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Full Length Research Paper

Wound healing properties of *Eucheuma cottonii* extracts in Sprague-Dawley rats

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Wounds are unavoidable events of life and arise due to agents that induce stress or injury. Wound has been a menace the world over and healing is a survival mechanism and represents an adaptation to the maintenance of normal anatomical structure and function. The objective of this study was to evaluate the potential wound healing properties of ethanolic and aqueous extracts of *Eucheuma cottonii*. A two centimeter diameter of skin excision wound was made on normal rats. Treatment with honey (100 mg/kg body weight) was used as positive control and untreated rats as the negative control groups. Inflammation and proliferation phases of wound healing including wound contraction, re-epithelization and granulation tissue development were monitored. This study showed that both seaweed extracts increased the rate of wound contraction compared with the positive and negative control. Ethanolic extract of *E. cottonii* was more effective than the aqueous extract by 20% (P<0.05). Histopathological findings showed the ethanolic extracts enhanced epithelization and tissue granulation significantly compared with both control groups. *E. cottonii* possesses several antioxidant compounds, which may be responsible for the accelerated wound healing. The present study demonstrated that these seaweed extracts accelerated healing better if not comparable with honey.

Key words: Seaweed (*Eucheuma cottonii*), wound healing, histopathology.

INTRODUCTION

Acute wounds normally heal in a very orderly and efficient manner characterized by four distinct, but overlapping phases: hemostasis, inflammation, proliferation and remodeling. Inflammation is followed by attraction and proliferation of fibroblasts and collagen deposition, and finally remodeling by collagen cross-linking and scar maturation (MacKay and Alan, 2003). *Eucheuma cottonii* is an edible species of Pacific red seaweeds obtained from Malaysian North Borneo Sabah waters which is as a potential source of a variety of compounds like dietary fibers, vitamin C, α-tocopherol, minerals, fatty acid and protein (Matanjun et al., 2009; Jime'nez-Escrig and Sa'nchez-Muniz, 2000). The seaweed compounds antioxidant properties have been well reviewed (Hollman and Katan, 1999; Chew et al., 2008). Likewise, *E. cottonii* is a rich source of antioxidants (Matanjun et al., 2008), which can significantly prevent tissue damage stimulate the wound healing process (Rajasekaran et al., 2004) and is anti-inflammatory (Shanmugam and Mody, 2000). Hsu (2005) showed that plant extracts have been widely used in topical applications for wound healing and disease treatments, but the data on marine plant are lacking as medicinal. This study aims the wound healing potential of the marine seaweed *E. cottonii* extracts a rat model. Additionally the effects on collagen deposition, granulation tissue formation, epithelization and wound contraction

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Table 1. The Wound healing (%) of different groups of rats fed with *E. cottonii* extracts over a period of 15 days.

Group	Day 3	Day 6	Day 9	Day 12	Day 15
Er.t	8.6 ± 0.4[a]	37.9 ±8.6[a]	79.9 ± 1.5[a]	92.3 ± 4.0[a]	100.0 ± 0.0[a]
E70	9.0 ±0.1[a]	36.3 ± 9.8[a]	75.9 ± 5.9[a]	96.0 ± 2.0[a]	100.0 ± 0.0[a]
Wr.t	3.3 ± 0.8[b]	19.8 ± 5.0[a]	50.0 ± 6.8[b]	70.1 ± 4.9[a]	83.4 ± 2.0[b]
W70	4.2 ± 0.1[b]	27.4 ± 3.1[a]	67.4 ± 4.9[b]	77.6 ± 2.6[a]	88.2 ± 3.2[b]
HNY	8.2 ± 2.2[a]	34.0 ± 8.5[a]	58.3 ± 6.5[b]	72.7 ± 9.7[a]	93.7 ± 3.2[b]
WTR	0.6 ± 0.4[b]	23.4 ± 8.8[a]	35.4 ± 7.2[c]	40.3 ± 13.0[b]	52.6 ± 8.5[c]

Values are means ± standard deviation of 12 animals per group in the first week and 6 animals each group in the second week. Means with different superscripts within a column were significantly different at $P<0.05$. Er.t and E70, ethanolic extract groups; Wr.t and W70, aqueous extract groups; HNY, positive control group; WTR, negative control group.

rate are reported.

