Investigating Potential Wound Healing Properties of Polysaccharides Extracted from *Grewia mollis* Juss. and *Hoheria populnea* A. Cunn. (Malvaceae)

N. Pearman[1], S. R. Moxon[1,2,✉], S. M. Carnachan[3], M. E. Cooke[4], E. I. Nep[1,5], I. M. Sims[3], G. A. Morris[1], and A. M. Smith[1]

[1]*School of Applied Sciences, University of Huddersfield, Queensgate, Huddersfield HD1 3DH, UK*

[2]*School of Biological Sciences, University of Manchester, Oxford Road, Manchester, M13 9PT, UK*

[3]*The Ferrier Research Institute, Victoria University of Wellington, 69 Gracefield Road, Lower Hutt 5040, New Zealand*

[4]*School of Chemical Engineering, University of Birmingham, Edgbaston, B15 2TT, UK*

[5]*Faculty of Pharmaceutical Science, University of Jos, Jos, Nigeria*

✉Corresponding author

Tel: +44 (0) 161 306 0502

Email: samuel.moxon@manchester.ac.uk

Abstract

The *Malvaceae* family is a group of flowering plants that include approximately 244 genera, and 4225 species. *Grewia mollis*, and *Hoheria populnea* (lacebark), are examples of the *Malvaceae* family that are used in traditional medicine. For this study polysaccharide samples were extracted from the inner bark of *Grewia mollis* (unmodified (GG) and destarched grewia gum (GGDS)) and from the leaves of *Hoheria populnea* (lacebark polysaccharide (LB)). Wound healing properties of grewia gum and lacebark polysaccharides were investigated using 3T3 fibroblast cells cultured in supplemented DMEM. Deposition of collagen using van Gieson's stain, expression of the COL1A1 gene which encodes type I collagen using quantitative PCR, and chemotaxis using a scratch plate assay were analysed following treatment of cells with the test polysaccharides.

Quantitative PCR results indicated that all three polysaccharides increased the levels of COL1A1 mRNA, with GG showing the greatest fold change. Histological staining also indicated that the fibroblasts treated with GG deposited more collagen than control cells. Additionally, scratch assay data indicated that simulated cell 'wounds' treated with each polysaccharide showed increased wound closure rate over a 36 hour period post treatment, with GG exhibiting the greatest effect on wound closure. Analysis of the *Malvaceae* derived polysaccharides indicates that they could have a positive effect on mechanisms that are integral to wound healing, potentially providing greater scientific understanding behind their use in traditional medicine.

Keywords

Wound healing; fibroblasts; bioactive polysaccharides;

Introduction

An interest in traditional medicine as a potential source of new drug targets has been revived in recent years. Colloquial knowledge of the medicinal benefits of certain plant species exists; however many of the proposed medicinal properties have been over emphasized and lack scientific data to support the claims (Paterson, 2008). One major concern is that studies have been conducted using crude or poorly characterised materials rather than highly purified, well-defined components such as polysaccharides (Paterson, 2008). The Malvaceae comprise a diverse group of flowering plants that include approximately 244 genera, and 4225 species (Christenhusz, 2017); examples include *Grewia mollis* Juss. (grewia) being native to sub Saharan Africa, and *Hoheria populnea* A. Cunn. (lacebark) native to New Zealand, which are both used in traditional medicine (Al-Youssef et al., 2012, Burkill, 1997, Collier, 1941). It is reported that species belonging to the genus *Grewia* have been used historically to treat a multitude of health issues. More specifically, it has been reported that native populations in sub Saharan Africa utilised Grewia extracts either via ingestion or topical application to treat insect bites and stings, snake bites, diarrhoea, gonorrhoea, menorrhagia, and wounds (Aziz et al., 2018, Maroyi, 2011, Abubakar et al., 2007, Molander et al., 2015, Louppe et al., 2008). Additionally, mucilage from the leaves and bark of lacebark trees has been used traditionally by populations indigenous to New Zealand (Māori and Tuhoe people) as a topical treatment for wounds, burns and ulcers or taken internally to treat inflammation of the digestive and respiratory tracts (Sims et al., 2018, Collier, 1941, Brooker, 1987, The Herb Fedaration of New Zealand, 2016).

The structural and physical properties of polysaccharides extracted from the inner bark of *Grewia mollis* (grewia gum; GG), and from the leaves of *Hoheria populnea* (lacebark mucilage; LB) have been previously characterised (Fig. S1, Nep et al., 2016, Sims et al., 2018). GG has been shown to swell in aqueous conditions forming a highly viscous solution that is thermally stable below 200 °C (Nep et al., 2016). In its native form, GG contains approximately 12% (by weight) starch (Table 1) which is thought to contribute to the reported swelling properties. GG can be further processed to remove starch (destarched grewia gum; GGDS); analysis of unmodified grewia gum (GG), and destarched grewia gum (GGDS) indicates that, together with the loss of starch component and therefore glucose in GGDS, there is also an increase in the degree of *O*-acetylation from ~ 38% to 49% (Nep et al., 2016).

The non-starch polysaccharide component of GG is a pectic polysaccharide-like structure comprising a rhamnogalacturonan I (RG-I) type backbone of repeating →4]-α-D-Gal*p*A-[1→2]-α-L-Rha*p*-[1→ units with terminal β-D-Glc*p*A branches at *O*-3 of the Gal*p*A residues (Sims et al., 2017). Analysis of LB indicates a more structurally complex polysaccharide comprising a similar RG-I type backbone but with additional branching at *O*-4 of the Rha*p* residues to terminal Gal*p*A residues or oligosaccharides of 4-Gal*p* residues containing a terminal Gal*p*A residue (**Table 1**). It is not currently known whether LB comprises a single polymer with several structurally discrete domains or possibly comprises several structurally related polymers (Sims et al., 2018). Evidence from the structural analysis of LB indicates an additional polysaccharide or portion of the polysaccharide comprising a backbone of 2-Rha*p* residues, some of which are likely branched at *O*-3. The LB polysaccharide contains lower levels of *O*-acetylation (10%) than either GG or GGDS (Nep et al., 2016, Sims et al., 2018).

Table 1: Physico-chemical properties of the mucilage from lacebark leaves (LB), Grewia gum (GG) and destarched Grewia gum (GGDS); adapted from (Sims et al., 2018, Nep et al., 2016). Important differences are highlighted in italics.

