Chapter

The Potential Effect of Medicinal Plants for Cartilage Regeneration

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Abstract

Any trauma to a joint such as sports injury can lead to osteoarthritis especially injuries that include torn cartilage, dislocated joints and ligaments. In sports injury specifically, most of the ointments in the market are only applied after physical activity. Repair of the bone and cartilage continues to be a challenge. Autologous and allografts are the gold standard for the treatment of the bone and cartilage. They have an invasive, open surgical procedure that requires the tissue to be harvested from an alternative site within the patient. South Africa is rich in native flora that is currently tapped as medicine by traditional healers. However, little is known about the natural products of our native flora and their potential to serve as a remedy for sports injuries, fracture healing and osteoarthritis. The grand purpose of the project is to explore medicinal plants of South Africa as a potential source for bone and tissue engineering of articular cartilage.

Keywords: tissue engineering, anti-inflammatory, osteoarthritis, sports injury, medicinal plants

1. Introduction

Generally, with the extensive screening of plants used in traditional medicine, evidence of their rational use in treating infections, diseases, inflammation and other disorders has been provided [1–3]. Herbal extracts have extensively health benefits, and indigenous medicinal plants have been used traditionally as a major source of drugs for the treatment of various illnesses, including osteoarthritis (OA), asthma, cancer, heart disease, tuberculosis, swollen ankles and hypertension [4–6]. Extracted compounds of medicinal plants are usually used as inputs in toxicology, phytochemicals, pharmaceuticals and other chemical industries [3–5, 7–9]. Stem cell therapies involving cartilage regeneration and several current 3D bioprinting processes involve the use of synthetic and natural biological molecules such as growth factors to improve their proliferation and differentiation [9–11]. There is an ongoing search in the science community for alternatives of these growth factors and the existing synthetic materials, due to reports on their numerous negative effects and complete failure in cartilage regeneration [3, 12–14]. Several medicinal plant extracts have been suggested to stimulate adult stem cell proliferation and thus regeneration of damaged or diseased tissues. Many Chinese herbs have been found to exert adipogenic, osteogenic and chondrogenic effects on human mesenchymal stem cells (hMSCs). Dried root of *Drynaria fortunei* contains flavonoid

and triterpenoid found to promote increased bone cell viability, intracellular total protein as well as alkaline and acid phosphates. Naringin, the major component of *Rhizoma drynariae* extract, enhanced the proliferation of BM-derived hMSCs by regulating β-catenin and AMP-activated protein kinase (AMPK) [15–17]. *Foeniculum vulgare* is traditionally used in the estrogenic activity to enhance milk secretion, in birth facilitation and for the alleviation of dysmenorrhea. *Foeniculum vulgare* extract has been found to promote the proliferation and differentiation of BM-derived hMSC into osteoblasts. Additionally, an ethanol extract of *Ferula gummosa* (an Iranian traditional medicine) was observed to enhance proliferation and differentiation of BM-derived hMSCs into osteocytes. [18]. Studies in this section elaborate on the possible mechanisms and beneficial effects of herbal remedies in the engineering of articular cartilage and regenerative medicine.

