision wound in the literature [44]. After that, gentle washes with PBS were used to remove
210 floating cell debris. Then, PBS was replaced with OPS solutions (0, 10 and 400 μg/ml),
211 scratched areas were photographed (t=0 h), and cells were incubated for an additional 24 and
212 48 h at 37 °C prior to acquiring images of the scratched areas. The scratch area was measured
213 using the Image-J software. Digital photographs were obtained using an inverted microscope
214 (Olympus, Japan).

215 **2.5.3.2. *In vivo* wound healing in diabetic rats' model**

216 *Preparation of OPS hydrogel*

217 The hydrogel was prepared by dissolving the lyophilized OPS, at room temperature, in a 0.9%
218 sodium chloride sterile saline solution, to give a final concentration of 5 mg/ml. The mixture
219 was kept under agitation until a hydrogel was formed.

220 *Diabetes Mellitus induction, wound creation and treatment*

221 The animals, allowed to acclimatize for one week, were intraperitoneally injected with freshly
222 prepared solution of alloxan monohydrate dissolved in physiological saline solution, at a dose
223 of about 130 mg/kg body weight. The injected rats immediately received 20% glucose solution

for 6 h to prevent fatal hypoglycemia that often follows alloxan treatment. For the next 24 h, the rats were given a 5% glucose solution as beverage. Three days post alloxan injection, the Type 1 diabetes onset was assessed via measuring, through the tail vein, blood glucose level using a digital glucometer (On Call Vivid). Rats that had blood glucose levels above 200 mg/dl were enrolled in the experiment. The animal's glycemia was assessed every three days to check the maintenance of high blood glucose levels. Fifteen days after confirmation of diabetic induction, wounds were done as previously described by Maalej et al. [45]. In brief, rats were anesthetized using intraperitoneal injection of 50 mg/kg of ketamine. A metal punch was used to demarcate an area of skin for removal. Then, full-thickness oval wounds (2×1 cm) were created on the shaved rat's dorsal interscapular region by removing a patch of skin with a pair of surgical scissors.

All animals were randomly divided into three groups each of six rats. Group I was untreated and served as the control (just cleaning the diabetic wounds with a physiological saline solution). Group II was treated with a synthetic reference drug « *Cytol Centella cream* ® » and served as a reference standard (positive control), while Group III was treated with the prepared OPS hydrogel and served as the test group.

After rinsing wounds with the physiological saline solution, the test sample (OPS hydrogel) and the reference drug (*Cytol Centella cream*) were applied, in a fine layer covering the surface of the wound, every 2 days starting from the wound induction (day 1), until the first group was completely healed (day 14). Once treated or just cleaned with the physiological saline solution, the wounds were covered with a compression dressing.

All the rats were anaesthetized with ether, sacrificed on the 14th post-wounding day and the granulation tissue was excised from the sacrificed animals. A part of wet tissue was preserved for hydroxyproline estimation and another one was fixed in formalin 10% (v/v), embedded in paraffin and processed for histological observation.

Wound healing evaluation parameters

To evaluate the healing process of the 3 studied groups, we relied on two clinical macroscopic criteria, including qualitative (color of the wound) and quantitative (wound closure rates) criteria and one microscopic criterion (histological evaluation).

Chromatic study

The chromatic evaluation of the healing process was done through the photography, every 2 days, of wounds. This study consisted of attributing a chromatic code to the wound of each rat as follows: bright red=blood covering the wound; dark red=coagulation of blood in the epidermis; red=granulation tissue and pink=epithelialization phase [46].

Wound closure rate and epithelization time

The rate of wound closure of individual animal (n=6) from control and treated groups was used as an indicator of wound healing. Progressive decrease in the wound size was monitored periodically every 2 days interval using transparent graph sheet and a marker. The shapes of the wounds were scanned and uploaded to the computer. The wound surface areas were measured using Autodesk AutoCAD 2015 software application and then converted into percent values taking the size of the wound at the time of wounding as 100%. Wound closure, which indicates the formation of new epithelial tissue to cover the wound, was expressed as reduction in percentage of the original wound size using the following expression:

$$\text{Wound closure rate (\%)} = (A_1 - A_n)/A_1 \times 100$$

where A_1 and A_n are the initial wound area (day 1) and wound area on day n, respectively.

Falling of scab (dead-tissue remnants) without any residual raw wound was taken as end point of complete epithelialization and the days required for this were taken as period of epithelization [47].

Histological analyses

Tissue specimen samples from wound site of all studied groups were explanted and fixed in 4% paraformaldehyde solution, embedded in paraffin wax, and cut and stained with hematoxylin-eosin (HE) stain [48]. The sections were observed under a light microscope regarding fibroblast proliferation, collagen formation, angiogenesis, and epithelialization.

2.6. Statistical analysis

Data from all tests are presented as the mean ± standard deviation (SD). Student's t-test was applied to ascertain significant differences between groups. Differences were considered to be statistically significant at $p < 0.05$. All analyses were carried out in triplicate.

3. Results and Discussion

3.1. Biochemical and physicochemical characterization of OPS

3.1.1. Basic composition and content determination

The water-soluble polysaccharide OPS was obtained by hot water extraction from dried Tunisian okra (*Abelmoschus esculentus*) pods, enzymatic deproteinization, ethanol and CTAB precipitation. The physicochemical parameters characterizing OPS are summarized in Table 1. Under the reported conditions, the OPS extraction yield was 26 ± 2.5% of dried material. Although the extraction yield in this study is slightly lower than the reports of Nagpal et al. [49] (31.52%) and Li et al. [50] (29.4%), it is much higher than that of the previous studies of Elkhalifa et al. [51] (2.66%), Zhang et al [52] (7.9%), Wang et al. [5] (10.35%), Kpodo et al. [53] (14.6%) and Samavati [54] (16.9%). Such variations in the percentage yield of okra polysaccharide could be due to several factors, including the physical (hydrated or dried) and maturation states of pods, the cultivation region and the extraction method used [50]. As shown in Table 1, the chemical analysis results showed that the contents of total sugar, uronic acid and protein in OPS were 70.61 ± 3.55%, 27 ± 1.5% and 6.54 ± 0.12%, respectively.

3.1.2. Monosaccharide composition and molar ratios

GC analysis of the monosaccharide composition showed that OPS is a hetero - polysaccharide and consisted of four types of monosaccharides, namely rhamnose (Rha), galactose (Gal) and galacturonic acid (GalA), followed by minor amount of glucose (Glc) (Fig. 1). According to Table 2, the Gal and GalA contents (related to dry weight) in OPS were about 24%, followed by Rha (18%), while Glc has the lowest level of 1.25%, suggesting the pectic nature of the polysaccharide extracted from the okra pods. Low Glc content indicates that the present extraction protocol results in pectin fraction with minor amounts of co-extracted cellulose, hemicelluloses or starch [45]. Similarly, polysaccharides obtained from the pods [55], flowers [56] and leaves [57] of okra plants have been reported to contain pectic polysaccharides. According to the literature, among plant cell wall polysaccharides, pectins are characterized by high versatility and complexity of their structure. Indeed, in terms of its monosaccharide profile, pectin is a complex of several polysaccharides consisting mainly of galacturonic acid and rhamnose units, as well as a variety of neutral sugars including arabinose, galactose, and lesser amounts of other sugars [58]. Furthermore, pectic polysaccharides consisted of different domains, the most important of which are HG and RG-I often described, respectively, as the "smooth" and "hairy" regions [59]. RG-I regions contain arabinan or galactan side chains depending on pectin origin. RG-II is a much less common but strongly conserved branched region, composed by the repetition of four branches containing neutral and acid residues departing from a backbone of HG [60].

Molar ratios of pectin's monosaccharides can help to reveal more important structural information through the determination of the % of key pectin regions HG and RG-I. According to Denman and Morris [61], several sugar ratios are often used as an expression for the occurrence and properties of pectin. The ratio of pectin backbone sugar GalA to the neutral sugars involved in side chains (R_1) and which is useful tool to estimate the linearity of pectin.

Ratio 2 (R_2) is defined as the proportion of Rha to GalA, thus reflecting the contribution of RG-I segments to the entire pectin population. While R_3 value is indicative of the length of galactose side chains of RG. As shown in Table 2, OPS exhibited high R_3 value (1.3) which indicates high branching of the RG-I segment. Furthermore, the RG-I molar content of OPS was found to be greater (94%), as compared to HG proportion (4.5%), thus indicating the abundance of "hairy" regions in the extracted pectin from Tunisian okra pods. As previously mentioned, the type of pectin is highly dependent on plant sources as well as extraction methods. Kpodo et al. [53], while conducting a comparative study on pectin characterization from six okra genotypes, showed that it is possible to tailor the structure of the extracted pectin by selecting the appropriate genotype, thus creating pectins with an extensive spectrum of functionalities. For the extraction of okra polysaccharides, it was demonstrated that most of the water extracted polysaccharides belong to RG-I type [6,62]. In summary, okra pods extraction resulted in an RG-I enriched OPS with short galactose-containing side chains.

3.1.3. Homogeneity and molecular weight determination

Molecular size characteristics of the isolated OPS were evaluated by size exclusion chromatography (SEC) coupled to multiangle laser light scattering and weight average (Mw), number average (Mn) molecular weights and polydispersity index (Mw/Mn) were determined. The obtained elution profile (Fig. 2) revealed narrow Mw distribution representing one polymer population of high molecular weight as indicated by the presence of one peak. The Mw and Mn values of OPS RG-I were 3.127×10^3 and 3.083×10^3 kDa, respectively. The Mw of OPS was comparable to okra RG-I polysaccharide showing a Mw larger than 2.99×10^3 kDa [10], but higher than those previously reported for pectin samples isolated from six okra genotypes which were of Mw ranging from 0.7 to 1.7×10^3 kDa [41]. Chen et al. [63] reported higher average Mw of pectin from *Abelmoschus esculentus* of 5.94×10^3 kDa. Ma et al. [64] reported, when studying the Mw of three polysaccharides extracted from okra under different conditions, that

macromolecules were mainly concentrated in the fraction extracted at 60 °C, and the polysaccharide might be degraded at higher temperature or under acidic pH extraction. The polydispersity index value of OPS was 1.03, indicating its narrow molecular distribution and uniform dispersity in an aqueous solution.

3.1.4. FTIR and ^{13}C NMR spectra of OPS

The FTIR spectrum of OPS, showing typical signals of a polysaccharide, was depicted in Fig. 3A. The broad and strong area of absorption between 3100 and 3500 cm^{-1} due to O–H stretching absorption is attributed to the vibrational modes of inter- and intramolecular hydrogen bonds [65]. The band around 2950 cm^{-1} refers to C–H absorption. The absorptions around 1601 and 1418 cm^{-1} were due to the stretching vibrations of C=O and –COOH, respectively, confirming the presence of GalA residues in the sample [63]. Two strong absorbance peaks between 1074 and 1039 cm^{-1} are characteristics of glycosidic linkages between sugar units.

The structural features of OPS were further elucidated by ^{13}C NMR spectral analysis. In a ^{13}C spectrum, the signals derived from α-anomeric carbons usually appear in the 95-101 ppm region while most of the β-anomeric carbons will appear in the range 101-105 ppm [66]. The ^{13}C NMR spectrum of OPS, displayed in Fig. 3B, showed peaks around 177.99 ppm indicative of the carbonyl group (C=O) of (GalA) [67]. The two signals at 106.78 and 101.07 ppm were assigned to the anomeric C-1 of (Gal) and (GalA) residues, respectively. Peaks in the range of 18-20 ppm can be assigned to the methyl groups of the rhamnose residue. The ^{13}C NMR spectrum of OPS was consistent with the previously reported spectra of RG-I regions extracted from six okra genotypes [53]. Moreover, such ^{13}C NMR values confirm the results obtained by the GC analysis showing the presence of some monosaccharides, such as GalA, Gal and Rha which are the principal OPS sugars.