	Lacebark leaf mucilage (LB)	**Grewia gum (GG)**	**Destarched Grewia gum (GGDS)**
Total Carbohydrate (weight %)	*74.7*	48.1	48.4
Starch (weight %)	-	*11.8*	-
Protein (weight %)	2.6	2.3	5.2
Ash (weight %)	12.1	10.2	8.0
Moisture (loss on drying) (weight %)	13.5	11.8	11.0
Arabinose (weight %)	0.2	0.5	0.2
Fucose (weight %)	0.3	-	-
Galactose (weight %)	*11.4*	0.2	0.2
Galacturonic acid (weight %)	*26.0*	16.3	17.7
Glucose (weight %)	-	*6.4*	2.1
Glucuronic acid (weight %)	14.5	12.1	13.9
Rhamnose (weight %)	*22.3*	12.3	14.2
Xylose (weight %)	-	0.3	0.1
Degree of *O*-acetylation (weight %)	*10*	38	49
Weight-average molar mass (g/mol)	2.31×10^6	nd	1.80×10^6
Radius of gyration (nm)	90	nd	81
Intrinsic viscosity (ml/g) in 0.1 M NaCl	nd	278	253

In this study, LB, GG and GGDS were evaluated as potential wound healing agents by investigating their impact on early healing mechanisms associated with cell migration and collagen synthesis in 3T3 fibroblasts. During healing, fibroblasts migrate from surrounding

tissue into the wound site after the period of inflammation (**Fig. 1**). Initially, fibroblasts produce matrix metalloproteinases to degrade any fibrin clots (Bainbridge, 2013). This is followed by synthesis of numerous extracellular matrix (ECM) polysaccharides and proteins, including type I collagen, thus instigating formation of new tissue (Fronza et al., 2009). This is a critical process in a mechanism called fibroplasia; whereby new fibrous tissue is generated by fibroblasts in the wound bed. During fibroplasia a change in fibroblast behaviour occurs with a shift away from proliferation towards a migratory phenotype. The phenotypic change results in a significant influx of fibroblasts into the wound bed and deposition of newly synthesised extracellular matrix (Velnar et al., 2009, McDougall et al., 2006, Witte and Barbul, 1997).

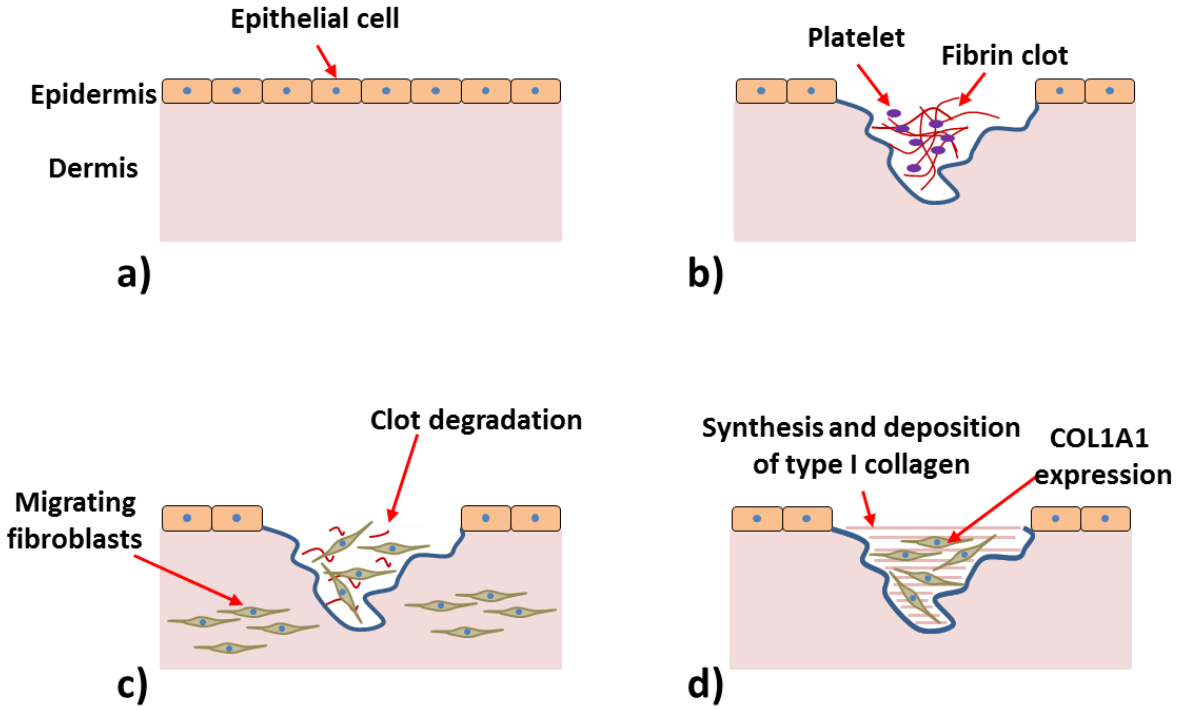


Figure 1: A simplified schematic demonstrating a) healthy, unwounded skin, b) wound formation and synthesis of a fibrin clot by platelets, c) migration of fibroblasts into wound site and degradation of fibrin clot, d) up-regulation of COL1A1 by fibroblasts resulting in synthesis and deposition of type I collagen in early formation of new tissue.

Collagens make up the majority of proteins in mammals (~30%) (Ricard-Blum, 2011) and contribute 70-80% of the dry weight of skin (Oikarinen, 1994); type I and III collagens are the most abundant. In relation to wound healing, COL1A1, a gene involved in type I collagen synthesis, is expressed in fibroblasts in the vicinity of the wound (deep dermal layer) from 16-24 hours after the damage has taken place (Scharffetter et al., 1989). The level of collagen I expression increases in fibroblasts, contributing to the granulation tissues over the next 6 days,

at which point the majority of migratory cells are expressing the gene. After 8 days the cells directly beneath the nascent epidermis express COL1A1 whereas the cells in the lower layers of the granulation tissue show very little expression. After 26 days there is very little expression of COL1A1 by any cells within the wound site (Scharffetter et al., 1989).

In the present study, 3T3 fibroblasts were cultured in media comprising the relevant polysaccharide dissolved in supplemented Dubecco's Modified Eagle Media (DMEM). The 3T3 cell line is commonly applied to models of early wound healing because they exhibit chemotaxis in response to simulated wounds, and can synthesise and deposit collagen acting as a platform for modelling fibroplasia *in vitro* (Fronza et al., 2009, Lipton et al., 1971, Peterkofsky, 1972). Reverse transcription PCR of COL1A1, histological staining of type I collagen deposits and scratch wound assays were conducted to investigate whether treatment with GG, GGDS and LB had an impact on early wound healing mechanisms. Polysaccharide extracts from the Malvaceae family have been used historically in wound healing with reports of positive results (Rawat et al., 2012). This study investigates if three (GG, GGDS and LB) structurally similar polysaccharides extracted from the Malvaceae family can elicit a response on key mechanisms of fibroplasia when subjected to *in vitro* wound healing assays.