1.1 Role of medicinal plants in chondrocytes

In South Africa, numerous plants used traditionally have been employed in tissue engineering of articular cartilage. Studies have observed medicinal plants such as *Pleurostylia capensis*, *Pterocarpus angolensis* and *Eucomis autumnalis*, having resveratrol playing proliferation and differentiation roles in tissue engineering of articular cartilage. High regulation of collagen type II has been observed chondrocytes treated with resveratrol [19]. This makes resveratrol potentially enhancing chondrocyte viability which can be applied in 3D bioprinting of cartilage constructs [20]. Recent publications show that bark and root water extracts of *Pterocarpus angolensis* plants in the stifle joints from the 3-month-old pig affect the accumulation of collagen type II in porcine articular cartilage in the middle zone. Cell culture experiments were designed to investigate the role of the bark and root water extracts of *P. angolensis* to induce the expression of collagen type II protein in porcine articular chondrocytes. Monolayer cells were treated with 15, 30 and 50 μg/ml of *P. angolensis* extract and hydrogen peroxide (2 μg/ml) for 4 days, and the untreated chondrocytes were used as controls. The results showed no significant difference in the cell index between the controls and chondrocytes that had been treated with the plant extracts at 15 and 30 μg/ml. A significant increase in the expression of collagen type II protein by the chondrocytes was observed and found to be optimal at a concentration of 30 μg/ml. There was an increase in the production of proteoglycans. However, the plant extracts at a 50 μg/ml induced apoptosis in the middle zone chondrocytes. In conclusion the findings of this study are of great importance in understanding the mechanisms through which *P. angolensis* enables the healing of breached tissue [21]. In our laboratory, an (unpublished) in vitro study has observed the enhancement of proliferation and osteogenic differentiation (by increasing alkaline phosphate activity) of C2C12 myoblast cells treated with *Pleurostylia capensis* crude extract. Furthermore, proliferation and lineage differentiation of *P. angolensis* and *E. autumnalis* in porcine adipose-derived mesenchymal stem cells (pADMSCs) have also been recorded in our work (Figure not shown). However, the potential use of medicinal plants with tissue engineering methods to treat the cartilage and bone is exciting, yet not fully realized, and is likely to be a future treatment strategy.

1.2 Medicinal plant extract in scaffolds

Signals, morphogens responding stem cells and scaffolds that are biomimetic of the extracellular matrix are the three paramount requirements in regenerative medicine [22, 23]. Currently, empirical formulations, medicinal plants and their bioactive compounds are being merged with polymers that can be used in tissue regeneration.

Many studies have tried to incorporate medicinal plants in the fabrication of different scaffolds for wound healing, bone fracture and cartilage regeneration.

Herbal plants have the potential in tissue engineering and regenerative medicine due to their minimal host inflammatory response, high level of tenability and the ability to progressively degenerate into non-cytotoxic components, which are either reabsorbed or removed from the biological system [24]. Recently, studies have shown that scaffolds treated with *Cissus quadrangularis* extract (known as Asthisandhani in Indian traditional medicine) exhibited significant differences with regard to hMSC proliferation, attachment and enhanced osteoblast differentiation properties compared with scaffolds not treated with the extract [25]. Young et al. [26] also incorporated *Terminalia bellirica* extract in a hydrogel composition for use in stem cell therapy. This extract was found to result in significantly higher rates of hMSC proliferation and cell attachment.

Similarly, we have evaluated natural polymer (chitosan and alginate) scaffolds incorporated with *E autumnalis* and *P. angolensis* extracts as done in [25] on pADMSCs for lineage differentiation. The attachment capacity was evaluated by incubating pADMSCs with herbal and non-herbal scaffold at different concentrations of 1, 3 and 5 mg/ml. The samples were further stained with 4′,6-diamidino-2-phenylindole and calcein green after 72 h according to the manufacturer's instructions. The pADMSCs incubated with herbal scaffolds showed significant differences with regard to proliferation and cell attachment compared to pADMSCs incubated with non-herbal scaffolds (**Figure 1**). A higher number of cells were obviously present and attached to the herbal scaffolds in DAPI staining (**Figure 1d** and **e**) than in non-herbal scaffold (**Figure 1f**). A similar condition was also observed in the calcein staining with herbal scaffold enhancing cell proliferation and attachment (**Figure 1g** and **h**) compared to in the non-herbal scaffold (**Figure 1i**).

The chondrogenic differentiation capacity of the herbal scaffolds was also evaluated using toluidine blue staining after 21 days in culture (**Figure 2**). Herbal scaffolds were found to enhance formation of chondrocytes (**Figure 2a** and **c**) compared to non-herbal scaffolds (**Figure 2e**). Herbal scaffolds also showed significant chondrogenic enhancement compared to the controls (**Figure 2b**, **d** and **f**).