MATERIALS AND METHODS

E. cottonii extract preparation

E. cottonii was collected from the coastal areas of Semporna (Sabah, Malaysin North Bomeo). In the laboratory, the fresh seaweed was cleaned and washed with distilled water. The seaweed was then dried at 40°C in a dark room for 3 days and then ground to a fine powder to pass through a 0.5 mm screen using a warring blending, and stored in air-tight containers covered with aluminum foil at -20°C. The seaweed extracts were prepared from the fine powder within a month using on a modified method of Ponce et al. (2003). Extraction was done at two different temperatures, (i) room temperature (25 ± 2°C) and (ii) 70°C for both the ethanol and water extracts.

The ethanolic extract ERT, was prepared from milled seaweed (200 g) using 1000 ml of 80% (v/v) ethanol at room temperature for 24 h under mechanical strrring and then filtered by W-hatman (No.4); filter paper . The residue was then mixed with another 1000 ml of 80% (v/v) ethanol and shaken for 24 h at 70°C followed by filtration (E70 ethanolic extract). Residue leftover from the filtration of E70 was then dissolved in 3000 ml distilled water, stirred at room temperature (25 ± 2°C) for 8 h and filtered to yield Wr.t. The remaining residue was then mixed with 3000 ml of distilled water, stirred at 70°C for 8 h in a water bath (70°C), and followed by a final filtration to yield W70. Subsequently, the ethanolic extracts. ERT and E70 were concentrated under vacuum at 40°C for 1 h. Similar concentration procedures were performed on Wr.t and W.70 at 70°C. All extracts were then dried in an oven at 40°C overnight to produce powdered extracts which were then stored in air-tight containers at, -20°C until use.

Experimental animals

Seventy two (10 to 12 weeks old) healthy male Sprague-Dawley rats weighing 300 to 350 g were allotted randomly into six groups of twelve animals each. The rats were acclimatized for one week before the experiment. The animals were housed in individual cages and kept in a ventilated room with the temperature regulated at 23 ± 2°C, with a 12 h light/ 12 h dark cycle. They were fed with commercial rat pellets and water. All experimental procedures and animal care had been approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University Putra Malaysia (UPM/FPV/PS/3.2.1.551/AUP-R-17).

Skin wounding and treatments

The rats were divided into treated and control groups. The positive and negative control groups were fed honey (Honey) and distilled water (W-TR), respectively. The remaining animals were fed daily with the different seaweed extracts dissolved in distilled water, at 100 mg/kg body weight using intragastric tube soon after the wound creation.

The rats were anaesthetized with ketamine-HCl and xylazine (150 and 10 mg/kg body weight) prior to wounding. The skin doral interscapular area wound site was prepared and shaved. A full skin thickness with circular area of 300 mm^2 (2 cm diameter) was excised. Immediately after wounding, the wound was washed with normal saline and closed with a film dressing.

Sampling and wound healing evaluation

Wounds were traced on 1 mm^2 graph paper on the day of wounding and planimetrically every three days until end of experimental period. Changes in wound area were calculated, giving an indication of the rate of wound contraction (Nayak et al., 2005). Six rats per group were sacrificed on the 7th day of wounding with ketamine overdose. The remainders were sacrificed using the same method on the 15th day post wound. A sagittal section splitting the wound into two equal halves was made to evaluate the extent of wound healing.

The cut tissues were rinsed with 0.2 M phosphate buffered saline (PBS, pH 7.4) and fixed with 4% paraformaldehyde. They were then embedded in wax after further processing, and cut to obtain 5 µm thick sections. These tissue sections were then mounted, stained with hematoxylin and eosin (H and E) stain and evaluated under light microscope. The tissue sections were evaluated for wound angiogenesis, collagen deposition, granulation tissue formation, epithelization and extent of wound contraction.

Statistical analysis

All results were expressed as means ± standard deviation, and analyzed using a one-way analysis of variance (ANOVA) and followed by Duncan multiple range tests. All procedures were performed at 95% confidence level using the Minitab 14.

Table 2. Period of epithelialization (day) in skin wounded rats fed with various extraction of *E. cottonii.*

Er.t	E70	Wr.t	W70	HNY	WTR
9.0 ± 0.4[a]	9 .2± 0.2[a]	14.8 ±0.7[b]	15.4 ±0.6[b]	12.1±0.8[b]	20.0±0.8[c]

Values are means ± standard deviation of 6 animals per group. Means with different superscripts were significantly different at P<0.05. Er.t and E70, ethanolic extract groups; Wr.t and W70, aqueous extract groups; HNY, positive control group; WTR, negative control group.

