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Wound healing properties of aqueous extracts of *Sargassum illicifolium*: An *in vitro* assay

Amal D. Premarathna[a], T.H. Ranahewa[a], S.K. Wijesekera[b], R.R.M.K.K. Wijesundara[a], Anura P. Jayasooriya[c,d], Viskam Wijewardana[a,e], R.P.V.J. Rajapakse[a,*]

[a] Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka
[b] Department of Zoology, Faculty of Natural Sciences, Open University, Kandy Regional Center, Polgolla, Sri Lanka
[c] Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka
[d] Department of Basic Veterinary Sciences, Faculty of Medical Sciences, University of West Indies, Trinidad and Tobago, West Indies
[e] International Atomic Energy Agency (IAEA) Vienna, Austria

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ABSTRACT

Background: Seaweeds contain biologically active molecules which are important in the pharmaceutical industry. The present study was aimed to investigate the effects of aqueous extract of seaweeds:*Sargassum illicifolium* (S.i) on the stimulation of mouse fibroblast cell proliferation and migration.

Materials and methods: *Sargassum illicifolium*-SW12, *Sargassum illicifolium*-SW13, and *Sargassum illicifolium*-SW23 were collected from Northern, North Western and Southern coastal sites of Sri Lanka respectively. To determine the cytotoxic of S.i extracts were used the MTT assay. The scratch wound healing assay was performed using the mouse fibroblasts (L929) cells that assess the cell proliferation and migration. The highest cell proliferation of S.i species aqueous extracts was measured by observing the change in the size of the scratch wound area.

Results: A concentration of S.i extracts 0.954 ± 0.04 μg/μl (Mean ± SE), ($P < 0.05$) had highest proliferative and migratory effect on L929 cells when compared with the control. Cell proliferation and/or migration was as follows: S.i-SW23 extracts (0.97 μg/μl, 205.4 ± 17.32%) was higher compared to extracts of S.i-SW12 (1.06 μg/μl, 175.8 ± 13.09%), S.i-SW13 (0.94 μg/μl, 178.8 ± 2.22%) and the control (100%). Scratch wound healing of S.i-SW23 extracts exhibited a significant enhancement within 24 h ($P < 0.05$) with a wound area of 97.83 ± 0.05% compared with the control (46.11 ± 0.54%). At 24 h S.i-SW23 extracts completely closed the wound.

Conclusions: Cell proliferation and migration were significantly faster in those treated with S.i-SW23 aqueous extracts when compared with the control group. Moreover, no cytotoxic effect of S.i aqueous extracts on L929 cell line were observed. This study suggests that S.i might be a potential therapeutic agent for skin wound healing by promoting fibroblast proliferation and migration. Further studies are highly warranted to identify active molecules in the extracts. Moreover, *in-vivo* studies would further verify wound healing therapeutic applications shown here.

1. Introduction

Seaweed is a rich source of bioactive compounds that produces a great variety of secondary metabolites which are important in the pharmaceutical industry [1,2]. Seaweeds were used in traditional medicine for many centuries and in recent years generated a growing interest in food industries [3]. Many efforts for alternative and new natural therapeutic strategies, have invested in marine algae as a prominent source of active biochemically compounds [4]. Marine algae has shown promising therapeutic effects as an antibiotic, anti-HIV, anticoagulant, anticonvulsant, antioxidant [5], anti-inflammatory, antinoceptive [6,7], wound healing, antiulcer [8], antitumor [9] and hepatoprotective [10] effects. Biologically active compounds of tannins, triterpenoids and alkaloids have been found to affect one or more phases of wound healing process [11,12]. The treatment of chronic wounds is sometimes arduous, with a prevalence of 4.5 per 1000.

Abbreviations: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; S.i, *Sargassum illicifolium*; L929, Mouse fibroblast cell; °C, Celsius; SE, Stranded Error

* Corresponding author.
E-mail address: jayanthar@pdn.ac.lk (R.P.V.J. Rajapakse).