Additionally, our anti-inflammatory assay for days 7, 14 and 21 using an interleukin 6 (IL-6) Elisa kit according to the manufacturer's instructions confirmed the anti-inflammatory nature of *E. autumnalis* and *P. angolensis*. Inflammation was significantly higher ($P < 0.01$) in cells cultured with interleukin 6 and non-herbal scaffolds than in herbal scaffolds. The herbal scaffolds suppressed the expression of IL-6 in the cultured pADMSCs.

The degradation of mineral deposition during the in vitro regeneration process in tissue engineering is very important. Hence, we tried to assess the in vitro degradation capacity of our scaffolds using scanning electron microscope (SEM). It was observed that our herbal scaffolds showed significantly higher and gradual releasing of materials into the culture environment than our non-herbal scaffolds. The in vitro mineral deposition was confirmed using Fourier transform infrared spectrometer (FT-IR) spectrum (**Figure 3a** and **b**) on day 14 of incubation with pADMSCs in culture. The FT-IR data for the herbal scaffolds (**Figure 3a**) has an open-chain bond —C═N— at peaks 1600.8 and 1416.4 which were reduced. The 1072.4 and 1029.8 peaks were longer and more pronounced. The peak bands after 824.15 that are assumed to be vibrations of P—O—H from $Ca_3(PO_4)_2$ seems to be extended to peak 450. In the case of the non-herbal scaffolds (**Figure 3b**), peak bands at 1600.8 and 1416.4 were longer and seen at 1593.7 and 1420, respectively. At 1072.4 it is almost absent and the peak band at 1015.7 is reduced.

The FT-IR analysis showed certain peaks which are in the same functional groups as alkyl carbonate, organic sulphate and phosphate ions [27, 28].