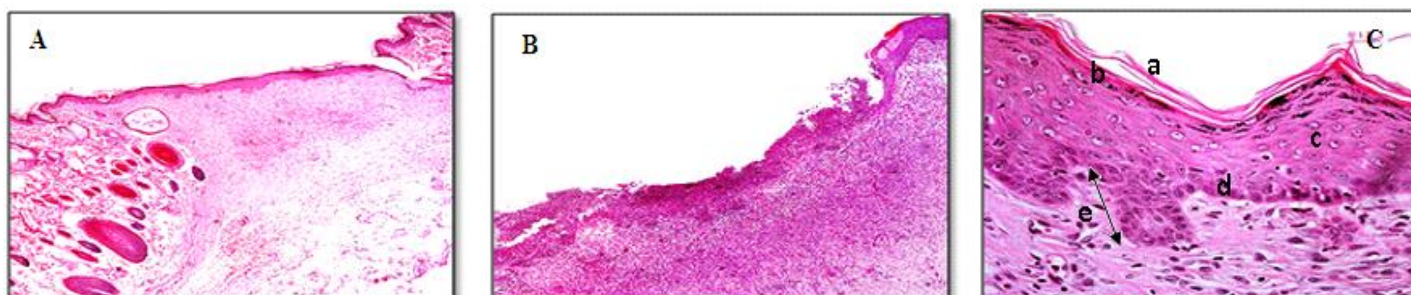

Figure 1. Epidermis photomicrographs in *Er.t* group: (A) 7th day after treatment (H&E stain; ×100), (B) 15th day after treatment (H and E stain; ×100) and (C) 15th day after treatment (H&E stain; ×400). Skin appear well rearranged epidermis with differetiated layers. (a) stratum corneum, (b) stratum granulosum (c) stratum spinosum, (d) stratum basale, (e) papillary layer.

RESULTS

Wound contraction

The rate of wound contraction were expressed as the percentage of wound area that had healed (Table 1). The percentage of wound contraction in all treatment groups I was significantly higher compared to the negative controon the 9th day (P<0.05). At this time the percentage of wound contraction ranged from 35.4±7.2% in Water group (negative control) to 79.9 ± 1.5% in ERT group. There was a significant difference (P<0.05) in percentage of wound closure between the control and treatment groups on the 15th day. On the 15th day rats of ERT and E70 groups completely healed, whereas rats of WRT group showed 83.4% and of W70 group, 88.2% healing, when compared to the negative control (52.6% healing) at the 15th day. The lowest percentage of wound healing was seen in negative control group.

Period of epithelization

Some of the phases of healing such as wound contraction and epithelialization run concurrently and independently (Kaushal et al., 2007). Epithelization is the process where epithelial cells around the margin of the wound or in residual skin appendages such as hair follicles and sebaceous glands lose contact inhibition and begin to migrate into the wound area by the process termed "epiboly" (Stenn and Paus, 2001). As can be seen from Table 2, groups of animal treated with the ethanolic extract took nine days for re-epithelization in contrast to twenty days taken by the control group. On the 15th day post treatment, all groups were completely covered with fibrin except for the negative control group. Therefore, it can be concluded that oral treatment with *E. cottonii* extracts on skin lesions accelerated the reepithelization and remodelling phases.

Histopathological studies analysis

Re-epithelization

Figures 1 to 6 show gross dermal lesions viewed under 100 and 400 magnifications on 7 and 15 days treatments. The absence of epidermis is