1. Materials and methods

1.1. Materials

Mouse 3T3 fibroblasts (p12) were used in this study (NIH 3t3 cells (LGC, Middlesex, UK). RNEasy and cDNA synthesis kits were purchased from Qiagen (UK) and Bio-Rad (UK) respectively. RT-PCR primers were purchased from Primer Design (UK). Unless stated, all other reagents were purchased from Sigma (UK) and used without further purification. Polysaccharides were extracted and purified as described previously (Nep et al., 2016, Sims et al., 2018).

1.2. Preparation of sample media

Prior to preparation of sample media GG, GGDS and LB were extracted and purified as per Nep et al., 2016 and Sims et al., 2018 (Fig. 2)

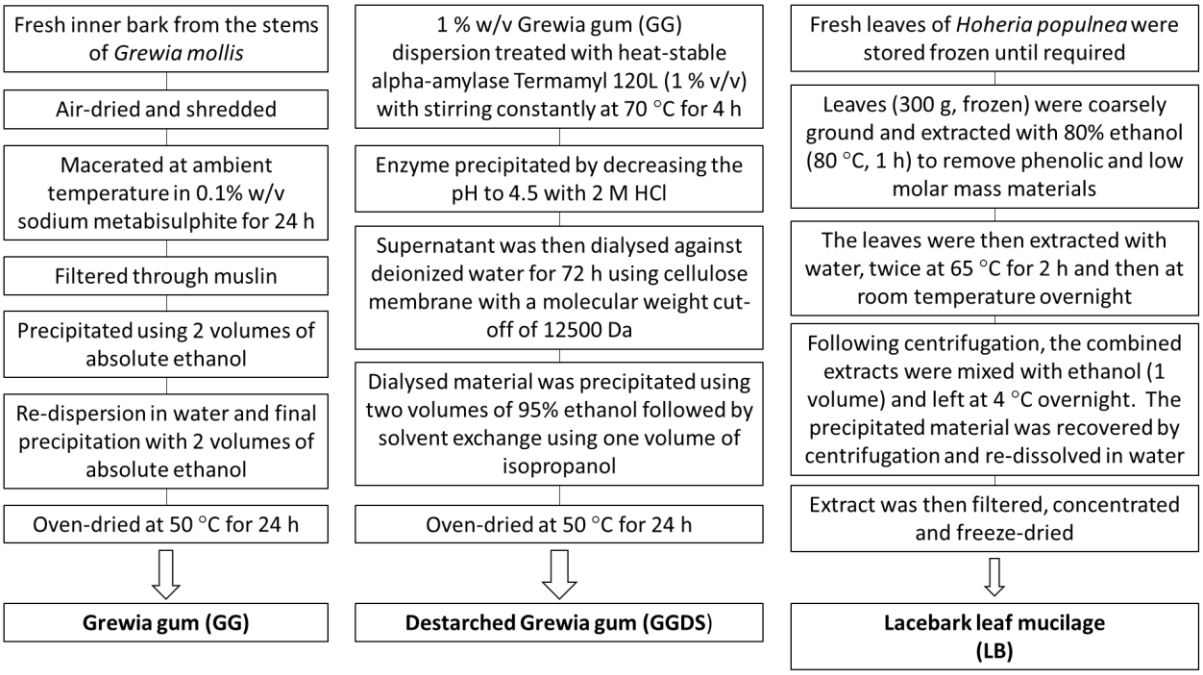


Figure 2 - Isolation and purification protocols for GG, GGDS and LB; for full details see (Nep et al., 2016, Sims et al., 2018)

After extraction and purification, sample media was prepared with 0.5 % (w/v) polysaccharide (GG, GGDS or LB) in supplemented DMEM (200 mM L-glutamine (5%), FBS (10%), HEPES (5%), PenStrep (2.5%) and amphotericin B (12.5 µg/mL)). At this concentration, all 3 polysaccharides formed a non-viscous solution allowing for extrusion through cell culture pipettes. Additionally, GG, GGDS and LB exhibited a minimal insoluble fraction at 0.5% (w/v). Prior to dissolution, the polysaccharide extracts were treated with ultra-violet (UV) light for 60 minutes before being added to the supplemented DMEM and then mixed overnight on a magnetic stirrer (20 °C, 1300 rpm). The media was centrifuged (3.0 rcf, 10 min) to remove any insoluble material (~10 %) and the supernatant, containing the soluble material, was then removed and stored at 4 ºC prior to use.

1.3. Histological Staining

3T3 fibroblasts cultured in DMEM supplemented with 200 mM L-glutamine (5%), FBS (10%), HEPES (5%), and PenStrep (2.5%) were seeded into 12 well plates at 1.25 x 10^5 cells/well. After 20 hours the media was replaced with supplemented DMEM containing 0.5% GG, GGDS, LB, and control (supplemented DMEM only); amphotericin B (12.5 µg/mL) was added to the sample media as an anti-fungal agent. After 24 and 48 h cultures were removed from the incubator (37°C, 5% CO_2) for histological staining.

The cells were fixed for 30 min using 125 µl of 10% formalin at room temperature. The media was removed and the cells were washed with PBS before staining with van Gieson's stain (0.05% acid fuchsin in saturated picric acid) to detect type I collagen deposition. Cells were incubated at room temperature for 30 minutes before removal of excess staining solution. Cultures were then washed with absolute ethanol to remove any non-specific staining.