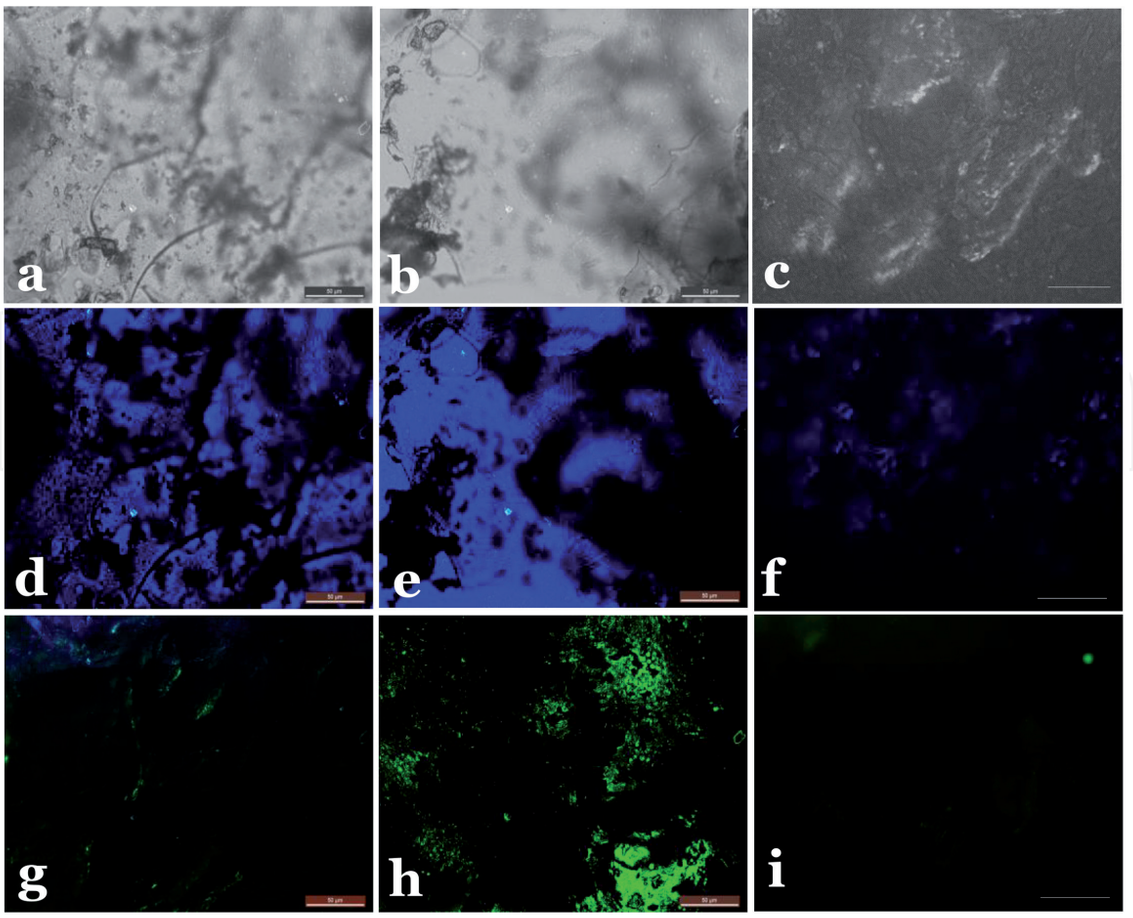


Figure 1.
Immunofluorescence staining of scaffolds (3 mg/ml) cultured for 72 h in adipose-derived porcine mesenchymal stem cells. (a–c) Images recorded under white field, (d–f) DAPI stain, (g–i) calcein stain, (a, d, g) Eucomis autumnalis scaffold, (b, e, h) Pterocarpus angolensis scaffold, and (c, f, i) cells cultured in non-herbal scaffold. Scale bar, 50 μm; magnification, 10×.

Furthermore, the presence of calcium, phosphate and carbonate compounds highlights the important relationship between intracellular calcium phosphate in osteoblasts and their role in mineralizing the extracellular matrix [29]. The long sharp peak at 1017.2 cm^{-1} also corresponds to silicate (Si) ions. Silicate and Cu ions are usually encountered in the presence of a hydrated surface layer of both bone crystal and synthetic apatite crystals, which contain varying concentrations of a wide variety of mineral ions that play important roles during bone and cartilage regeneration [30].

1.3 Medicinal plant extracts in wound healing

The skin is susceptible to injury and is the body tissue most exposed to damage. Wound healing is a normal biological process involving proliferation and redifferentiation of fibroblasts and keratinocytes [31, 32]. Significant advances have been made in the past years in wound healing so as to bring solutions for the treatment of chronic wounds and speeding up of acute healing. Several recent studies have found plants to be significant in controlling wound healing [33, 34].

Scrophularia striata, a well-known plant in Iranian traditional medicine, has anti-oxidative and anti-inflammatory properties. It is traditionally employed in wound healing due to these mentioned properties. Ghashghaii et al. [35] evaluated the wound healing potential of *S. striata* on cutaneous wounds in rats. Data from the study showed that rats treated with *S. striata* showed a significant decrease in the wound area, with a decrease in the number of lymphocytes,