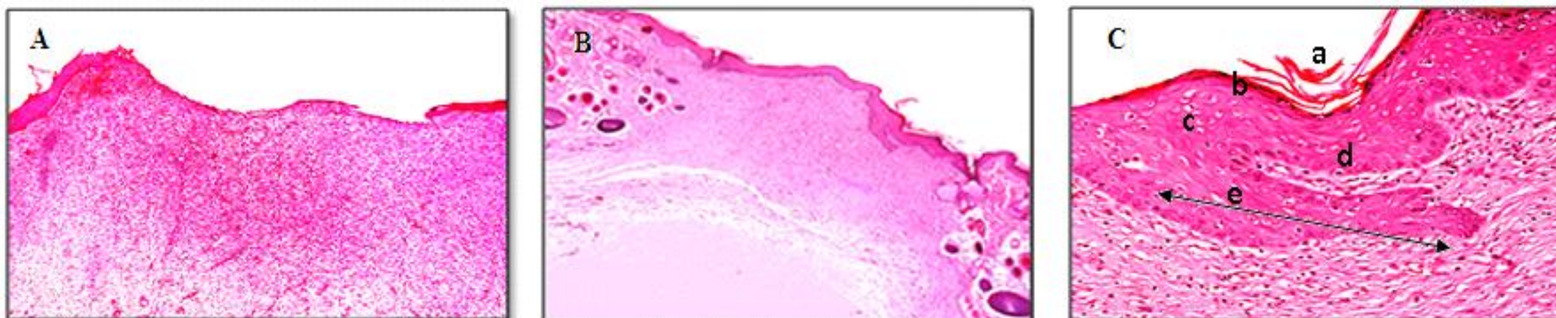

Figure 2. Epidermis photomicrographs in *E70* group: (A) 7th day after treatment (H and E stain; ×100), (B) 15th day after treatment (H and E stain; ×100) and (C) 15th day after treatment (H and E stain; ×400). Skin appear well rearranged epidermis. (a) stratum corneum, (b) stratum granulosum (c) stratum spinosum, (d) stratum basale, (e) papillary layer.

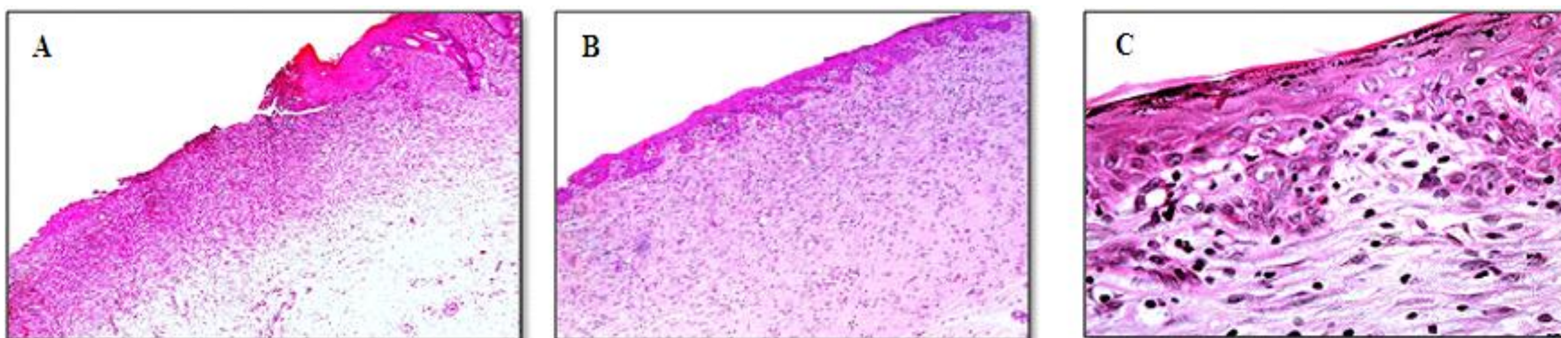

Figure 3. Epidermis photomicrographs in *Wr.t* group: (A) 7th day after treatment (H&E stain; ×100), (B) 15th day after treatment (H and E stain; ×100) and (C) 15th day after treatment (H&E stain; ×400). Regeneration epithelium is organized in few layers of keratinocytes.

noted in rats from all treatment groups on 7-day treatment. However, signs of epidermal regeneration were evident in groups that were treated with ethanolic extracts (Figure 1 A). The groups treated with ethanolic extract showed that fibroblastic nodule were well covered by regenerated epidermis and its highly differentiated epidermal layers. The regenerated epidermis had covered the wound completely by the 15th day of treatment. In wounds treated with aqueous extract of seaweed, the regenerated epidermis was organized in few layers of keratinocytes. Many mitoting bodies were observed in the stratum germinativum (Figures 2 and 3). Furthermore, the skin of wounded rats treated with honey regeneration was also organized in few layers of keratinocytes and differentiated epidermal layers were not complete. There was a good mitotic activity in the stratum germinativum. Scar formation was also evident in W70 and HNY groups (Figures 4 and 5B). In the negative control group primitive degree of granulation tissue formation was evident. There were no differentiated epidermal layers (Figure 6).