1.4. *Scratch Wound Assay*

3T3 fibroblasts were cultured to confluency in 6 well plates. A simulated wound was introduced by using a sterile pipette tip to physically dissociate a population of cells from the centre of the well. The cells were then washed with PBS, and sample media added (containing 0.5% GG, GGDS, LB, and a control (supplemented DMEM only). The sample media contained 10 µg/ml mitomycin C to prevent proliferation and promote fibroblast migration into the wound site creating an *in vitro* model of fibroblast migration in fibroplasia. Closure of the wound was tracked by photographing the plates at set time points (0, 8, 24 and 36 h), and analysing the images using Image J (National Institutes of Health, Bethesda, USA). Two analyses were conducted from cell images. Wound closure rate was determined by calculating the total wound area recovered by fibroblasts and extrapolating it as a function of time using Equation 1, where 'a' represents wound area at 0 h and 'b' represents wound area at 36 h. Additionally, percentage closure was calculated by comparing the area of the original wound (0 h) to the wound area at each specific time point (8, 24 and 36 h) using Equation 2, where 'a' represents wound area at 0 h and 'b' represents wound area at 8, 24 or 36 h.

$$\textit{wound closure rate } (\mu m^2/h) = (a-b) \div 36 \qquad \textbf{Equation 1}$$

$$\textit{\% wound closure} = [1 - (b \div a)] \times 100 \qquad \textbf{Equation 2}$$

1.5. Quantitative PCR

3T3 fibroblasts in supplemented DMEM were seeded into 6 well plates at 2.5 x 10^5 cells/well. After 20 hours the media was replaced with supplemented DMEM containing 0.5% GG, GGDS, LB, and control (supplemented DMEM only). At set time points (4, 8, 24, 48, and 72 h) RNA was extracted from the cultures using RNeasy kit, and reverse transcribed into cDNA using iScript™ cDNA synthesis kit as per the manufacturer's instructions. The resulting cDNA

was used for qPCR analysis to assess the level of expression of type I collagen (COL1A1) using precision OneStepPLUS SYBR Green Dye with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping gene. Primer sequences are shown in **Table 2**. Gene expression was quantified using the Pfaffl method (Pfaffl, 2001).

Table 2: Forward and reverse primers

Gene	Forward Primer	Reverse Primer
Collagen Type 1	CTGTTCTGTTCCTTGTGTAACTGTGTT	GCCCCGGTGACACATCAA

1.6. Statistical Methods

Data was analysed using a one-tailed t-test with equal variances assumed. All statistical analyses were performed on data in triplicate using Microsoft Excel and a p-value < 0.05 considered significant. Data plotted represent mean values with error bars indicating standard deviation. Where relevant, p-values obtained in t-tests are included in the data to highlight significant differences.

2. Results and Discussion

In this study, potential wound healing properties of the polysaccharides extracted from *Grewia mollis* Juss. and *Hoheria populnea* A. Cunn. were investigated by analysing their effects on COL1A1 transcription, collagen deposition, and cell migration in 3T3 fibroblasts, which play a key role in multiple wound healing processes *in vivo*. Approximately 24-48 hours post-injury fibroblasts migrate into the wound site and degrade clotted fibrin via matrix metalloproteases (Li and Wang, 2011). Fibroblasts then synthesise key ECM components such as type I collagen in order to replace degraded clots with new ECM (Bainbridge, 2013).

2.1. Histological Staining

Histology using van Gieson's stain was conducted to qualitatively assess the levels of collagen deposited by 3T3 fibroblasts following treatment with each polysaccharide. Van Gieson's stain dyes collagen red and can be utilised to specifically stain for collagen deposits (Majima et al., 2000). Bright field images of stained cultures indicated that fibroblasts treated with GG deposited more collagen than control fibroblasts, with dense bundles of collagen apparent at 48 h in the GG-treated cultures (**Fig. 3**), Similar deposition of collagen was observed in a

wound healing study using type-2 diabetic rats treated with a crude methanolic extract, containing polysaccharides, from the Malvaceae plant, *Sida cordifolia* Linn (Pawar et al., 2016) and is also consistent with *in vivo* results using *Hibiscus rosa-sinensis* Linn where extracts have been reported to positively influence mechanisms such as fibroblast migration and collagen synthesis at a wound site (Shivananda Nayak et al., 2007, Bhaskar and Nithya, 2012, Mondal et al., 2016). Conversely, staining of cells treated with GGDS and LB indicated that the levels of collagen deposition were similar to that of the control at both 24 and 48 h post-treatment.