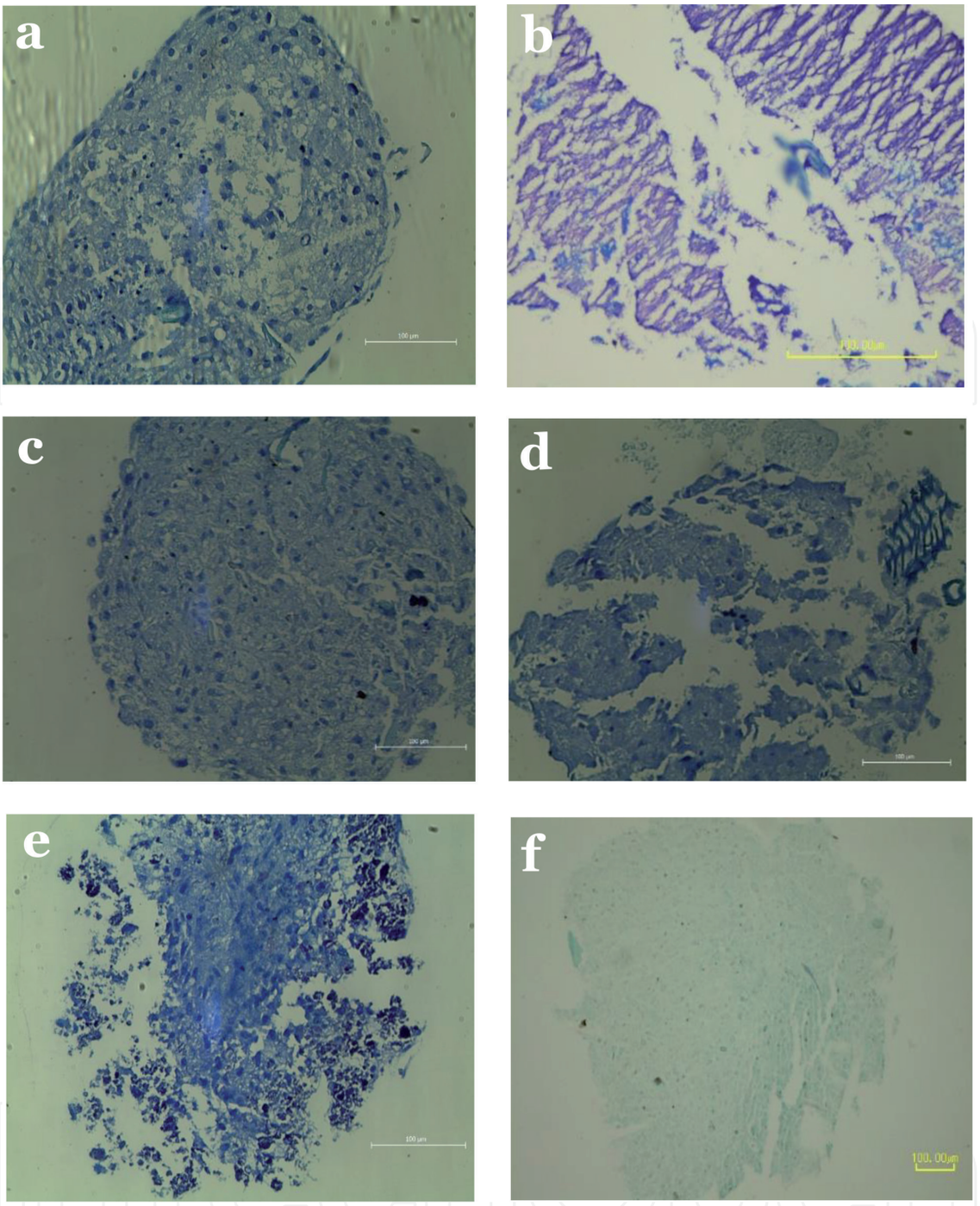


Figure 2.
Chondrogenic evaluation of the three experimental groups of the scaffold (3 mg/ml) with pADMSCs micro-mass pellet stained with toluidine blue at day 21 of treatment. (a) E. autumnalis herbal scaffold, (b) transforming growth factor (TGF)-beta 1 10 ng/ml positive control, (c) P. angolensis scaffold, (d) bone morphogenetic protein-2 (BMP-2, 10 ng/ml), (e) non-herbal scaffold and (f) negative control, pADMSCs without treatment. Scale bar, 100 μm; magnification, 10×.

enhanced number of fibroblasts and epithelial formation that resulted to early maturity of the collagen fibres compared to other groups. The study generally showed that application of *S. striata* on wounds resulted in substantial contraction and faster wound healing, which makes *S. striata* a potential subject for the treatment of wounds in animals and human beings.