Granulation tissue development

In the groups treated with ethanolic extract there was a mass of newly formed fibrous tissue

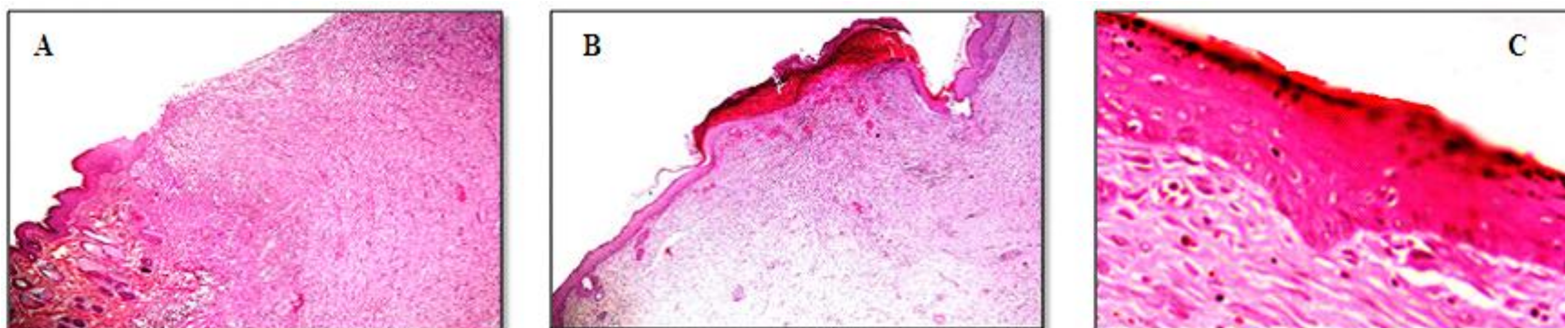

Figure 4. Epidermis photomicrographs in *W70* group: (A) 7th day after treatment (H and E stain; ×100), (B) 15th day after treatment (H&E stain; ×100) and (C) 15th day after treatment (H and E stain; ×400). Regenerated epithelium is organized in a few layers of keratinocytes.

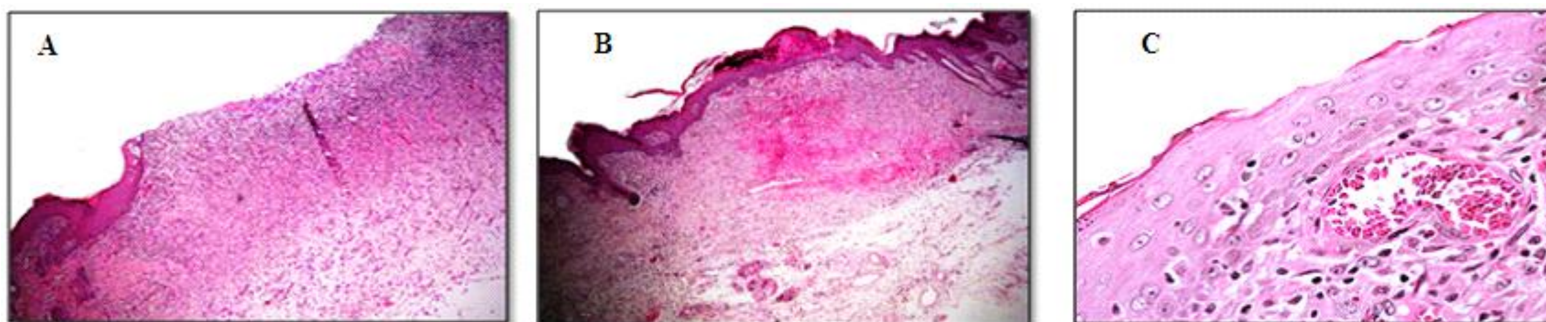

Figure 5. Epidermis photomicrographs in *HNY* group: (A) 7th day after treatment (H&E stain; ×100), (B) 15th day after treatment (H and E stain; ×100) and (C) 15th day after treatment (H and E stain; ×400). Regeneration epithelium is organized in few layers of keratinocytes.