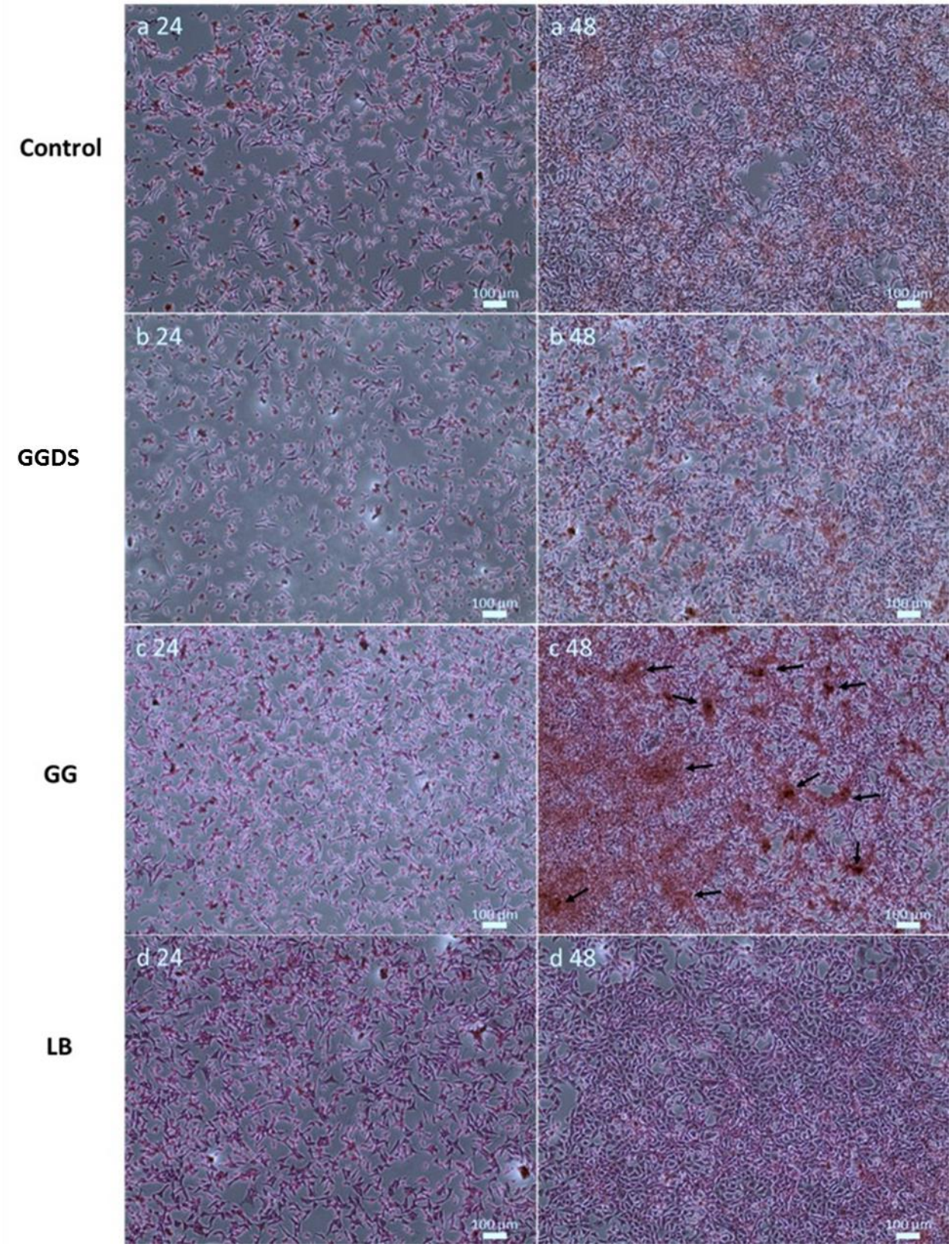
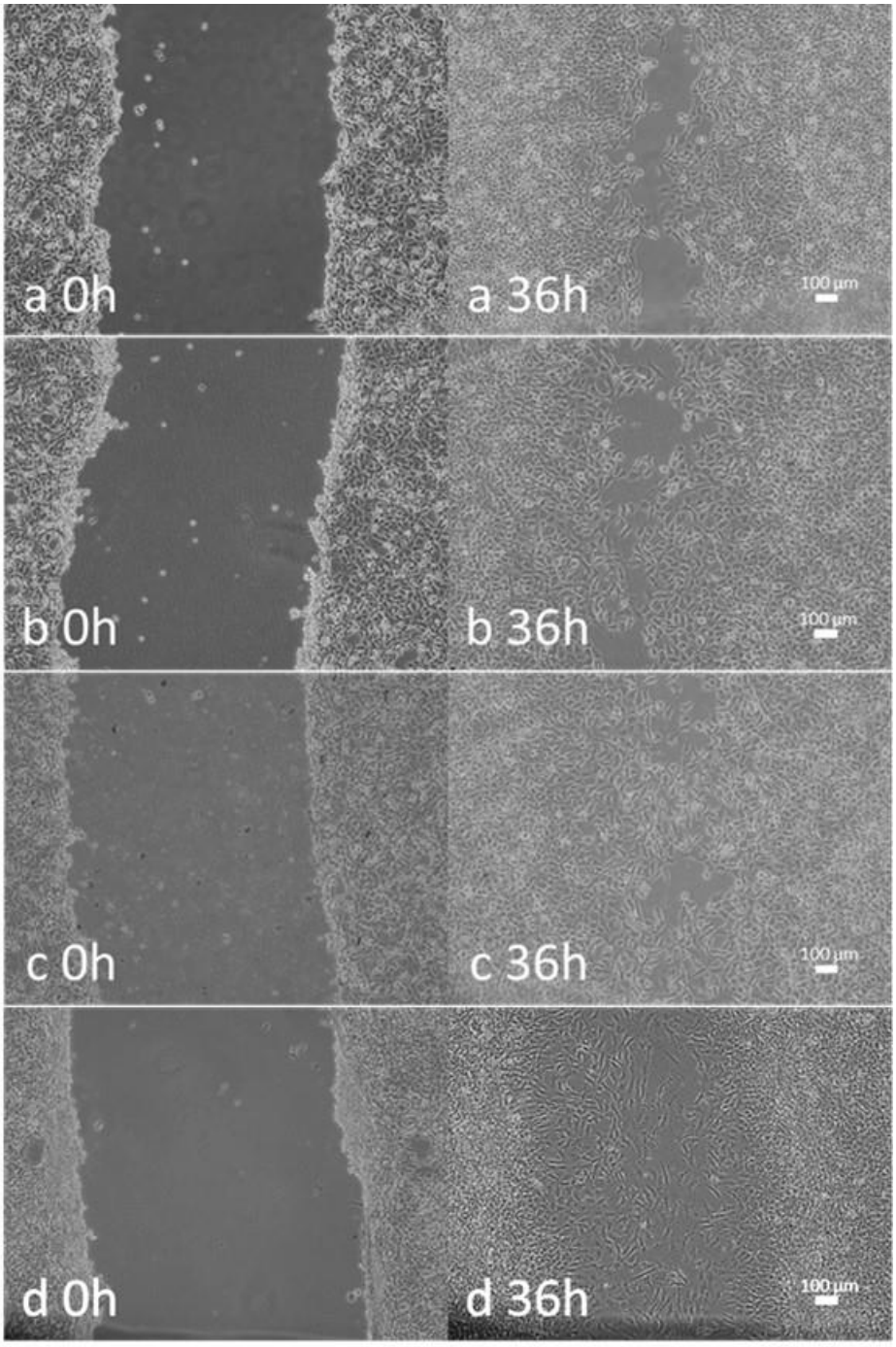


Figure 3: Collagen staining of 3T3 fibroblast cultures treated with sample media (0.5% w/v test polysaccharide) at 24 and 48 hours post-treatment for control (a), GGDS (b), GG (c), and LB (d). Arrows indicate collagen deposition.

228 *2.2. Scratch Wound Assay*

229 Possible wound healing properties of the polysaccharide extracts were assessed using a scratch
230 assay (**Fig. 4**) which probes the ability of cells to migrate into a simulated wound area (Yarrow
231 et al., 2004).



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233 **Figure 4:** Scratch assay using 3T3 fibroblasts showing control with mitomycin C at 0 hours
234 and 36 h (a), 0.5% GGDS (b) 0.5% GG (c) and 0.5% LB (d).

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The scratch assay is designed to simulate wounds of similar size, however, as Fig. 5 demonstrates, variances occur in initial wound size. Hence, measures of wound closure taking account of the variance in initial wound size were employed. Rate of wound closure was analysed to determine how quickly cells migrated into and reoccupied the wound site following injury (Fig. 6) in order to better highlight any differences in wound recovery between each sample. Additionally, the overall % wound closure was calculated to evaluate healing in relation to the initial wound size (Fig. 7).