Additionally, *Anogeissus leiocarpus*, a Ghanaian traditional plant, has been evaluated for wound healing activities in albino Wistar rats. A study of the wound healing effect of *A. leiocarpus* extract gave an interesting result. The plant formulation showed a progressive decrease in wound area with time [36, 37]. At day 15, the

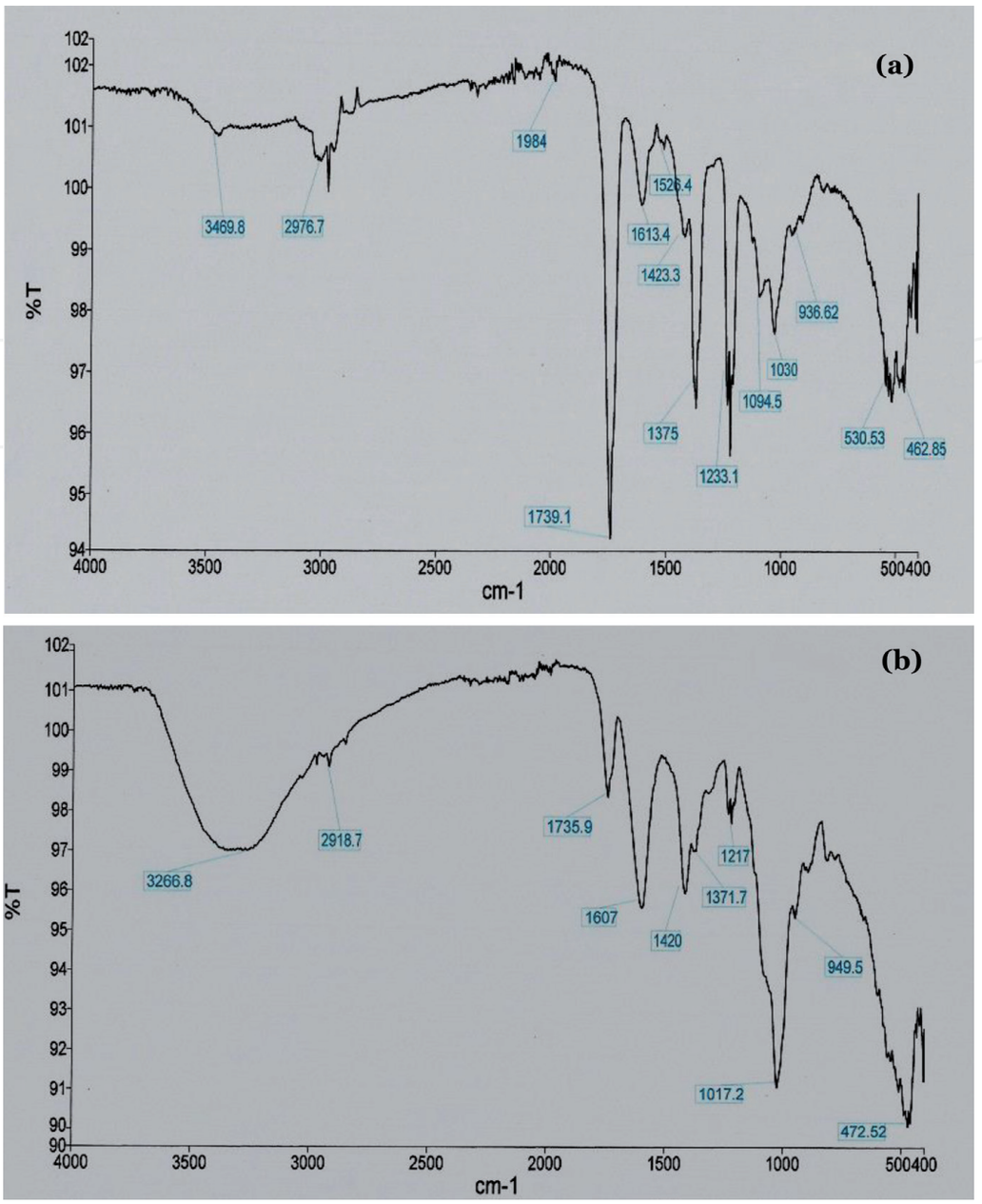
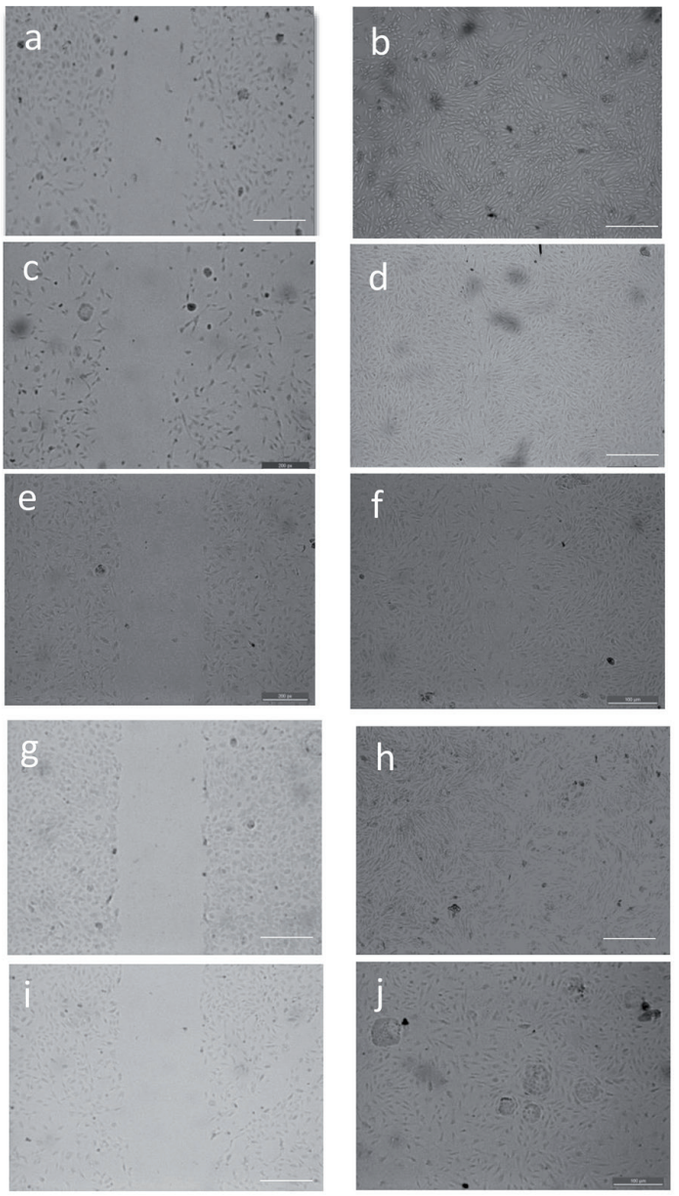