consisting of fibroblasts and collagen producing fibrocytes together with congested blood vessel in the dermis in the 15th day post treatment. There were only a few inflammatory cells, in papillary layer of the dermis (mainly plasma cells) (Figures 7A and B). In wounded rats treated with aqueous extract of seaweed, the underlying granulation tissue consists of huge amount of newly formed blood vessels that appear slightly congested. There was an infiltration of subacute to chronic inflammatory cells mainly lymphocytes and plasma cells (Figures 7C and D). Furthermore, in skin wounded rats treated with honey underlying tissue consists of granulation tissue with moderate inflammatory reaction, plasma cells and macrophages (Figure 7E). In negative control group there was necrotic tissue infiltrated by different type of inflammatory cells, mostly lymphocytes, macrophages (Figure 7F).

DISCUSSION

The normal healing cascade begins with an orderly process of hemostasis and fibrin deposition, which leads to an inflammatory cell cascade, characterized by neutrophils,

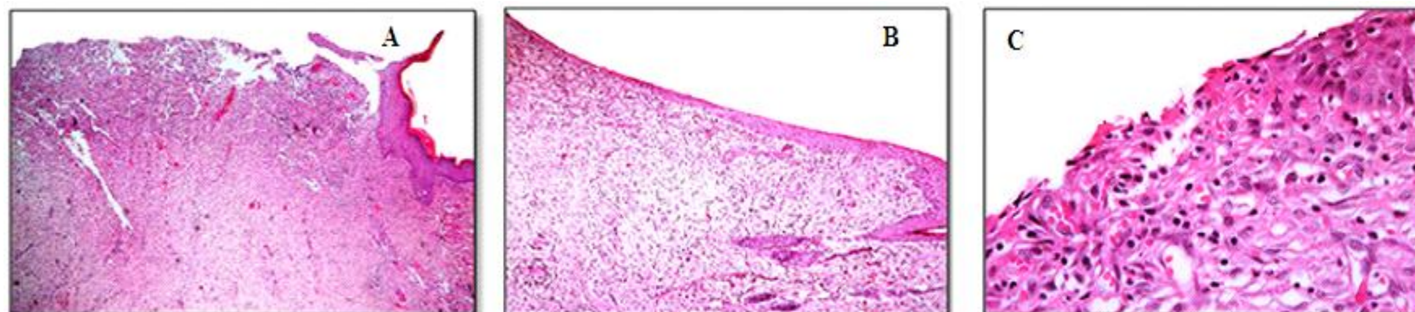

Figure 6. Epidermis photomicrographs in *WTR* group: (A) 7th day after treatment (H&E stain; ×100), (B) 15th day after treatment (H and E stain; ×100) and (C) 15th day after treatment (H and E stain; ×400). In most parts, no regenerating epithelium is evident.