3.2. Rheological characterization

The rheological behavior of OPS solutions was investigated by oscillatory sweep measurements at 25 °C, of the storage modulus (G′), loss modulus (G″) as a function of frequency (f) in the linear domain and the steady-state viscosity (η) vs shear rate (Fig. 4). Measurements were performed at constant pH and ionic strength to avoid any fluctuation induced by these parameters.

In Fig. 4A, in the domain of OPS concentration investigated, both G′ and G″ were nearly independent of frequency with the G' values almost larger than G'' over the applied frequency, which is consistent with a gel-like behavior and good storage stability of the OPS solution, even at low concentration (0.5%). Indeed, Mezger [68] reported that materials having highest values of G' modulus show highest stability over time.

Such gelling effect is the consequence of the properties OPS macromolecular chain to generate in aqueous solution an interconnected three-dimensional network, due to the high molecular weight of OPS (exceeding 10^6 Da) combined with its polyelectrolyte character induced by the presence of ionizable carboxylic groups [69]. At pH 7, the carboxylic groups were mostly in the ionic form (COO^-), promoting the polymer chains expansion in the coiled form and increase in their hydrodynamic volume, which favors the entanglement of the polymer chains. An increase in the magnitude of G' with the increasing concentration of OPS is observed, which is expected, since the increase in the polymer concentration will increase the number of chains junction and entanglement density. This led to a higher cohesion in the polymer network formed by coiled pectin chains thanks to the set-up of intermolecular interaction through hydrogen bonding between pectin chains. It was established that gel formation in RG-I rich in galactan side chains results from interactions between galactan chains. The 2D-correlation FTIR spectroscopy [70] together with molecular modelling [71] showed that in such gels, galactan adopts a regular helical structure and chains interact in a side-by-side antiparallel fashion.

The steady-state viscosity (η) as a function of shear rate (Fig. 4B) displayed a typical shear-thinning behavior of pectin gel which is attributed to the disentanglement and alignment of pectin along the shear direction under the low shear force, causing a gradual decrease in viscosity, which agrees with literature data [70]. Such shear-thinning (pseudo plastic) behaviour has been widely observed for many food gums [71]. At higher shear rates, the breakdown of intermolecular junctions became faster than the rate of network reformation and caused the observed decrease in apparent viscosity [60, 70].

The zeta potential of 0.05% okra pectin in aqueous solutions at different pH values is presented in Fig. 4C. In the pH range from 2.0-12.0, the zeta potential remained negative, with an increment in the absolute value as pH is going up. This indicated that OPS chains bear negative ionic groups over this pH range, and their ionic degree is more pronounced with increasing pH. This evolution is presumably due the carboxylic groups of OPS that turned more dissociated as the pH is increasing [72].

It has been reported that rheological properties of pectic polysaccharides depend on both intrinsic and extrinsic factors, including the number and distribution pattern of free carboxyl groups, the molecular weight and types of pectin, pectin concentration, ion concentration, pH, temperature and ionic strength [71]. Other factors like extraction conditions, contribution of proteins, drying method and extraction method may also influence the rheological properties of these polysaccharides [73].

3.3. Antioxidant potential *in vitro*

In an attempt to accelerate the wound healing process, many investigations have been carried out using an antioxidant strategy, as the excessive production of reactive oxygen species (ROS) promotes the imbalance between antioxidants and oxidants, thus leading to a slow tissue regeneration and healing process. Particularly, besides the impaired vessel formation, damage

from ROS are also among many factors that can hinder diabetic wounds healing, and thus results in severe complication of diabetes [74].

For wound management, biopolymers-based dressings with antioxidant properties have appealed increasing attention to minimize the damage caused by reactive chemical species, especially in the case of diabetic chronic wounds.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals are relatively stable free radicals that are often used to evaluate the antioxidant radical scavenging activity of various compounds. As illustrated in Fig. 5A, the DPPH radical scavenging activity of OPS increased gradually as the concentration increased, presenting a dose-dependent relationship. It exhibited a value of 63.5 ± 1.98 % at a concentration of 0.4 mg/ml. The IC_{50} values of OPS and gallic acid (GA) were 0.32 and 0.048 mg/ml, respectively. The results showed that OPS owned a stronger antioxidant activity than those of previously reported okra polysaccharides [5,55,64].

The FRAP (ferric reducing antioxidant power) assay takes advantage of electron-transfer reaction and is considered a common way to evaluate the antioxidant potential. The reducing power of OPS was assessed based on its ability to reduce Fe^{3+} to Fe^{2+} and the results in ferrous sulfate equivalent ($EFeSO_4$) is presented in Fig. 5B. OPS displayed a reducing potential of the ferric ion of 1.28 ± 0.018 mmol $EFeSO_4.7H_2O$/g. Chen et al. [63] revealed that okra polysaccharides extracted by using ultrasound at different frequencies exhibited FRAP values lower than 21 μmol Fe^{2+}/g sample.

According to both antioxidant assays, OPS exhibited a potent antioxidant activity *in vitro* which may be likely due to the presence of the RG-I pectic domain, as previously reported. Indeed, according to Zhang et al. [75], uronic acid content might be an important molecular feature for the antioxidant properties of polysaccharides. Li et al. [76] demonstrated that acidic polysaccharide fractions with the highest uronic acid content showed stronger free radical scavenging activities than the neutral polysaccharide fraction. Similarly, Wu et al. [77] showed

that acidic polysaccharides, which contained amounts of uronic acid, exhibited stronger antioxidant activity. Besides, Rao et al. [78] found that three types of uronic acid exhibited strong antioxidant effect in the order of polygalacturonic acid > glucuronic acid > galacturonic acid, suggesting degree of polymerization may impart the activity. According to the previous report of Ma et al. [64], the pectin of OPW-60 containing the highest branching rate and the lowest linearity and thus which may contain more hydrogen bonding sites, exhibited the strongest antioxidant capacity.

Interestingly, OPS demonstrated the potential to act as a natural antioxidant which would broaden the development and utilization of okra resources. Moreover, since antioxidants assist in controlling oxidative stress at the chronic wound site, thereby accelerating the healing process, OPS-based hydrogel could contribute to the conception of an appropriate environment for wound healing through the protection of wound tissue from oxidative damage.

3.4. Biocompatibility analysis

The safety of OPS was evaluated on normal HEK-293 cells cultivated with different concentrations of okra polysaccharide ranging from 10 to 1000 μg/ml, and the MTT assay was employed to determine cell viability. The results in Fig. 6 show that, compared to the cell viability of the control group, the co-culture with OPS for 48 h did not lead up to a remarkable decrease of the cell viability which remains higher than 91%. The results clearly illustrated that OPS possessed good cytocompatibility and non-toxicity to human normal cells at concentrations up to 1 mg/ml. It is well-established in the literature that pectins may be considered as a biocompatible and non-toxic biopolymer. According to da Costa Amaral et al. [78], two pectin fractions from gabiroba pulp exhibited a cytotoxic effect, even in low concentrations, against human glioblastoma cancer cells, while no cytotoxicity was observed in normal fibroblast cells.

3.5. Wound healing activity

In this study, the potential wound healing property of the polysaccharide (OPS) extracted from the Tunisian okra pods was investigated by analyzing its effects, *in vitro* through the cell migration assay, which play a key role in the wound healing process, and *in vivo* through the assessment its efficiency to accelerate wound healing using alloxan-induced diabetic rat model.

3.5.1. Scratch wound assay *in vitro*

The scratch assay was utilized to evaluate the *in vitro* potential of OPS in cell migration and proliferation, into the wounded area (Fig. 7). Our findings indicated that the polysaccharide stimulated cell proliferation and migration compared to the control, and thereby decreased the cell free gap (distance) in a concentration dependent manner (Fig. 7A). Indeed, cells at the wound edges remove toward the surface traversing layers and lead to the re-epithelialization of the wound surface. The highest wound closure rate was 99.09% compared to control (23.96%), after 48 h of incubation (Fig. 7B).

According to the efficiency of OPS to promote the cell proliferation, a key phenomenon in the re-epithelialization process during wound healing [79], together with its non-toxicity and antioxidant capacity we have found, it was of interest to study, *in vivo*, the healing capacity of this polysaccharide on treating chronic wounds.

3.5.2. Effect of OPS hydrogel on diabetic wound rats

Alloxan is one of the most known diabetogenic agents often used to produce a model of type I diabetes mellitus in experimental studies involving diabetes [79]. In the present study, the average blood glucose of rats suffered from alloxan injection reached above 200 mg/dl, along with polyuria and visible body weight decrease. Such symptoms indicated the successful establishment of the diabetic model. In our wound healing rat model, a single full thickness oval skin wound (about 160 mm^2) was made on the shaved dorsal interscapular region of each diabetic rat. The wound healing potential of the OPS hydrogel was evaluated on the basis of

some parameters, including the rate of wound closure, wound color, state of inflammation and epithelialization time along with the histological evaluation of the healed tissues.

The evaluation of the wound closure by monitoring the skin wound area is a parameter widely used, since it is easily accessible and handled and has clinical applicability. Fig. 8A showed the representative photos of wounds in different rats' groups at days 1, 3, 5, 10, 12 and 14. As shown, macroscopic follow-up of the hydrogel OPS-treated wound area, throughout the treatment period, did not show any signs of infection and inflammation, such as the presence of pus, secretion or a foul odor, nor a delay in the healing process. In contrast, in the untreated group (Group I), wound healing was significantly delayed because of the impaired blood circulation in diabetic rats, which still show, at the end of the experiment (day 14), an open wound with red rounding tissues.

After local application of OPS hydrogel and *Cytol Centella ® cream*, a dark red coloration was observed on the third day for both treated groups (Groups II and III) which gives evidence of the initiation of the healing process through the formation of blood clots with cellular debris. On the 5^{th} day, a healthy and brown in color wounds were observed in both treated groups due to plasma exudation with formation of superficial crusts at the site of the wound until the 10^{th} day. Nevertheless, wounds that received the reference drug *Cytol Centella ® cream* revealed a thicker, harder and darker crust around the wound area than those that received the OPS hydrogel, leading to a drier wound. It has been previously reported that crusts act as a physical barrier to protect the wound from external infections and germs. However, less dry the wound was, rapidly the epidermal cells migrate, and hence the epithelialization process can be accelerated [37,73]. On day 12, in OPS hydrogel-treated rats, the crust began to detach with growth of new skin tissue to let appear a pink blade coloration, and complete closure of the wounds was noticed on the 14^{th} day. However, all untreated animals still show, at the end of the experiment, an open wound with red rounding tissues.