Figure 3.
The FT-IR spectrum of the scaffolds in culture with pADMSC cells at day 14 to confirm biomineralization. The analysis was done using the KBr method in the range of 400–4000 cm^{-1}. (a) Herbal scaffold and (b) non-herbal scaffold.

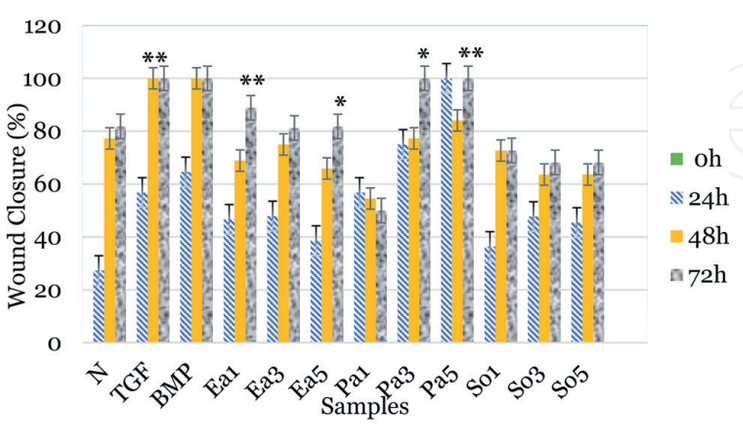
mixture containing 100 mg/ml aqueous extract and 10% w/w powdered ointment of *A. leiocarpus* showed 100% healing similar to the standard antibiotic (2% w/w penicillin).