Interestingly, results showed the migration of 3T3 fibroblasts into simulated wounds occurred on a similar timeframe as reported *in vivo* , with the majority of cellular migration observed after 24 hours (Scharffetter et al., 1989). Similar results have also been reported for Aloe extracts *in vitro* (Fox et al., 2017). Significant increases in wound closure rate were observed for cells treated with each polysaccharide extract over the 36 hour culture period compared with control cultures (**Fig. 6**). However, the most significant difference was observed in cultures treated with LB (345 vs 235 $\mu m^2/h$, LB vs control, $p = 0.003$). No significant differences in closure rates were observed between each experimental sample (LB vs GG, LB vs GGDS, GG vs GGDS). Despite significantly higher wound closure rates for cultures treated with all three polysaccharide extracts, the only significant increase in overall % wound closure was observed in cell populations treated with GG (**Fig. 7)**. In order to determine % closure, the initial wound area (0 h) was compared to wound areas at set time points (8, 24 and 36 h) following wound generation and subsequent treatment with the polysaccharide extracts. The % wound closure in cells treated with GG was significantly greater than the control after 24 and 36 hours (90.1%, $p = 0.034$ and 95.7%, $p = 0.040$, respectively). Samples treated with LB and GGDS showed evidence of increased % closure but the differences were not significantly different than the control at a level of 95% confidence.

During the scratch assay, all samples were cultured with media containing mitomycin C to inhibit proliferation (Lee et al., 2001). Wound closure is, therefore, most likely to occur as a consequence of fibroblast migration into the simulated wound site. Significant increases in wound closure rate for all three polysaccharide-treated cell populations provides evidence that GG, GGDS and LB have a positive impact on fibroblast migration. However, as this only resulted in significant increases in overall % closure for wounds treated with GG, it could be argued that GG has the greatest effect on fibroblast migration. Interestingly, GG also appeared to produce the greatest response in terms of type I collagen deposition. This alludes to a

potential mechanism whereby GG significantly increases migration of fibroblasts into a wound and positively influences deposition of type I collagen.

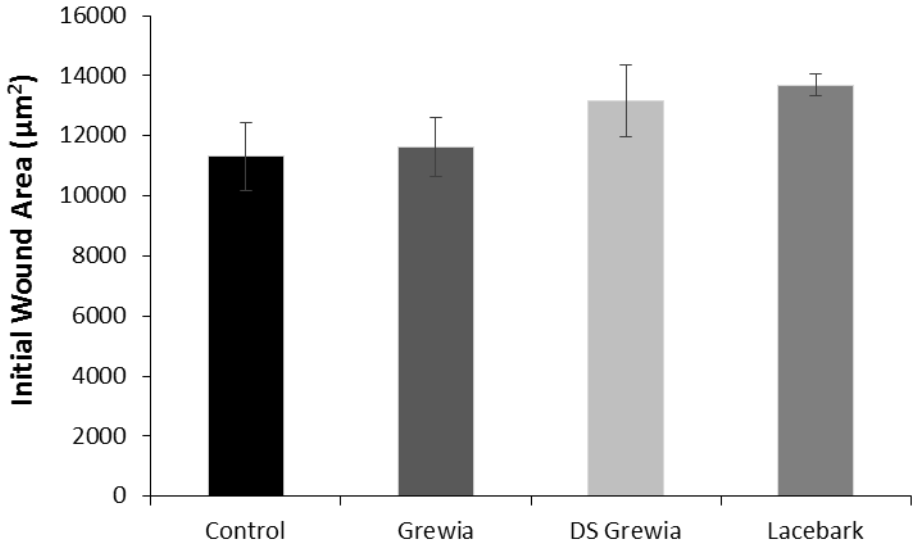

Figure 5: Average wound area at 0 h for cell populations treated with grewia (GG), DS grewia (GGDS), lacebark (LB) and untreated controls.

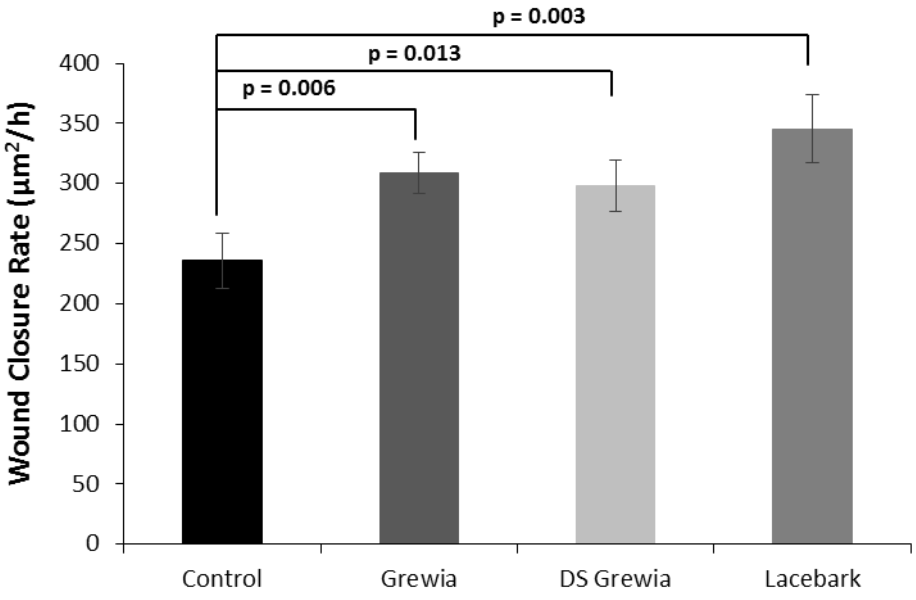

Figure 6: Wound closure rates over a 36 hour period for fibroblasts treated with grewia (GG), DS grewia (GGDS), lacebark (LB) and untreated controls.

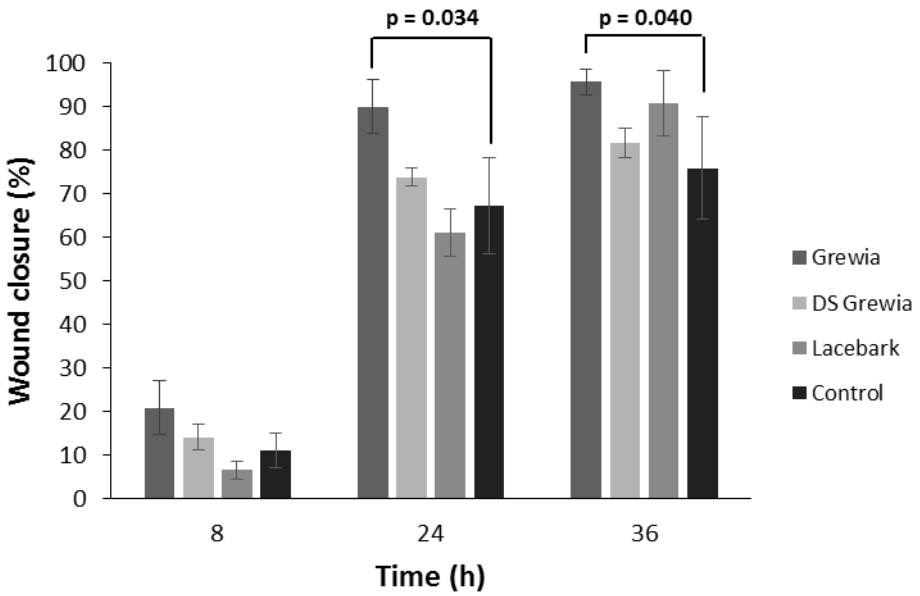

Figure 7: Wound closure expressed as a percentage of wound size relative to the size of the initial wound (0 h) for fibroblasts treated with grewia (GG), DS grewia (GGDS), lacebark (LB) and untreated controls.