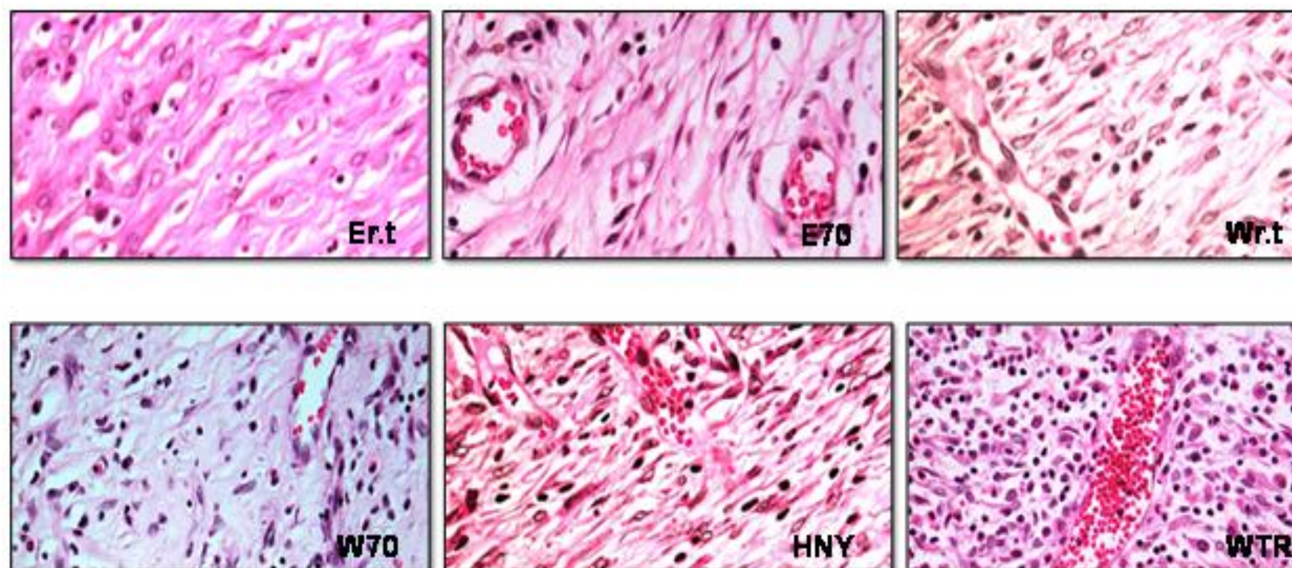

Figure 7. Granulation tissue of the wounded skin treated orally with seaweed extracts (Er.t, E70, Wr.t, W70), honey (HNY) and distilled water (WTR) on the 15th day post treatment (H and E stain; ×400).

macrophages and lymphocytes within the tissue (Mast et al., 1992). This is followed by attraction and proliferation of fibroblasts and collagen deposition, and finally remodeling by collagen cross-linking and scar maturation. In the end of healing, the amount of inflammatory cell

decreases and the fibroblast increases. The fibroblast is the connective tissue cell responsible for collagen deposition that is needed to repair the tissue injury (Ross, 1969). Ethanolic and aqueous extracts of *E. cottonii* from different temperatures were compared with honey as positive control group. The healing properties of honey have been known since immemorial time and have recently gained recognition from the scientific community (Molan, 2000; Dweck, 2009). Some authors (Subrahmanyam, 1998; Middelkoop et al., 2004) demonstrated honey has been used successfully in wound treatment even when compared to some drugs such as silver sulfadiazine (SSD). The mechanism of the anti-bacterial and anti-inflammatory effects of honey has been understood in previous research (Al-Waili and Boni, 2003). In this study, ethanolic extracts of *E. cottonii* had better wound healing promoting property than honey. There was not any scar in healed sites at ethanolic extract treated groups (Figures 1B and 2B). Scar was seen in groups treated with aqueous extract of seaweed and honey (Figures 4B and 5B).

The ethanolic extract demonstrated significantly better and faster healing properties compared to the aqueous extract. The ethanolic extract contains some pigments (fucoxanthin, astaxanthin, carotenoid) and phenolic compounds (phenolic acid, flavonoid, tannins) with potential antioxidant activities (Chandini et al., 2008), which may be responsible and favorable for faster wound healing (Devipriya and Shyamaladevi, 1999). This finding is in agreement with other researcher's findings which showed flavonoids (Tsuchiya et al., 1996) and triterpenoids (Nayak et al., 2007) present in plant extracts promote the wound healing process mainly due to their astringent and antimicrobial properties. Yoshie et al. (2003) were identified flavonids such as catechol, quercitrin and myricetin in seaweeds. Flavonoids in ethanolic extract of seaweed may be responsible for wound contraction and increased rate of epithelization. The aqueous extract exhibited the weakest antioxidant activity and had low concentrations of polyphenols. However, Trombetta et al. (2006) showed that the polysaccharides isolated from aqueous extract of *Opuntia ficus-indica* have been demonstrated in animal models as active principles responsible for facilitating healing of wounds. In this present paper we reported the wound healing potential of ethanolic and aqueous extracts of *E. cottonii.* We observed that orally application of *E. cottonii* extracts, enhanced cutaneous healing, but it was faster in ethanolic extract which appeared completed in 15 days. There was no significant difference among different levels of alcoholic extract (Er.t and E70). The histopathology finding is highly significant in skin wounds treated with the extract than in control group. Thus, we suggest that the ethanolic extract of *E. cottonii* could be used as a dietary nutritional supplement to promote human health and prevent oxidation-related diseases, due to its antioxidant property. This study demonstrated that the yet-to-be-determined active compound(s) could be extracted from seaweed using ethanol-water (80:20 v/v) solvent system. Further studies with purified components are required to appreciate the complete mechanism of wound healing activity of ethanolic extract of *E. cottonii*.