Furthermore, a study has used *Moringa* extract incorporated with nanofibrous polyacrylonitrile for wound healing. Data from the study showed that *Moringa* influenced the healing properties of the material. At days 1, 4 and 7 of the wound dressing experiment, the percentage wound closure of the rat was the highest for the nanofiber containing 0.5 g of *Moringa* leaf extract (35, 87 and 95%, respectively) compared to the positive control medical gauze (29, 75 and 93%, respectively) [38].

Similarly, our study also evaluated the wound healing capacity of *E. autumnalis* and *P. angolensis* using the subcutaneous porcine adipose-derived stem cells up to 72 h, as done in [39, 40] with a slight modification. Percentage wound healing closure was calculated using the equation: initial area of wound—nth day area of wound/



A



B

Figure 4.
*In vitro wound closure appearance of the adipose subcutaneous cells after treatment with herbal and non-herbal scaffold media at 5 mg/ml. (A) 0 h (a, c, e, g and i) and 72 h (b, d, f, h and j). (a–b) Scaffold with E. autumnalis extract; (c–d) scaffold with P. angolensis extract; (e–f) negative control, scaffold without extract; (g–h) positive control, TGF 10 ng/ml; and (i–j) positive control, BMP-2 10 ng/ml. Scale bar, 100 μm; magnification, 10×. (B) Wound healing percentage (%) at 0, 24, 48 and 72 h of treatment with 1, 3 and 5 mg/ml of E. autumnalis and P. angolensis extract scaffold media. The data are expressed as mean ± standard deviation from six independent experiments, ** ($p < 0.01$) and * ($p < 0.05$).*

initial area of wound × 100. Data from our in vitro study (**Figure 4**) showed that the herbal extracts influenced the in vitro healing capacity of the cellulose/alginate polymer scaffolds. The healing capacity was found to be significantly higher ($P < 0.01$) in *P. angolensis* (**Figure 4A (a–b)**, **B** Pa3 and Pa5) at 24 and 72 h, respectively, compared with the non-herbal scaffold (**Figure 4A (e–f)**, **B** So3 and S05). The *E. autumnalis* extract performed well and was statistically significant (**Figure 4A (c–d)**, **B** Ea1 ($P < 0.01$) and Ea5 ($P < 0.05$)) at 72 h, respectively, but was low compared to the positive controls (**Figure 4A (g–j)**, **B** TGF ($P < 0.01$) and BMP-2, respectively). Our data so far depicted that herbal extracts improved the wound healing capacity with the incorporated natural biopolymers.

2. Conclusion

Numerous polymeric constructs have been used in combination with growth factors for engineering and regeneration of tissues. This combination of polymer and growth factors for tissue repair depends largely on using biodegradable materials that can stimulate specific cellular responses at a molecular level which should be suitable, simple and cost-effective. Our data in this section offers pharmacological evidence on the potential use of the mentioned plant extracts in bone fracture, cartilage regeneration and wound treatment. In fact, medicinal plants found to have anti-inflammatory properties may partake in host modulatory therapy for various inflammatory diseases as proposed in [3, 39].

We would like to state that the herbs and all the substances in this study are for cartilage defects of grades 1, 2 and 3 according to outerbridge scale. Therefore, if congenital or after trauma large cartilage case is presented, then operative treatment is advised.

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