519 The quantified wound areas of each group in different time points were shown in Fig. 8B. As
520 compared to the other counterparts, the application OPS hydrogel exhibited the optimal wound
521 healing activity during the whole experimental period, which is in accordance with Fig. 8A.
522 Both the reference (*Cytol Centella* ®) and OPS hydrogel-treated groups resulted in a faster
523 reduction of the wound diameter than the diabetic control group (Group I), with approximately
524 52 and 40% of wound contraction achieved on the 5^{th} day, respectively. Moreover, OPS
525 hydrogel was more efficient than the standard *Cytol Centella* ® *cream* on healing contraction
526 of diabetic rats ($p<0.05$). Indeed, 100% of wound healing was recorded at the end of experiment
527 in OPS-treated group, against 89.08 and 80.3% for *Cytol Centella*-treated and untreated diabetic
528 groups, respectively.
529 Images taken from tissue biopsies of wound sections of each group on the 14^{th} post excision
530 day, after hematoxylin-eosin (HE) staining, showed a rapid re-instatement of normal tissue
531 structures following the application of OPS hydrogel, in comparison to the reference drug-
532 treated group and the control diabetic group (Fig. 9). In wounds of diabetic animals, a delay in
533 healing was evident as epidermal morphology was irregular and incomplete with localized
534 tissue destruction (Fig. 9A). The dermis of the untreated diabetic wound revealed the presence
535 of a moderate number of inflammatory cells (yellow arrow) which represent chronic stage of
536 inflammation together with a pronounced hyperemia of capillary blood vessels (red arrows).
537 Moreover, we note the presence of a foreign body reaction (star) which may be probably the
538 result of the gauze fibers adherence to the healing tissues and then their penetration in the dermis
539 of the wound's tissue after being enclosed by newly grown tissue. The granulation tissue
540 demonstrated an aggregation of macrophages with moderate collagen fibers (blue arrows). The
541 application of the reference drug and OPS hydrogel was able to restore the healing process in
542 these diabetic rats, with better results observed following the treatment with OPS hydrogel.
543 Indeed, a fully re-epithelization with a well-structured layer of epidermis, faster keratinization,

higher collagen density and newly formed blood vessels (black arrows) occupying the dermal could be observed.

All these features demonstrated that the wound healing effect of OPS hydrogel was better than the well-known healer *Cytol Centella ® cream*, which is in accordance with the chromatic study.

Several factors have been reported to contribute in promoting the wound healing process, including the antioxidant property, mitogenic effect, moisturizing ability, etc. [74]. Antioxidant dressings have attracted widespread attention, particularly for chronic or hard-to-heal wounds treatment that often stagnate in the inflammatory phase due to of oxidative stress. Indeed, antioxidants have been demonstrated to improve the wound microenvironment by removing excessive reactive oxygen and thus reducing oxidative stress and accelerating healing [75]. Interestingly, the antioxidant character of OPS might be among the most efficient contributing factors for improved diabetic wound healing. Nonetheless, the lack of antimicrobial activity may be regarded as the main disadvantage of the application of the pectic OPS hydrogel as wound dressing. Indeed, besides oxidative stress, microbial infection is also considered among the factors delaying the wound-healing process and thus should receive careful attention in chronic wound treatment. Accordingly, antibacterial and biofilm-preventing activities in the area of wound healing is an interesting property that should be displayed by an efficient wound dressing to be a convincing candidate for the healing of chronic wounds. Bustamante-Torres et al. [80] reported that despite their versatile properties, including biodegradability, nontoxicity and biocompatibility, biopolymers usually have no antimicrobial properties by themselves, except for the chitosan [81] (Olmos & González-Benito, 2021). Therefore, antimicrobial agent incorporation into the natural polymeric matrix emerged as a possible alternative to overcome this drawback and to impart antimicrobial properties to the biocomposite, thus improving its wound healing performance [82]. Interestingly, combination of pectic OPS with other synthetic

569 or natural polymers, such as chitosan, may be explored to reduce the disadvantages of each
570 individual polymer by itself, leading to composite dressings with increased antibacterial
571 activities towards chronic wounds, in which each biopolymer can contribute to different stage
572 of wound healing.

573 Besides, the gelling ability of some wound dressings play a major role in maintaining a
574 favorable wound microenvironment. Polymers-based hydrogel dressings have demonstrated to
575 possess unique features, including their capacity to maintain the wound-bed with ideal humidity
576 [76]. This means that, thanks to their swelling property, hydrogels can absorb and retain a
577 minimum amount of fluid from a heavily exuding wound and at the same time donate moisture
578 to dry tissue thus promoting autolytic debridement. They have also been shown to provide a
579 three-dimensional network structure for cell adhesion, migration and proliferation as well as
580 transportation of cytokines, nutrients and metabolic waste, which make the wounds heal faster
581 [77]. It should also be noted that the mechanical characteristics of hydrogels are critical in
582 pharmacological and biological applications. In this study, the influence of frequency on
583 viscoelastic characteristics of pectic OPS confirms its high gelling ability at a concentration of
584 0.5%. Its shear-thinning behavior characterized by decreasing viscosity with increasing shear
585 rate is an important feature in the use of a gelling skin care product [77x]. Indeed, it has been
586 reported that products having high viscosity at low shear force show a firm, stable and well-
587 bodied products, with good standup properties, in contrast, the viscosity decrease at higher
588 shear force allowed the product when applied to be absorbed into the skin easily [77y]. In
589 addition, the zero-shear viscosity of OPS solutions, showing a material behavior at a minimum
590 stress and which is considered as an important parameter in assessing the capacity of use and
591 storage of a material [77y], was found to be higher than 10 Pa.s, suggesting that pectic OPS gel
592 might be considered strong enough to have good stability during its storage. These above-
593 mentioned rheological properties highlight that pectic OPS-based hydrogel fulfill the

mechanical properties required for a product to be suited for applications as wound healing biomaterial.

To sum up, the development of multifunctional wound healing materials with biological properties and which meet the growing consumer interest in bio-based products is a major challenge for the pharmacological and dermato-cosmetic sectors of applications. Particularly, plant-based biopolymers for efficient application as dressing to wound healing are believed to offer effective, affordable, and accessible forms of treatment. Nowadays, they are viewed as an efficient alternative for wound healing thanks to their widespread availability besides their promising medicinal value.

Finally, based on the above findings reported, we should mention that the OPS hydrogel, developed in this study, meets the basic requirements of an efficient wound dressing, such as bioavailability, biocompatibility, non-toxicity, biodegradability, hydrophilicity and easy-handling, and more importantly good mechanical characteristics, antioxidant activity, cell migration and proliferation promoting properties, moisturizing ability and histocompatibility. As compared to other hydrogels, the main advantages of OPS are that it is very inexpensive being easily extracted from an available nutritive vegetable crop, easy to produce, and is easily applied to the wound thanks to its good mechanical properties.

Interestingly, the present study can provide a useful and efficient healing biomaterial, particularly a pectic polysaccharide based-hydrogel for diabetic chronic wound management, extracted for the first time from the Tunisian okra pods.

4. Conclusions

In this study, a pectic polysaccharide was extracted and purified from okra, which was identified to contain RG-I domain. Its molecular weight was estimated to be 3.127×10^3 kDa. Antioxidant potential of OPS, especially in relation to its ability to sequester DPPH radicals and to reduce the ferric ion, was also observed. In addition, after treatment with OPS hydrogel,

in alloxan-induced diabetic rats, the wound healing process was considerably accelerated by stimulating re-epithelialization, cell migration and proliferation and blood vessels formation.

In summary, the biological activities presented by OPS highlight the potential of this okra-derived polysaccharide to be applied in pharmacological and dermato-cosmetic sectors, and suggest, particularly, its potential as a hydrogel for use in diabetic wound dressing sector whose market is continuously growing and promising. Furthermore, this study may offer a new route for utilizing Tunisian okra, an easily available nutritive vegetable crop, as a valuable source for the large-scale production of value-added biomaterials, which would certainly broaden its development and utilization.

CRediT authorship contribution statement

Hana Maalej: Conceptualization; Investigation; Methodology; Writing original draft; Data curation. **Amina Maalej**: Investigation; Methodology; Formal analysis. **Asma Bayach**: Investigation. **Agata Zykwinska**: Investigation; Data curation; Methodology; Writing-review & editing. **Sylvia Colliec-Jouault**: Supervision; Resources; Writing-review & editing. **Corinne Sinquin**: Investigation; Methodology. **Laetitia Marchand**: Investigation; Methodology. **Naourez Ktari**: Investigation. **Sana Bardaa**: Investigation. **Riadh Ben Salah**: Resources. **Mohamed Chamkha**: Supervision; Resources. **Sami Boufi**: Investigation. **Moncef Nasri**: Supervision; Resources.

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.

Acknowledgments

The present work is supported by the Tunisian Ministry of Higher Education and Scientific Research (MHESR), Tunisia.

References

[1] M.H. Romdhane, H. Chahdoura, L. Barros, M.I. Dias, R.C.G. Corrêa, P. Morales, I.C. Ferreira, Chemical composition, nutritional value, and biological evaluation of Tunisian okra pods (*Abelmoschus esculentus* L. Moench), Molecules. 25 (2020) 4739. https://doi.org/10.3390/molecules25204739

[2] L. Bai, P. Zhu, W. Wang, M. Wang, The influence of extraction pH on the chemical compositions, macromolecular characteristics, and rheological properties of polysaccharide: The case of okra polysaccharide, Food Hydrocoll. 102 (2020) 105586. https://doi.org/10.1016/j.foodhyd.2019.105586

[3] C. Wang, Y.B. Yu, T.T. Chen, Z.W. Wang, J.K. Yan, Innovative preparation, physicochemical characteristics and functional properties of bioactive polysaccharides from fresh okra (*Abelmoschus esculentus* (L.) Moench), Food Chem. 320 (2020) 126647. https://doi.org/10.1016/j.foodchem.2020.126647

[4] S. Habtemariam, The chemical and pharmacological basis of okra (*Abelmoschus esculentus* (L.) Moench) as potential therapy for type 2 diabetes, Medicinal Foods as Potential Therapies for Type-2 Diabetes and Associated Diseases, Elsevier: Amsterdam, The Netherlands, 2019, pp. 307-332.

[5] K. Wang, M. Li, X. Wen, X. Chen, Z. He, Y. Ni, Optimization of ultrasound-assisted extraction of okra (*Abelmoschus esculentus* (L.) Moench) polysaccharides based on response surface methodology and antioxidant activity, Int. J. Biol. Macromol. 114 (2018) 1056-1063. https://doi.org/10.1016/j.ijbiomac.2018.03.145

[6] K. Alba, P.T. Nguyen, V. Kontogiorgos, Sustainable polysaccharides from Malvaceae family: Structure and functionality, Food Hydrocoll. 118 (2021) 106749. https://doi.org/10.1016/j.foodhyd.2021.106749

[7] Y. Liu, Y. Ye, X. Hu, J. Wang, Structural characterization and anti-inflammatory activity of a polysaccharide from the lignified okra, Carbohydr. Polym. 265 (2021) 118081. https://doi.org/10.1016/j.carbpol.2021.118081

[8] I.F. Olawuyi, W.Y. Lee, Structural characterization, functional properties and antioxidant activities of polysaccharide extract obtained from okra leaves (*Abelmoschus esculentus*), Food Chem. 354 (2021) 129437. https://doi.org/10.1016/j.foodchem.2021.129437

[9] Q. Yuan, Y. He, P.Y. Xiang, S.P. Wang, Z.W. Cao, T. Gou, D.T. Wu, Effects of simulated saliva-gastrointestinal digestion on the physicochemical properties and bioactivities of okra polysaccharides, Carbohydr. Polym. 238 (2020) 116183. https://doi.org/10.1016/j.carbpol.2020.116183

[10] J. Liu, Y. Zhao, Q. Wu, A. John, Y. Jiang, J. Yang, B. Yang, Structure characterisation of polysaccharides in vegetable "okra" and evaluation of hypoglycemic activity, Food Chem. 242 (2018) 211-216. https://doi.org/10.1016/j.foodchem.2017.09.051