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REFERENCES

Al-Waili NS, Boni NS (2003). Natural honey lowers plasma prostaglandin concentrations in normal individuals. J. Med. Food, 6: 129-133.

Chandini SK, Ganesan P, Bhaskar N (2008). *In vitro* antioxidant activities of three selected brown seaweeds of India. Food Chem., 107(2): 707-713.

Chew YL, Lim YY, Omar M, Khoo KS (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT, 41: 1067-1072.

Devipriya S, Shyamaladevi CS (1999). Protective effect of quercetin induced cell injury in the kidney. Indian J. Pharmacol., 31: 422–24.

Dweck AC (2009). The internal and external use of medicinal plants. Clinics in Dermatol., 27(2): 148-158.

Hollman PC, Katan MB (1999). Dietary flavonoids: intake, health effects and bioavailability. Food Chem. Toxicol., 37: 937-942.

Hsu S (2005). Green tea and the skin. J. Am. Acard. Dermatol., 52: 1049-1059.

Jime'nez-Escrig A, Sa'nchez-Muniz FJ (2000). Dietary fiber from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. Nutri. Res., 20: 585-598.

Kaushal MN, Kutty GC, Rao M (2007). Wound healing activity of NOE-aspirin: A pre-clinical study. Nitric Oxide, 16: 150-156.

MacKay D, Alan LM (2003). Nutritional Support for Wound Healing. Alternative Med. Rev., 8: 359-377.

Mast BA, Cohen IK, Diegelmann B, Lindblad WJ, Eds WB (1992). In Wound Healing, Biochemical and Clinical Aspects, Saunders Company, Philadelphia, p. 344.

Matanjun P, Mohamed S, Mustapha NM, Muhammad KH, Ming CH (2008). Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. J. Appl. Phycol., 20: 367-373.

Matanjun P, Mohamed S, Mustapha NM, Muhammad K (2009). Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. J. Appl. Phycol., 21: 1-6.

Middelkoop E, Bogaerdtb AJ, Lammec EN, Hoekstrab MJ, Brandsma K, Ulrich MMW (2004). Porcine wound models for skin substitution and burn treatment. Biomaterials, 25: 1559-1567.

Molan PC (2000). Establishing honey as a recognized medicine. J. Am. Apitherapy Soc., 7(1): 7-9.

Nayak BS, Anderson M, Pinto Pereira LM (2007). Evaluation of wound-healing potential of *Catharanthus roseus* leaf extract in rats. Fitoterapia, 78: 540-544.

Nayak BS, Vinutha B, Geetha B, Sudha B (2005). Experimental evaluation of *Pentas lanceolata* flowers for wound healing activity in rats. Fitoterapia, 76(7-8): 671-675.

Ponce NMA, Pujol CA, Damonte EB, Flores ML, Stortz CA (2003). Fucoidans from the brown seaweed Adenocystis: Extraction methods, antiviral activity and structural studies. Carbohydr. Res., 338: 153-165.

Rajasekaran NS, Nithya M, Rose C, Chandra TS (2004). The effect of finger millet feeding on the early responses during the process of

wound healing in diabetic rats. Biochimica et Biophysica Acta, 1689: 190-201.
Ross R (1969). Wound healing. Sci. Am., 220: 40-50.
Shanmugam M, Mody KH (2000). Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. Curr. Sci., 79(12): 1672-1682.
Stenn KS, Paus R (2001). Controls of hair follicle cycling. Physiol. Rev., 81: 449-494.
Subrahmanyam M (1998). A prospective randomized clinical and histological study of superficial burn wound healing with honey and silver sulfadiazine. Burns, 24: 157-161.
Trombetta D, Puglia C, Perri D, Licata A, Perogolizzi S, Lairiano ER, De Pasquale A, Saija A, Bonina FP (2006). Effect of polysaccharides from *Opunta ficus-indica* cladodes on the healing of dermal wounds in the rat. Phytomed., 13: 289-294.
Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M (1996). Comparative study on the antibacterial activity of phytochemical flavanones against methicillinresistant Staphylococcus aureus. J. Ethnopharmacol., 50: 27-34.
Yoshie SY, Hsieh YP, Suzuki T (2003). Distribution of flavonoids and related compounds from seaweeds in Japan Accession number. J. Tokyo Univ. Fish, 89: 1-6.