2.3. Quantitative PCR

Quantitative PCR results indicated that treatment of 3T3 fibroblasts with all three polysaccharide extracts resulted in increased levels of COL1A1 mRNA within 8 hours of treatment, with GG-treated cultures showing the greatest increase ($2.5x10^3$ fold increase compared to the control at 8 h post inoculation). At 24 hours post inoculation, expression of COL1A1 mRNA in LB-treated cultures had reduced to levels similar to the control; after 48 hours this was also true for GGDS-treated cultures. Levels of expression in GG-treated cultures had dropped below that of the control after 48 hours and by 72 hours the expression levels in GGDS-treated samples were also lower than the control (**Fig. 8**).

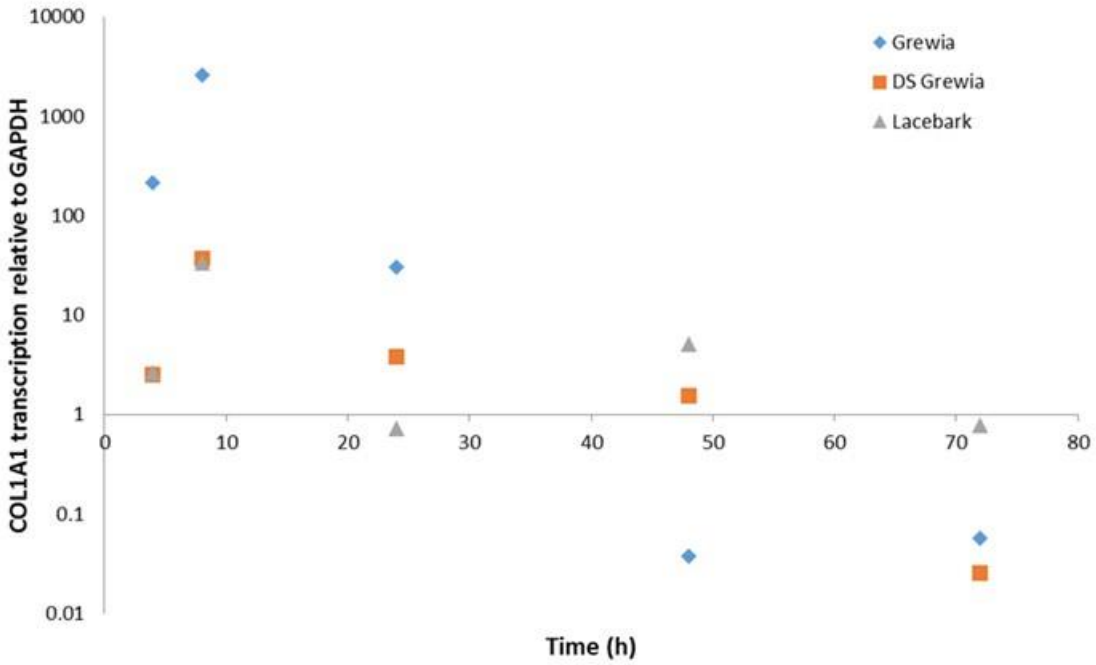

Figure 8: COL1A1 transcription relative to GAPDH for 3T3 fibroblasts treated with 0.5% Grewia (GG), 0.5% DS Grewia (GGD and 0.5% lacebark (LB) at 4, 8, 24, 48 and 72 hours.

Histological staining of type I collagen, while being qualitative, appears to correlate with the data generated from the qPCR (**Fig. 3** and **Fig. 8** respectively). More specifically, the highest level of collagen deposition was observed in cell populations treated with GG, which also produced the highest level of COL1A1 expression in treated fibroblast populations. All cell populations treated with polysaccharide extracts showed a greater level of COL1A1 mRNA transcription than the control after 8 hours, with GG exhibiting the greatest increase at this time point ($2.5x10^3$ fold increase). It is, therefore, possible that GG, GGDS and LB have a direct impact on collagen synthesis in fibroblasts by stimulating increased COL1A1 transcription. This potential mechanism is further strengthened by histological data suggesting the observed increase in COL1A1 gene expression which leads directly to collagen deposition by fibroblasts when treated with GG. It is worth noting, however, that the observed increase in collagen is related to the levels of collagen deposited into the ECM and any collagen released into the supernatant would be washed away during the assay. Therefore, only collagen attached to the surface of the plate or the cells themselves will be stained using this method. However, in the context of wound healing, collagen depositions into the surrounding ECM are of greater significance (Jorgensen, 2003).