[11] N. Sengkhamparn, R. Verhoef, H.A. Schols, T. Sajjaanantakul, A.G. Voragen, Characterisation of cell wall polysaccharides from okra (*Abelmoschus esculentus* (L.) Moench), Carbohydr. Res. 344 (2009) 1824-1832. https://doi.org/10.1016/j.carres.2008.10.012

[12] A.G. Voragen, G.J. Coenen, R.P. Verhoef, H.A. Schols, Pectin, a versatile polysaccharide present in plant cell walls, Struct. Chem. 20 (2009) 263-275. https://doi.org/10.1007/s11224-009-9442-z

[13] K. Banaś, J. Harasym, Natural Gums as Oleogelators, Int. J. Mol. Sci. 22 (2021) 12977. https://doi.org/10.3390/ijms222312977

[14] G.A. Martău, M. Mihai, D.C. Vodnar, The use of chitosan, alginate, and pectin in the biomedical and food sector—biocompatibility, bioadhesiveness, and biodegradability, Polymers, 11 (2019) 1837. https://doi.org/10.3390/polym11111837

[15] F. Naqash, F.A. Masoodi, S.A. Rather, S.M. Wani, A. Gani, Emerging concepts in the nutraceutical and functional properties of pectin—A Review, Carbohydr. Polym. 168 (2017) 227-239. https://doi.org/10.1016/j.carbpol.2017.03.058

[16] O. Zaitseva, A. Khudyakov, M. Sergushkina, O. Solomina, T. Polezhaeva, Pectins as a universal medicine, Fitoterapia, 146 (2020) 104676. https://doi.org/10.1016/j.fitote.2020.104676

[17] H. Yamada, H. Kiyohara, T. Matsumoto, Recent studies on structures and intestinal immunity modulating activities of pectins and pectic polysaccharides from medicinal herbs, in: Pectins and pectinases, Wageningen Academic Publishers Wageningen, 2009, pp. 293-304.

[18] F.A.B.I.O.L.A. Munarin, M.C. Tanzi, P.A.O.L.A. Petrini, Advances in biomedical applications of pectin gels, Int. J. Biol. Macromol. 51 (2012) 681-689. https://doi.org/10.1016/j.ijbiomac.2012.07.002.

[19] M. Tummalapalli, M. Berthet, B. Verrier, B.L. Deopura, M.S. Alam, B. Gupta, Composite wound dressings of pectin and gelatin with aloe vera and curcumin as bioactive agents, Int. J. Biol. Macromol. 82 (2016) 104-113. https://doi.org/10.1016/j.ijbiomac.2015.10.087.

[20] G. Giusto, C. Vercelli, F. Comino, V. Caramello, M. Tursi, M. Gandini, A new, easy-to-make pectin-honey hydrogel enhances wound healing in rats, BMC complement. Med. Ther. 17 (2017) 1-7. https://doi.org/10.1186/s12906-017-1769-1

[21] A. Synytsya, P. Poučková, M. Zadinová, Y. Troshchynska, J. Štětina, A. Synytsya, V. Král, Hydrogels based on low-methoxyl amidated citrus pectin and flaxseed gum formulated with tripeptide glycyl-l-histidyl-l-lysine improve the healing of experimental cutting wounds in rats, Int. J. Biol. Macromol. 165 (2020) 3156-3168. https://doi.org/10.1016/j.ijbiomac.2020.09.251

[22] J. Koehler, F.P. Brandl, A.M. Goepferich, Hydrogel wound dressings for bioactive treatment of acute and chronic wounds. Eur. Polym. J. 100 (2018) 1-11. https://doi.org/10.1016/j.eurpolymj.2017.12.046

[23] A. Francesko, P. Petkova, T. Tzanov, Hydrogel dressings for advanced wound management, Curr. Med. Chem. 25 (2018) 5782–5797. https://doi.org/10.2174/0929867324666170920161246

[24] S. Cheng, H. Wang, X. Pan, C. Zhang, K. Zhang, Z. Chen, ... & X. Qi, Dendritic hydrogels with robust inherent antibacterial properties for promoting bacteria-infected wound healing, ACS Appl. Mater. Interfaces. 14 (2022) 11144-11155. https://doi.org/10.1021/acsami.1c25014

[25] X. Qi, X. Tong, S. You, R. Mao, E. Cai, W. Pan, & J. Shen, Mild hyperthermia-assisted ROS scavenging hydrogels achieve diabetic wound healing, ACS Macro. Lett. 11 (2022) 861-867. https://doi.org/10.1021/acsmacrolett.2c00290

[26] S. You, Y. Huang, R. Mao, Y. Xiang, E. Cai, Y. Chen, & X. Qi, Together is better: poly (tannic acid) nanorods functionalized polysaccharide hydrogels for diabetic wound healing, Ind. Crops. Prod. 186 (2022) 115273. https://doi.org/10.1016/j.indcrop.2022.115273

[27] Z.N. Ilmi, P.A.C. Wulandari, S.A. Husen, D. Winarni, M.A. Alamsjah, K. Awang, P. Pudjiastuti, Characterization of alginate from *Sargassum duplicatum* and the antioxidant effect of alginate–okra fruit extracts combination for wound healing on diabetic mice, Appl. Sci. 10 (2020) 6082. https://doi.org/10.3390/app10176082

[28] D. Gao, Y. Zhang, D.T. Bowers, W. Liu, M. Ma, Functional hydrogels for diabetic wound management, APL Bioeng. 5 (2021) 031503. doi: 10.1063/5.0046682

[29] E. Rezvani Ghomi, S. Khalili, S. Nouri Khorasani, R. Esmaeely Neisiany, S. Ramakrishna, Wound dressings: Current advances and future directions, J. Appl. Polym. Sci. 136 (2019) 47738. https://doi.org/10.1002/app.47738

[30] S.A. Shah, M. Sohail, S. Khan, M.U. Minhas, M. De Matas, V. Sikstone, M. Kousar, Biopolymer-based biomaterials for accelerated diabetic wound healing: A critical review, Int. J. Biol. Macromol. 139 (2019) 975-993. DOI: 10.1016/j.ijbiomac.2019.08.007

[31] Y. Liang, M. Li, Y. Yang, L. Qiao, H. Xu, B. Guo, pH/glucose dual responsive metformin release hydrogel dressings with adhesion and self-healing via dual-dynamic bonding for athletic diabetic foot wound healing, ACS Nano. 16 (2022) 3194-3207. https://doi.org/10.1021/acsnano.1c11040

[32] Z. Qian, H. Wang, Y. Bai, Y. Wang, L. Tao, Y. Wei, H. Liu, Improving chronic diabetic wound healing through an injectable and self-healing hydrogel with platelet-rich plasma release, ACS Appl. Mater. Interfaces. 12 (2020) 55659-55674. https://doi.org/10.1021/acsami.0c17142

[33] A. Maalej, Z. Bouallagui, F. Hadrich, H. Isoda, S. Sayadi, Assessment of *Olea europaea* L. fruit extracts: Phytochemical characterization and anticancer pathway investigation, Biomed. Pharmacother. 90 (2017) 179-186. https://doi.org/10.1016/j.biopha.2017.03.034

[34] A. Maalej, I. Dahmen-Ben Moussa, F. Karray, M. Chamkha, S. Sayadi, Olive oil by-product's contribution to the recovery of phenolic compounds from microalgal biomass: biochemical characterization, anti-melanogenesis potential, and neuroprotective effect, Biomass Convers. Biorefinery, (2022) 1-13. http://dx.doi.org/10.1007/s13399-022-02640-9

[35] M. Hamdi, S. Hajji, S. Affes, W. Taktak, H. Maâlej, M. Nasri, R. Nasri, Development of a controlled bioconversion process for the recovery of chitosan from blue crab (*Portunus segnis*) exoskeleton, Food Hydrocoll. 77 (2018) 534-548. https://doi.org/10.1016/j.foodhyd.2017.10.031

[36] M. Dubois, K.A. Gilles, J.K. Hamilton, P.T. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350-356. https://doi.org/10.1021/ac60111a017

[37] T. Bitter, A modified uronic acid carbazole reaction, Anal. Biochem. 4 (1962) 330-334. https://doi.org/10.1016/0003-2697(62)90095-7

[38] A.G. Lloyd, K.S. Dodgson, R.G. Price, F.A. Rose, I. Polysaccharide sulphates, Biochim. Biophys. Acta, 46 (1961) 108-115. https://doi.org/10.1016/0006-3002(61)90652-7

[39] A. AOAC, Official methods of analysis of AOAC international, 17th ed., 2000.

[40] C. Kjeldahl, A new method for the determination of nitrogen in organic matter, Z. Anal. Chem. 22 (1883) 366-382. http://dx.doi.org/10.1007/BF01338151

[41] J.P. Kamerling, G.J. Gerwig, J.F.G. Vliegenthart, J.R. Clamp, Characterization by gas-liquid chromatography-mass spectrometry and proton-magnetic-resonance spectroscopy of pertrimethylsilyl methyl glycosides obtained in the methanolysis of glycoproteins and glycopeptides, Biochem. J. 151 (1975) 491-495. https://doi.org/10.1042/bj1510491

[42] W. Brand-Williams, M.E. Cuvelier, C.L.W.T. Berset, Use of a free radical method to evaluate antioxidant activity, LWT-Food Sci. Technol. 28 (1995) 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

[43] I.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Anal. Biochem. 239 (1996) 70-76. https://doi.org/10.1006/abio.1996.0292

[44] M.J. Salierno, A.J. García, A. Del Campo, Photo-activatable surfaces for cell migration assays, Adv. Funct. Mater. 23 (2013) 5974-5980. https://doi.org/10.1002/adfm.201300902

[45] H. Maalej, D. Moalla, C. Boisset, S. Bardaa, H. Ben Ayed, Z. Sahnoun, N. Hmidet, Rhelogical, dermal wound healing and in vitro antioxidant properties of exopolysaccharide hydrogel from *Pseudomonas stutzeri* AS22, Colloids Surf. B. 123 (2014) 814-824. https://doi.org/10.1016/j.colsurfb.2014.10.017

[46] I. Teot, S. Meaume and O. Dereure, Plaies et cicatrisations au quotidien, Editions Sauramps médical, 2001, pp. 351.

[47] A. Fikru, E. Makonnen, T. Eguale, A. Debella and G. Abie Mekonnen, Evaluation of *in vivo* wound healing activity of methanol extract of *Achyranthes aspera L*., J. Ethnopharmacol. 143 (2012) 469-74. https://doi.org/10.1016/j.jep.2012.06.049

[48] J.F.A. McManus and R.W. Mowry, Staining Methods, Histologic and Histochemical, Harper 7 Raw, New York, Evanston, London, 1965.

[49] M. Nagpal, G. Aggarwal, M. Jindal, A. Baldi, U.K. Jain, R. Chandra, J. Madan, Ultrasound, microwave and Box-Behnken Design amalgamation offered superior yield of gum from *Abelmoschus esculentus*: Electrical, chemical and functional peculiarity, Comput. Electron. Agric. 145 (2018) 169-178. https://doi.org/10.1016/j.compag.2017.12.036

[50] Y. Li, X. Wang, X. Lv, X. Wang, X. Wang, J. Cui, M. Yan, Extractions and rheological properties of polysaccharide from okra pulp under mild conditions, Int. J. Biol. Macromol. 148 (2020) 510-517. https://doi.org/10.1016/j.ijbiomac.2020.01.163

[51] A.E.O. Elkhalifa, E. Al-Shammari, M. Adnan, J.C. Alcantara, K. Mehmood, N.E. Eltoum, S.A. Ashraf, Development and Characterization of Novel Biopolymer Derived from *Abelmoschus esculentus* L. Extract and Its Antidiabetic Potential, Molecules, 26 (2021) 3609. https://doi.org/10.3390/molecules26123609

[52] T. Zhang, J. Xiang, G. Zheng, R. Yan, X. Min, Preliminary characterization and anti-hyperglycemic activity of a pectic polysaccharide from okra (*Abelmoschus esculentus* (L.) Moench), J. Funct. foods, 41 (2018) 19-24. https://doi.org/10.1016/j.jff.2017.12.028

[53] F.M. Kpodo, J.K. Agbenorhevi, K. Alba, R.J. Bingham, I.N. Oduro, G.A. Morris, V. Kontogiorgos, Pectin isolation and characterization from six okra genotypes, Food Hydrocoll. 72 (2017) 323-330. https://doi.org/10.1016/j.foodhyd.2017.06.014

[54] V. Samavati, Polysaccharide extraction from *Abelmoschus esculentus*: Optimization by response surface methodology, Carbohydr. Polym. 95 (2013) 588-597. https://doi.org/10.1016/j.carbpol.2013.02.041

[55] B. Xiong, W. Zhang, Z. Wu, R. Liu, C. Yang, A. Hui, Z. Xian, Preparation, characterization, antioxidant and anti-inflammatory activities of acid-soluble pectin from okra (*Abelmoschus esculentus* L.), Int. J. Biol. Macromol. 181 (2021) 824-834. https://doi.org/10.1016/j.ijbiomac.2021.03.202

[56] W. Zhang, Q. Xiang, J. Zhao, G. Mao, W. Feng, Y. Chen, T. Zhao, Purification, structural elucidation and physicochemical properties of a polysaccharide from *Abelmoschus esculentus* L (okra) flowers, Int. J. Biol. Macromol. 155 (2020) 740-750. https://doi.org/10.1016/j.ijbiomac.2020.03.235

[57] I.F. Olawuyi, J.J. Park, D. Hahn, W.Y. Lee, Physicochemical and Functional Properties of Okra Leaf Polysaccharides Extracted at Different pHs, Chemistry, 4 (2022) 405-418. https://doi.org/10.3390/chemistry4020030

[58] A. Kaczmarska, P.M. Pieczywek, J. Cybulska, A. Zdunek, Structure and functionality of Rhamnogalacturonan I in the cell wall and in solution: A review, Carbohydr. Polym. (2021) 118909. https://doi.org/10.1016/j.carbpol.2021.118909

[59] A.E. Spadoni, S. Karboune, L. Liu, Structural Characterization of Pectic Polysaccharides in the Cell Wall of Stevens Variety Cranberry Using Highly Specific Pectin-Hydrolyzing Enzymes, Polymers, 13 (2021) 1842. https://doi.org/10.3390/polym13111842

[60] S. Vidal, T. Doco, P. Williams, P. Pellerin, W.S. York, M.A. O'Neill, P. Albersheim, Structural characterization of the pectic polysaccharide rhamnogalacturonan II: evidence for the backbone location of the aceric acid-containing oligoglycosyl side chain, Carbohydr. Res. 326 (2000) 277-294. https://doi.org/10.1016/S0008-6215(00)00036-7

[61] L.J. Denman, G.A. Morris, An experimental design approach to the chemical characterisation of pectin polysaccharides extracted from *Cucumis melo* Inodorus, Carbohydr. Polym. 117 (2015) 364-369. https://doi.org/10.3390/polysaccharides1010002

[62] N. Sengkhamparn, E.J. Bakx, R. Verhoef, H.A. Schols, T. Sajjaanantakul, A.G. Voragen, Okra pectin contains an unusual substitution of its rhamnosyl residues with acetyl and alpha-linked galactosyl groups, Carbohydr. Res. 344 (2009) 1842-1851. https://doi.org/10.1016/j.carres.2008.11.022

[63] Y. Chen, J.G. Zhang, H.J. Sun, Z.J. Wei, Pectin from *Abelmoschus esculentus*: Optimization of extraction and rheological properties, Int. J. Biol. Macromol. 70 (2014) 498-505. https://doi.org/10.1016/j.ijbiomac.2014.07.024

[64] L.Y. Ma, R. Xu, H.F. Lin, M.Y. Xie, S.P. Nie, J.Y. Yin, Structural characterization and antioxidant activities of polysaccharides from okra (*Abelmoschus esculentus* (L.) Moench) pericarp, Bioact. Carbohydr. Diet. Fibre. 26 (2021) 100277. https://doi.org/10.1016/j.bcdf.2021.100277

[65] R. Gnanasambandam, A. Proctor, Determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy, Food. Chem. 68 (2000) 327-332. https://doi.org/10.1016/S0308-8146(99)00191-0

[66] S.W. Cui, Food carbohydrates: chemistry, physical properties, and applications, CRC press, (2005).

[67] Y. Tamaki, T. Konishi, M. Fukuta, M. Tako, Isolation and structural characterisation of pectin from endocarp of *Citrus depressa*, Food. Chem. 107 (2008) 352-361. https://doi.org/10.1016/j.foodchem.2007.08.027

[68] T.G. Mezger, in T.G. Mezger (Ed.), The rheology handbook for users of rotational and oscillatory rheometers, Coatings Compedia, 2006, p. 80-88.

[69] C.R. Vithanage, M.J. Grimson, P.R. Wills, P. Harrison, B.G. Smith, Rheological and structural properties of high-methoxyl esterified, low-methoxyl esterified and low-methoxyl amidated pectin gels, J. Texture stud. 41(2010) 899-927. https://doi.org/10.1111/j.1745-4603.2010.00261.x

[70] H. Zhang, J. Chen, J. Li, C. Wei, X. Ye, J. Shi, S. Chen, Pectin from citrus canning wastewater as potential fat replacer in ice cream, Molecules, 23 (2018) 925.

[71] S.Y. Chan, W.S. Choo, D.J. Young, X.J. Loh, Pectin as a rheology modifier: Origin, structure, commercial production and rheology. Carbohydr. Polym. 161 (2017) 118-139. https://doi.org/10.1016/j.carbpol.2016.12.033

[72] B. Bindereif, H.P. Karbstein, K. Zahn, U.S. van der Schaaf, Effect of conformation of sugar beet pectin on the interfacial and emulsifying properties, Foods, 11 (2022) 214.

[73] B.E. Morales-Contreras, W. Rosas-Flores, J.C. Contreras-Esquivel, L. Wicker, J. Morales-Castro, Pectin from Husk Tomato (*Physalis ixocarpa* Brot.): Rheological behavior at different extraction conditions, Carbohydr. Polym. 179 (2018) 282-289. https://doi.org/10.1016/j.carbpol.2017.09.097

[74] A. Zhang, Y. Liu, D. Qin, M. Sun, T. Wang, X. Chen, Research status of self-healing hydrogel for wound management: A review, Int. J. Biol. Macromol. 164 (2020) 2108-2123. https://doi.org/10.1016/j.ijbiomac.2020.08.109

[75] T. Zhang, H. Liu, X. Bai, P. Liu, Y. Yang, J. Huang, X. Min, Fractionation and antioxidant activities of the water-soluble polysaccharides from *Lonicera japonica* Thunb, Int. J. Biol. Macromol. 151 (2020) 1058-1066. https://doi.org/10.1016/j.ijbiomac.2019.10.147

[76] J. Li, Y. Liu, L. Fan, L. Ai, L. Shan, Antioxidant activities of polysaccharides from the fruiting bodies of *Zizyphus Jujuba* cv. Jinsixiaozao, Carbohydr. Polym. 84 (2011) 390-394. https://doi.org/10.1016/j.carbpol.2010.11.051

[77] H. Wu, T. Min, X. Li, L. Li, F. Lai, Y. Tang, X. Yang, Physicochemical properties and antioxidant activities of acidic polysaccharides from wampee seeds, Int. J. Biol. Macromol. 59 (2013) 90-95. https://doi.org/10.1016/j.ijbiomac.2013.04.020

[78] da Costa Amaral, S., Barbieri, S. F., Ruthes, A. C., Bark, J. M., Winnischofer, S. M. B., & Silveira, J. L. M. (2019). Cytotoxic effect of crude and purified pectins from Campomanesia xanthocarpa Berg on human glioblastoma cells. *Carbohydrate polymers*, *224*, 115140.

[79] R.S.P. Rao, G. Muralikrishna, Water soluble feruloyl arabinoxylans from rice and ragi: Changes upon malting and their consequence on antioxidant activity, Phytochem. 67 (2006) 91-99. https://doi.org/10.1016/j.phytochem.2005.09.036

[80] G. Stojkov, Z. Niyazov, F. Picchioni, R.K. Bose, Relationship between structure and rheology of hydrogels for various applications, Gels. 7 (2021) 255. https://doi.org/10.3390/gels7040255

[81] A. ROȘU, S. Bistriceanu, C. IBĂNESCU, O.M. Daraba, M. Lungu, Rheological research of some polysaccharide gels loaded with Nigella Sativa extracts, Cellul. Chem. Technol. 47 (2013) 359-367.

Table 1: Yield and chemical components of OPS

	OPS
Yield (g/100 g dry okra powder)	26 ± 2.5
Moisture (%)	7.03 ± 0.04
Protein (Kjeldahl) (%)	6.54 ± 0.12
Fat (%)	3.33 ± 0.09
Carbohydrates (%)	70.61 ± 3.55
Sulfates (%)	1.51 ± 0.1
Uronic acid (%)	27 ± 1.5
Ash (%)	8.98 ± 0.03
% C	34.92 ± 1.54
% H	5.72 ± 0.12
% N	1.06 ± 0.01
% S	0.35 ± 0.05

The % is related to dry weight. Data are expressed as means ± standard deviation (SD; n = 3).

Table 2: Monosaccharide composition of OPS

	OPS
Rhamnose[a]	18.25% ± 0.35 (28.46)
Arabinose[a]	ND[b]
Galactose[a]	24.45% ± 0.49 (37.25)
Glucose[a]	1.25% ± 0.07 (1.30)
Galacturonic acid[a]	24.6% ± 0.28 (32.99)
Glucuronic acid[a]	ND[b]
R_1[c]	0.5
R_2[c]	0.9
R_3[c]	1.3
R4[c]	1.3
HG[c]	4.5%
RG-I[c]	94%
HG/RG-I[c]	0.04

The % is related to dry weight
[a] Each value was expressed the mean ± standard deviation (n = 3).
In parentheses, data is presented as mol% of each sample.
[b] ND: not detectable or lower than the limit of quantification.
[c] According to reference (Denman and Morris, 2015),
R1 = GalA/(Rha + Ara + Gal), R2 = Rha/GalA, R3 = Gal/Rha,
HG = GalA – Rha, RG-I = 2Rha + Ara + Gal.

Figure Captions

Figure 1: GC analysis of monosaccharide components of okra polysaccharide.

Figure 2: HPSEC-MALS elution pattern of OPS.

Figure 3: Infrared spectrometry **(A)** and ^{13}C NMR analysis **(B)** of OPS.

Figure 4: (A) Storage modulus (G′) and loss modulus (G″) *vs.* frequency at different concentrations, **(B)** viscosity vs shear rate in presence of NaCl (0.9%), and **(C)** Zeta-potential vs pH of OPS solution.