The observed changes in behaviour of 3T3 fibroblasts following treatment with the polysaccharide extracts could be explained by cell-polysaccharide interactions which are influenced by the chemical structure of each extract. The polysaccharides from Grewia and lacebark have a common rhamnogalacturonan I-type (RG I-type) backbone with uronic acid side chains and different levels of acetylation. It is thought that these materials bind to growth factors secreted by cells and protect them from degradation during the wound healing process (Munarin et al., 2012). While the three polymers tested are structurally similar, the effect of each polymer on 3T3 fibroblasts was observed to be different. The levels of COL1A1 up-regulation and increases in cell migration appear to be similar between cells treated with GGDS and LB, while GG has a more pronounced effect (**Figs. 4-7**). All polymers increased transcription of COL1A1 after 8 hours. Additionally, all polymers appear to positively impact cell migration but only significantly increased % wound closure was observed following treatment with GG. Interestingly, one notable difference between GGDS, LB and GG is that GG has starch associated with the polymer molecule, while the other two do not (Nep et al., 2016). Starch in the GG samples is likely to swell, and create a "scaffold" which facilitates these wound healing processes. It may also be competing with the GG for water, and therefore be modifying the physical behaviour. Indeed, it has previously been reported that removing starch from GG results in a decrease in mechanical properties (Nep et al 2016). Furthermore, Wittaya-areekul and Prahsarn (2006) reported that starch-polysaccharide composite materials for wound healing applications have reduced water uptake and increased tensile strength. This could also play a role in healing by simulating an *in vivo*-like wound environment, specifically the localised increase in mechanical strength at the wound site as a result of collagen deposition (Baie and Sheikh, 2000). This could be a direct result of upregulation of the Col1A gene stimulated by supplementing with GG. Moreover, it is probable that the GG also influences the mechanical properties of the wound. It is widely reported across multiple fields that better recapitulation of the *in vivo* environment during *in vitro* testing positively impacts cell responses (Edmondson et al., 2014, Metcalfe and Ferguson, 2007, Mazzoleni et al., 2009, Hutmacher, 2010). Therefore, any mechanical replication of an *in vivo* wound healing environment due to the presence of GG could potentially have a positive impact on cellular responses to simulated wounds. Another important difference between the grewia and lace bark polysaccharides is the degree of O-acetylation (see **Table 1**), previous studies on acemannan extracted from Aloe vera suggests that deacetylation decreases bioactivity including type-1 collagen expression (Chokboribal et al., 2015), which is consistent with GG performing better than LB.

4. Conclusion

Analysis of the *Malvaceae* derived polysaccharides indicates they have positive effects on mechanisms that are integral to fibroplasia in wound healing. GG arguably demonstrated the greatest effect on simulated wound closure in cell cultures 36 hours post treatment, as well as showing a 2.5 x 10^3 fold increase in COL1A1 mRNA transcription over the control at 8 hours post treatment; in conjunction to this, histological staining data appears to corroborate these findings. The results presented in this study provide a greater scientific understanding behind the use of *Malvaceae* derived polysaccharides in traditional medicine.

5. Acknowledgements

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6. Author Contributions

G. A Morris and A. M. Smith designed the experiments presented in this study. I. M. Sims, S. M. Carnachan and E. I. Nep performed the extraction, purification and chemical characterisation of lacebark and grewia. N. Pearman, S. R. Moxon and M. E. Cooke carried out all cell culture analyses detailed in this manuscript. N. Pearman and S. R. Moxon contributed equally to preparation of the manuscript. All authors proofed, critically revised and approved the manuscript prior to submission.

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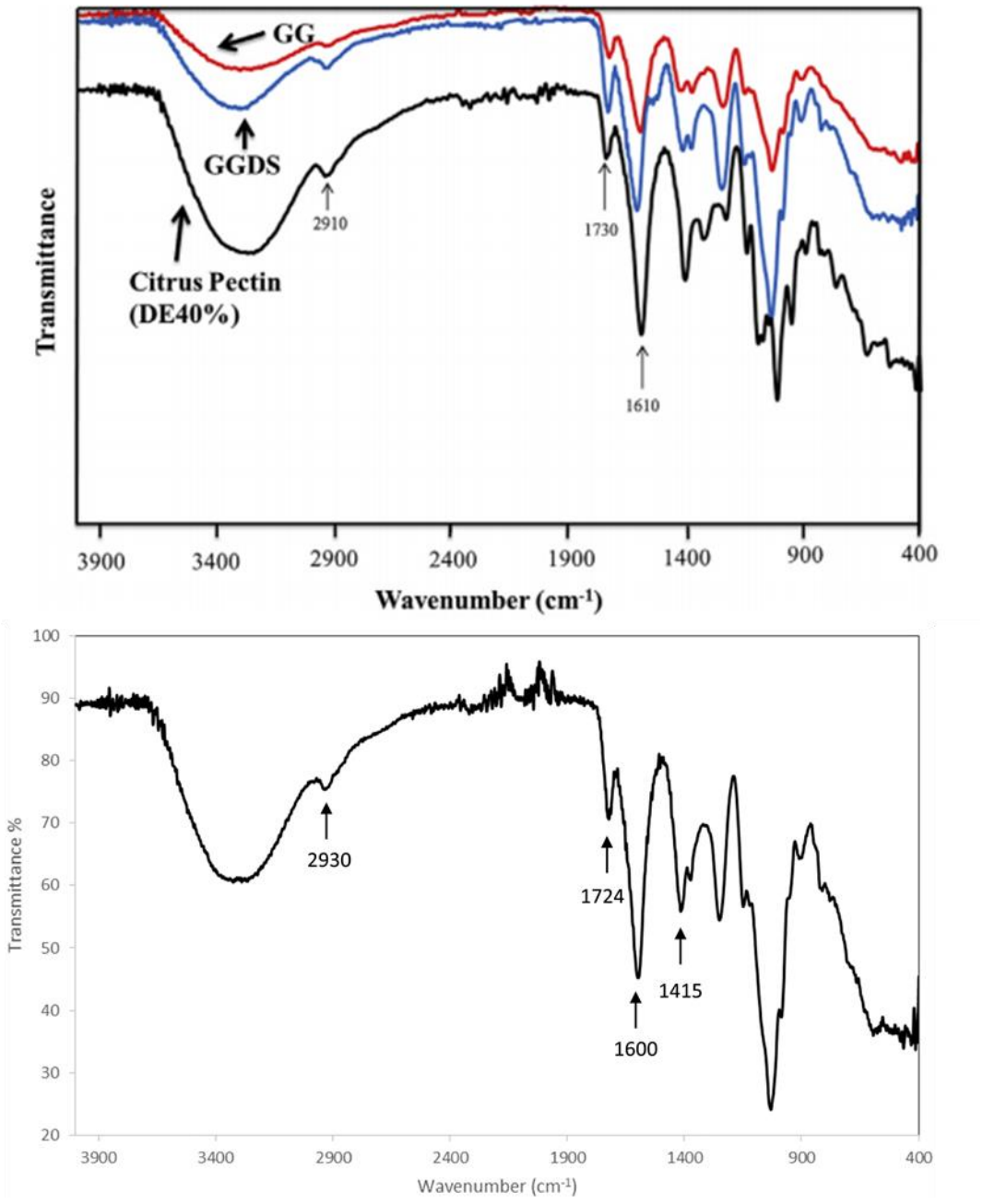
8. Supplementary Material



Supplementary Figure 1 - Fourier-transform infrared spectroscopy of GG and GGDS (above) and LB (below), reproduced from Nep et al., 2016 and Sims et al., 2018 with permission from Elsevier publishing.