Figure 5: Antioxidant potential of OPS: (**A)**: Scavenging effect on DPPH radicals; (**B)**: Ferric reducing antioxidant power (FRAP).

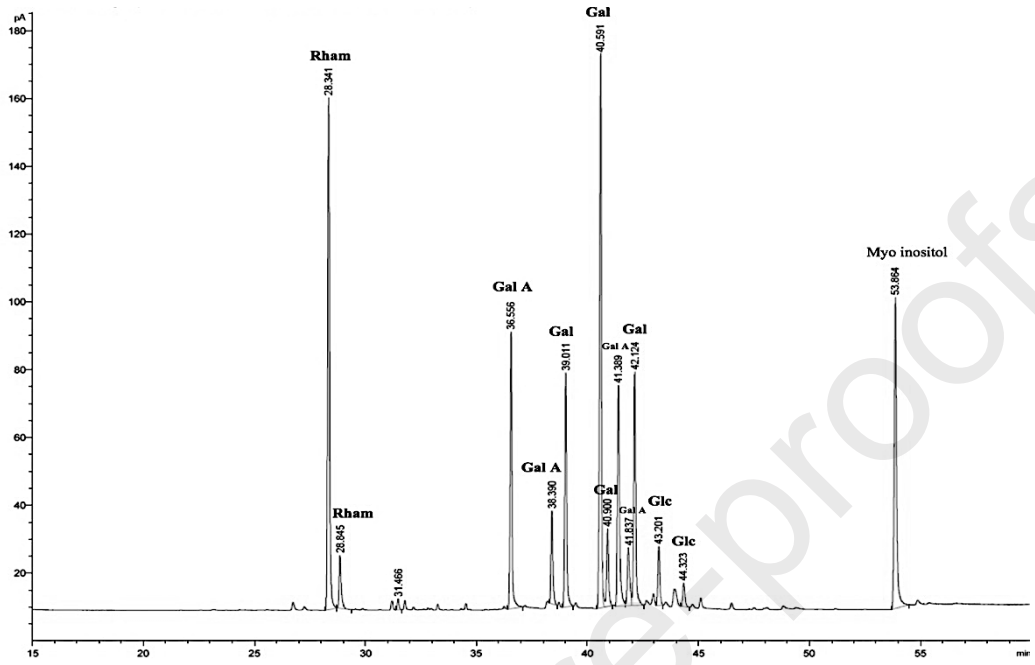
Figure 6: Effects of OPS on HEK-293 cells viability: **(A):** MTT analysis; **(B):** cellular density after 48 h incubation without (i) or with 1 mg/ml OPS (ii).

Figure 7: Scratch assay using B16 cells **(A)** and wound closure expressed as a percentage of wound size relative to the size of the initial wound **(B).**

Figure 8: (A) Representative images of full-thickness skin defects and **(B)** the wound healing rate (%) for different group treatment on wounds at days 1, 3, 5, 10, 12 and 14.

Figure 9: Histological HE staining analysis of wounded skin tissue sections in the diabetic untreated **(A)**, *Cytol Centella*-treated **(B)** and OPS hydrogel-treated **(C)** groups on the 14^{th} day post-wounding (100× magnification).

Fig.1



Rham: rhamnose; GalA: galacturonic acid; Gal: galactose; Glc: glucose

Fig.2

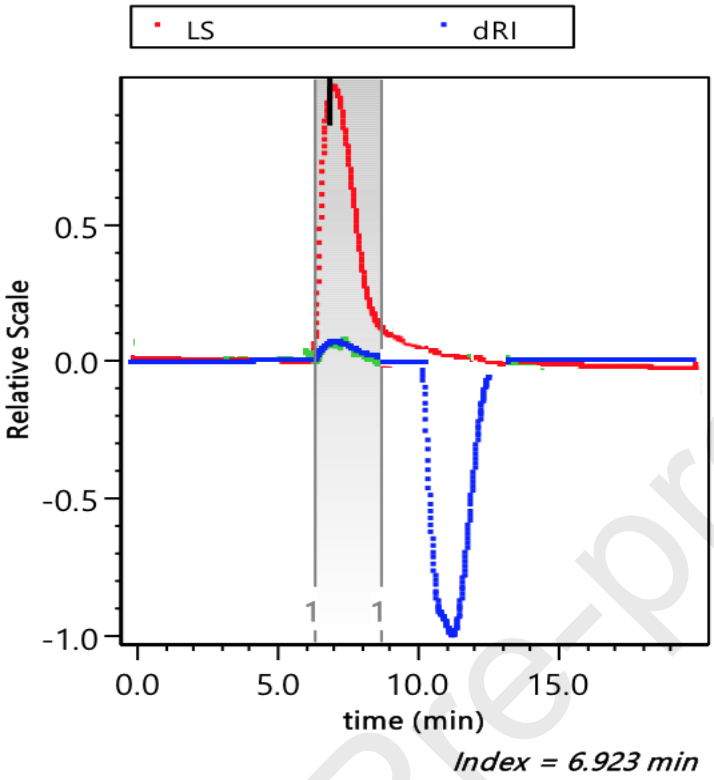
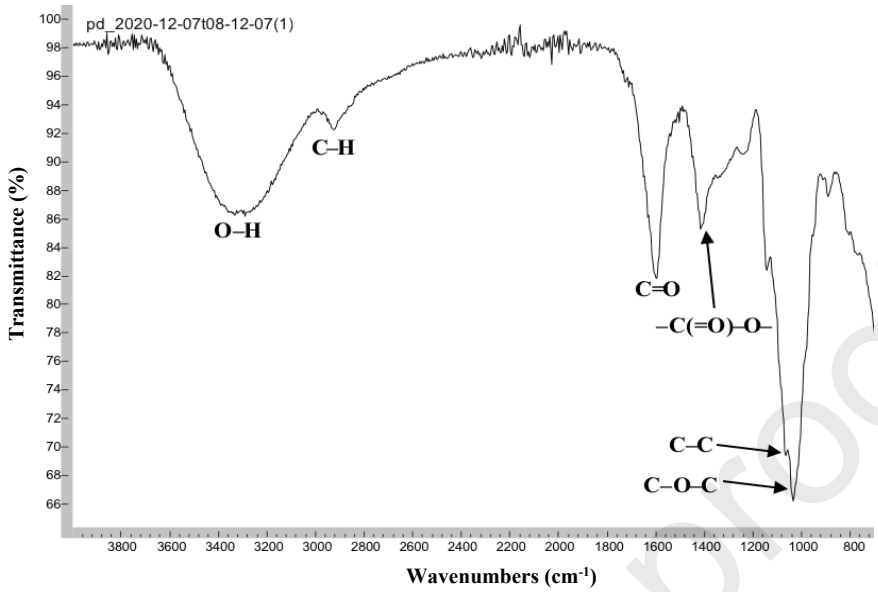
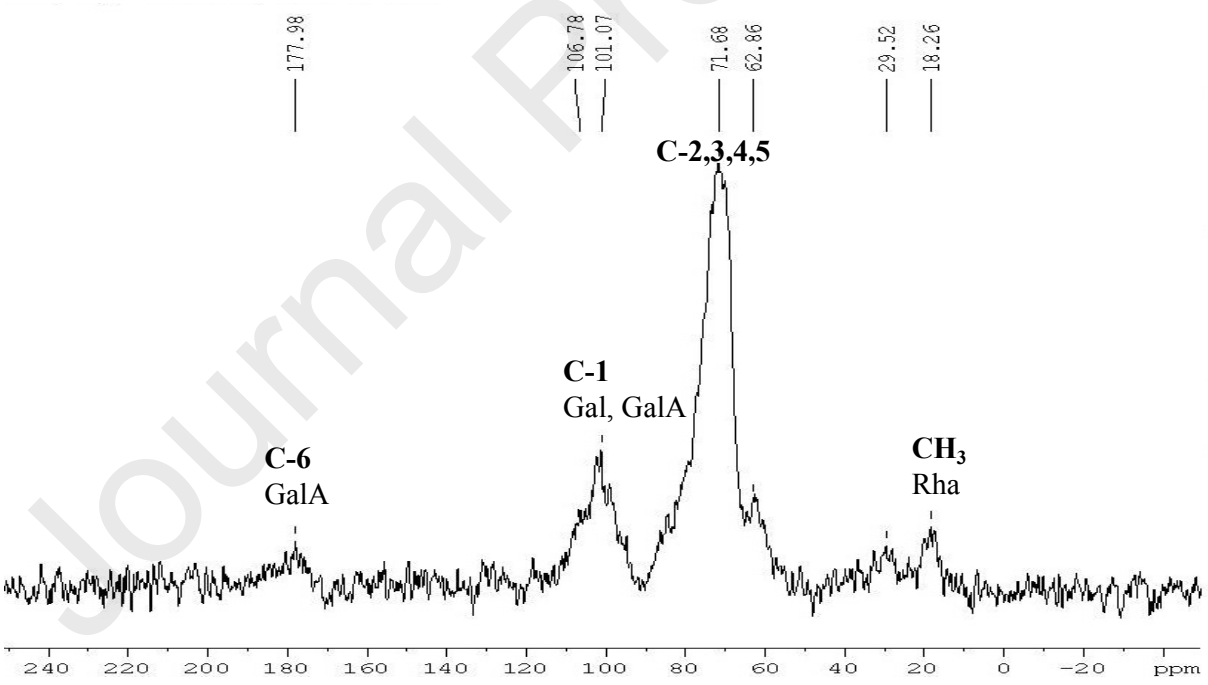


Fig.3

A

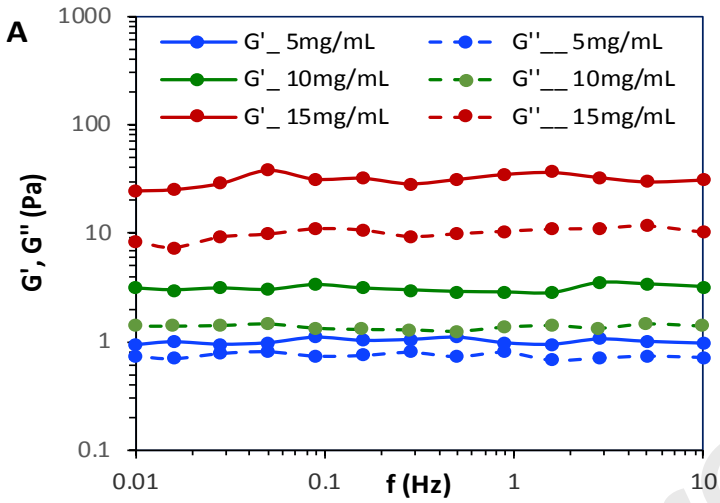


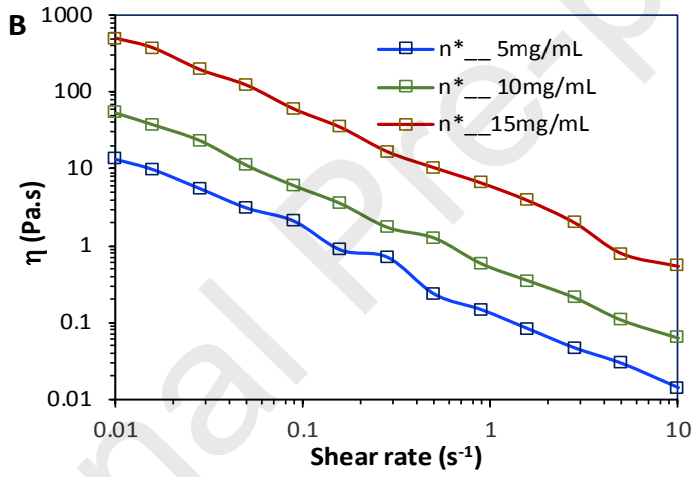
B



GalA: galacturonic acid; Gal: galactose; Rha: rhamnose

Fig.4





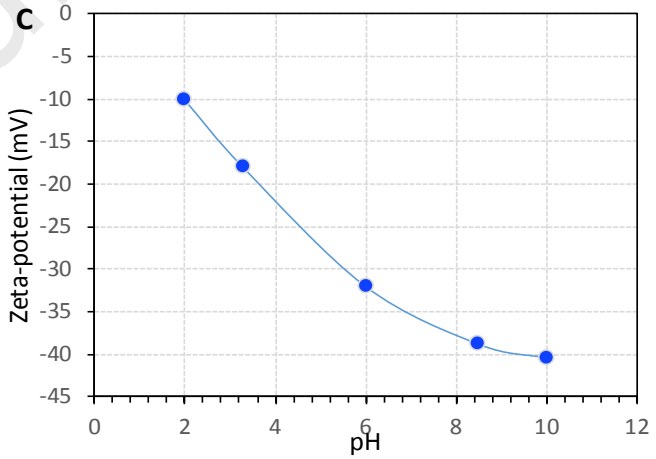
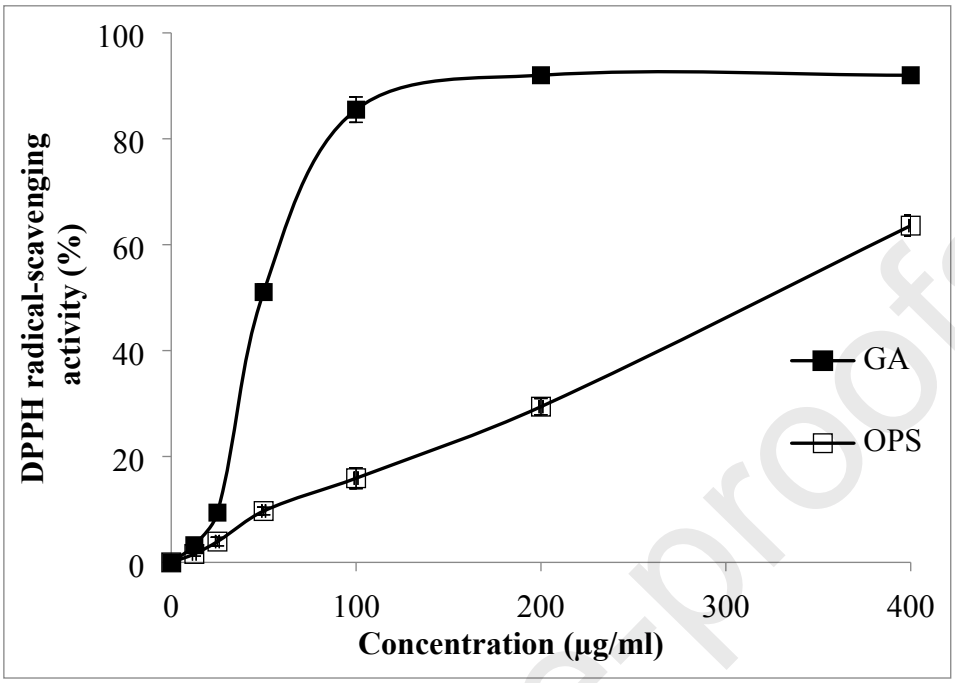
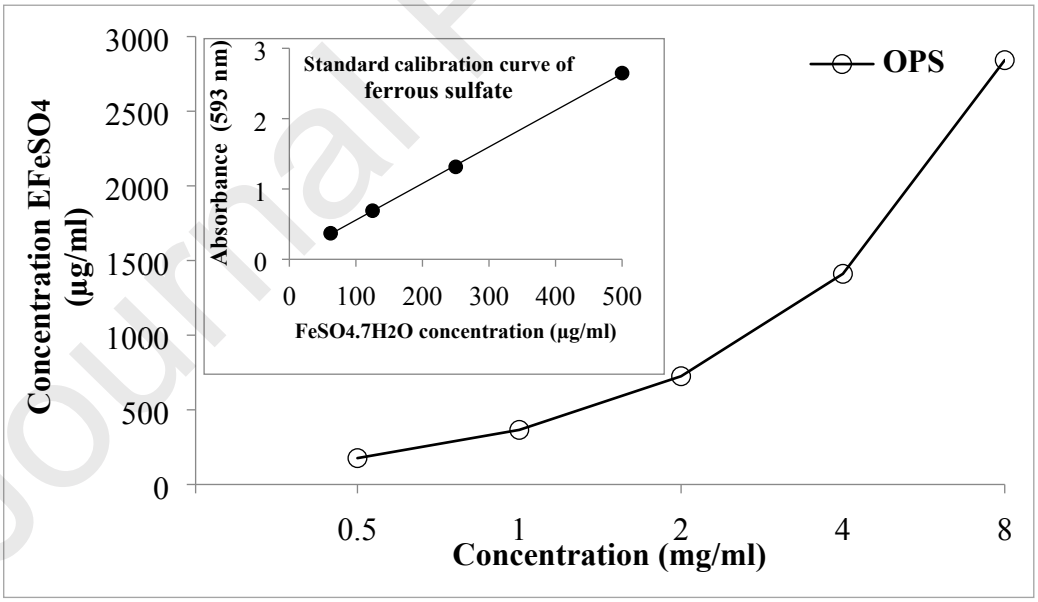


Fig.5

A



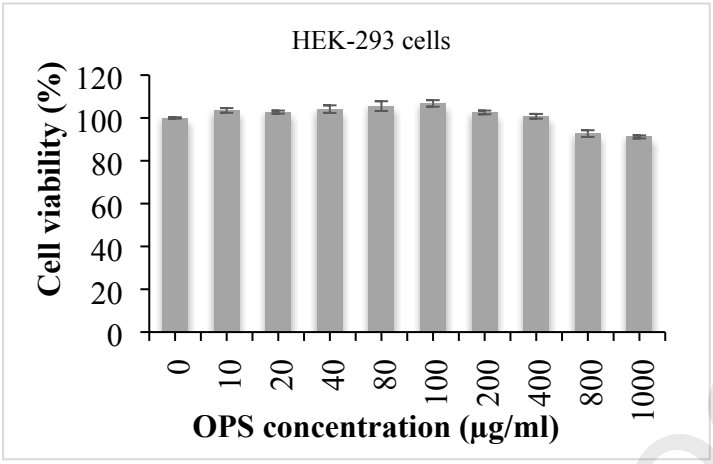
B



Gallic acid (GA) was used as positive control; $EFeSO_4$: Equivalent $FeSO_4$
Values are means ± SD (n = 3)

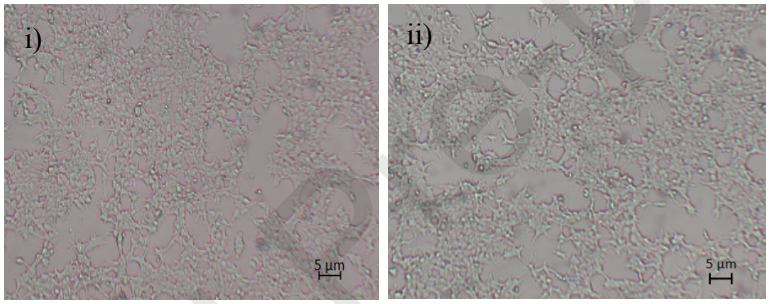
Fig.6

A



B

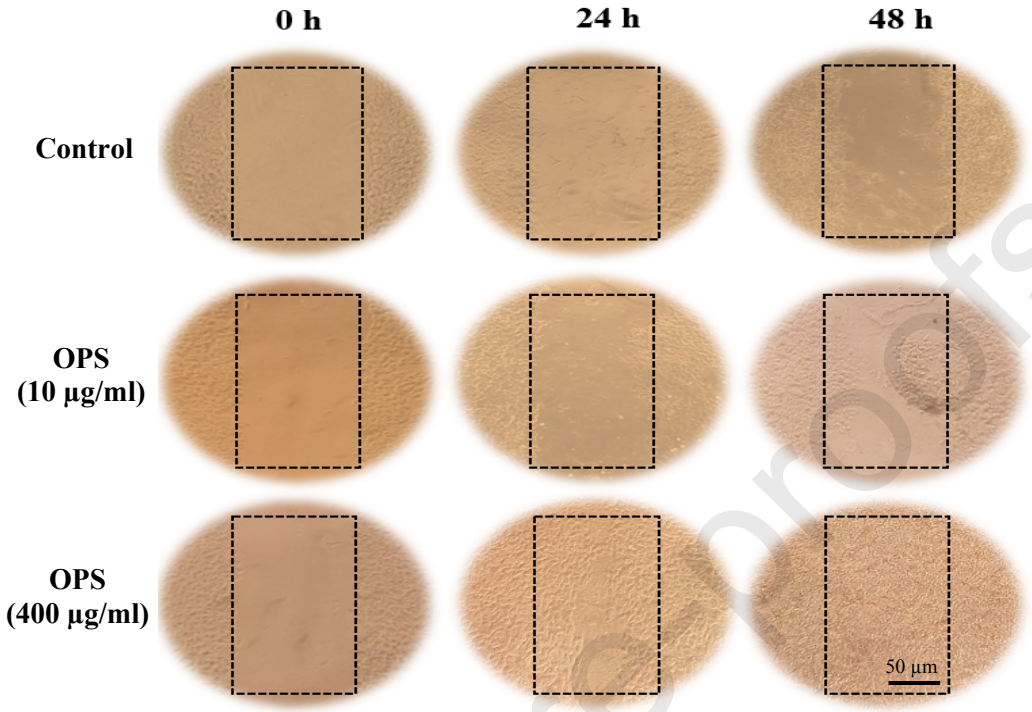
Cellular density



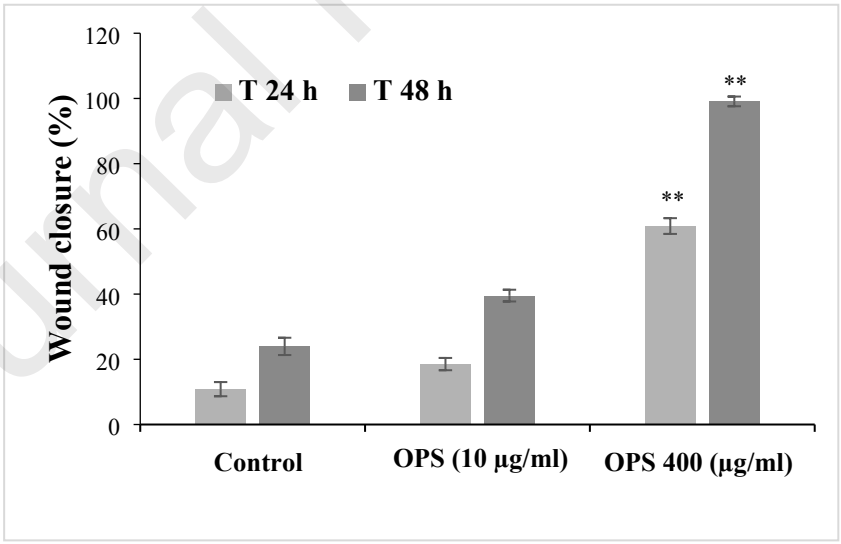
HEK293 cells (1 x 10^5 live cells/ml) were cultured in different concentrations of OPS (0–1000 µg/ml) for 48 h. The cells treated with medium alone were considered 100% viable (control). Cell morphology was observed under inverted phase contrast microscope (magnification 40x).

Fig.7

A



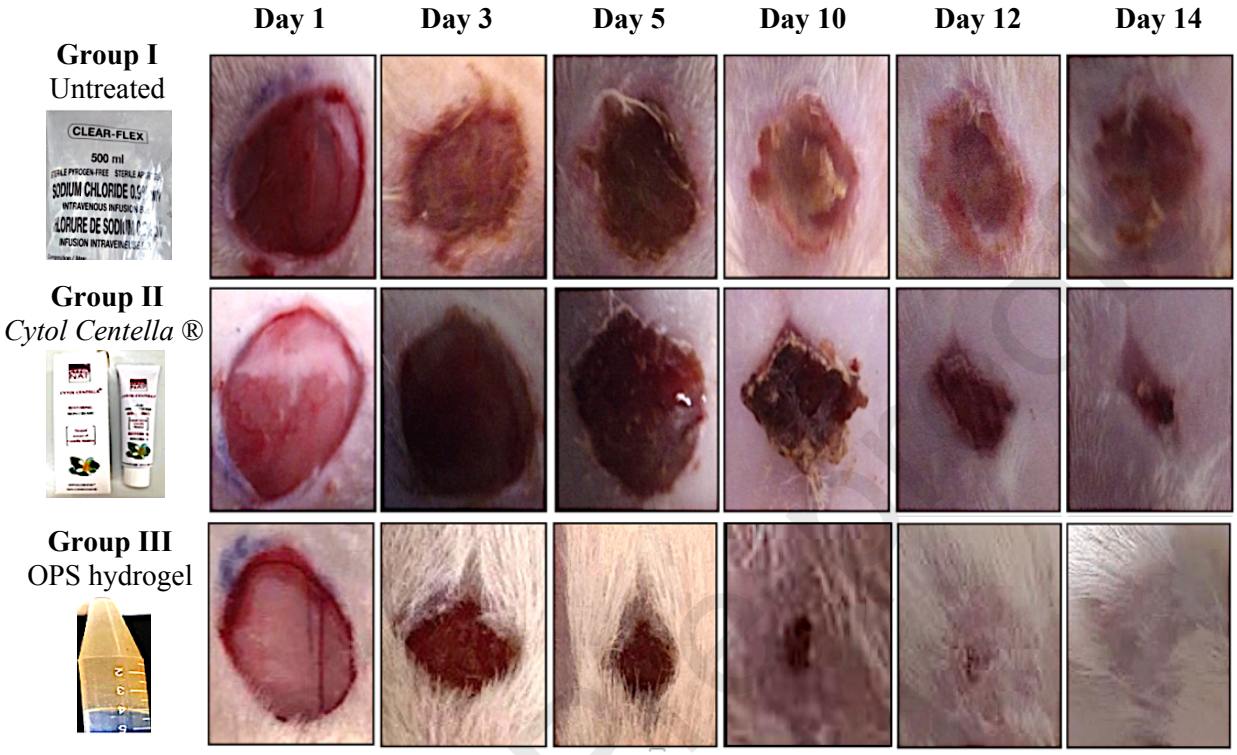
B



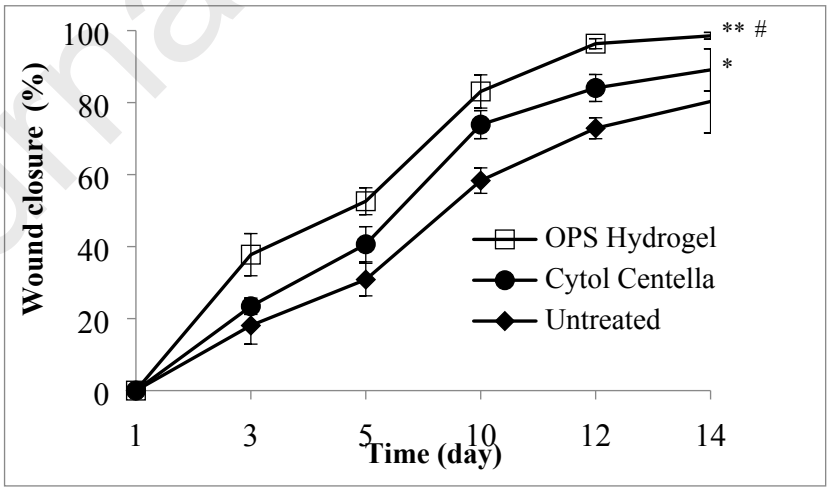
Cells were treated with 10 and 400 µg/ml of OPS for 24 and 48 h and untreated cells were used as control. Percentage of wound closure was measured and presented on a histogram using Image-J software. $^{*}P < 0.05$ and $^{**}P < 0.005$ vs untreated cells (Control). Percentage of wound healing was measured and presented on a histogram using Image-J software.

Fig.8

A

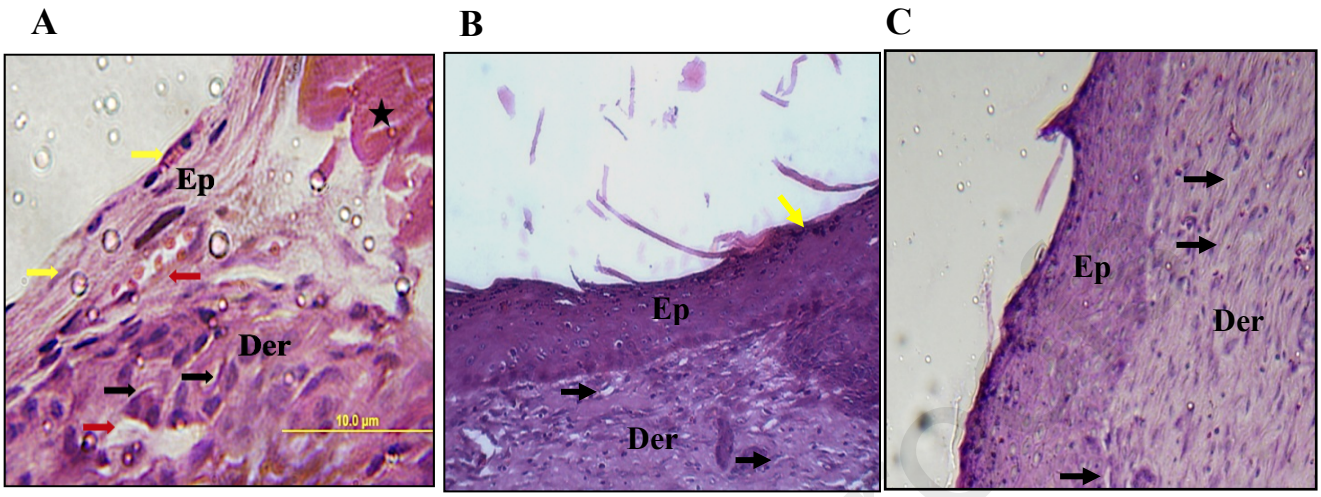


B



The photographs of the wounds are representative of six rats in each group;
The data represent the mean of six rats.
*P < 0.05 and **P < 0.005 vs untreated diabetic group (Group I)
#P < 0.05 vs the reference group (Group II)

Fig.9



A, B and C correspond to untreated, *Cytol Centella*-treated and OPS hydrogel-treated groups;

Ep: epidermis ; Der: dermis ; ➞ : inflammatory cells ; ➞ : blood vessels ; ➞ : vessel's congestion ; ★ : inflammatory cells

Conflict of Interest

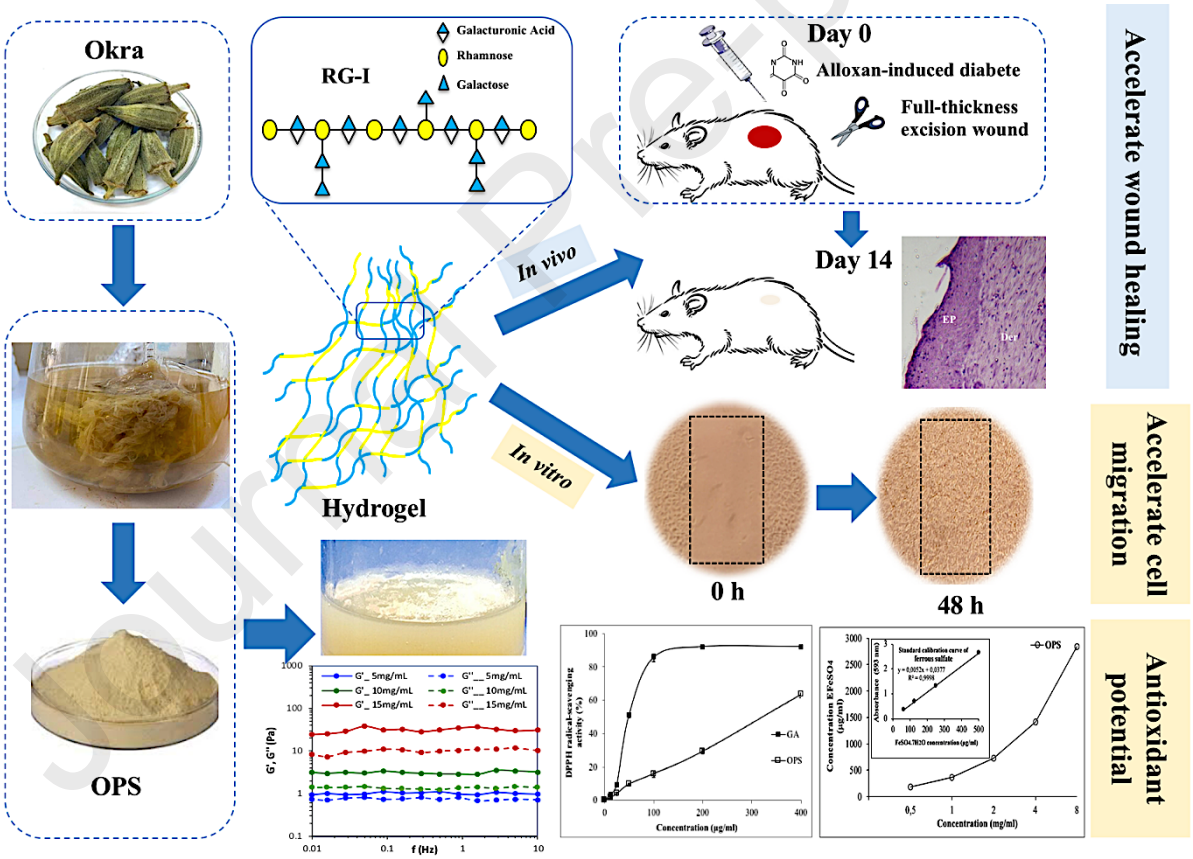
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical Abstract



Highlights

•RG-I pectic polysaccharide (OPS) was extracted and purified from okra pods.

•Physico-chemical features of OPS were elucidated by FTIR, GC, SEC-MALLS and NMR.

• OPS solution exhibited a gel-like behavior.

•OPS demonstrated notable antioxidant capacity and stimulated cell migration and proliferation.

•OPS hydrogel effectively accelerated wound healing in alloxan-induced diabetic rats.