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Chronic wounds affects the life quality especially in older adult population [13] and cutaneous wound appears more frequently in diabetic's patients [14,15] leading to a painful condition. Therefore, new natural products would be useful as therapeutic or even in the prevention of chronic wounds. The wound is the result of accidental damage or a surgical procedure. It is particularly susceptible to bacterial and other infections, and provides an entry point for systemic infections [16]. Wound healing is a specific process leading to the restoration of injured tissues [17]. Wound healing process can be described as regeneration of the injured connective tissue of wounds in order to fill the wound gap followed by proliferation and migration of dermal and epidermal cells, and matrix synthesis [18]. Wound healing drugs are still unsatisfactory because of their low availability, high cost, and several detrimental side effects [12]. Therefore, medicinal plant derived drugs are under great demand due to common belief that they are safe, reliable, clinically effective, low cost and better tolerated by patients [19]. Therefore, the findings in this study could provide baseline information and accumulating evidence suggests that bioactive compounds extracted from *S.ilicifolium* have an effective wound healing activity by promoting the cellular proliferation and migration of fibroblast cells. However, this valuable asset is less exploited so far. In the current study we investigated the effects of seaweed extracts in wound healing in-vitro and presents data that warrants further in-vivo experiments.

2. Materials and methods

2.1. Seaweeds sample collection

Seaweeds *Sargassum illicifolium* species J.Agardh 1848 (Ochrophyta: Phaeophyceae :Fucales: Sargassaceae) were collected on January 2015 from the coastal algae beds Kankasannthure (N09° 48.592′E, 080° 02.546′), Negombo (N07° 12.170′E, 079° 48.570′) and Ahangama (N05° 58.006′E, 080° 22.482′) in Sri Lanka. The seaweeds were authenticated at the "National Herbarium of Peradeniya Botanical Garden" and a voucher specimen representing, *Sargassum ilicifolium* (Kankasannthure, Specimen NO; SW12), *Sargassum ilicifolium* (Negambo, Specimen NO; SW13) and *Sargassum ilicifolium* (Ahangama, Specimen NO; SW23) were deposited in the Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya for future references. Seaweeds were washed thoroughly first in seawater, then in tap water and finally, in distilled water to remove all sand particles, impurities and epiphytes. Then the seaweed samples were dried at 40 °C for four days until constant weight was obtained. The samples were made as a 0.5 mm particle size powder with an electric grinder (Herbal Grinder CS-700, China) and stored at −20 °C.

2.2. Preparation of seaweed aqueous extract

100 g of seaweed powder was soaked in 500 ml of distilled water and it was kept for 1 h at 40 °C in an ultrasound sonicator (Branson 2510, Danbury, USA) to permit full extraction of the active ingredients. Then the samples were shaken in a roller (Denley-spiramix 5, UK) at RT. After three days the preparation was filtered using nylon mesh 0.50 µm to the extracts. The filtered extracts were centrifuged (Beckman Avanti, UK) at 15,000 rpm for 10 min at 4 °C and the supernatant was used for the *in-vitro* experiment. Finally, the extracts were kept in the fridge in a closed container prior to use.

2.3. Cell culture

The Mouse fibroblast cell line (L929) was purchased from American Type Culture Collection (ATCC), USA. Cells were maintained at Roswell Park Memorial Institute medium (RPMI-1640), L- Glutamine and supplemented with 10% of Fetal Bovine Serum (FBS, Invitrogen, Gibco, UK), 2 g/l sodium bicarbonate, 10 ml/L penicillin and streptomycin (PSA), 5 ml/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Cell cultures were maintained at 37 °C in a humidified 5% CO_2 incubator (MCO.20AIC, Japan).

2.4. Cell viability assay

L929 cells were seeded in 96-well plates (Corning Glass-work, Corning, NY) at a concentration of 2×10^5 cells/well and incubated for 24 or 48 h to reach full confluence. Next, media (50 µl) containing aqueous extracts of different *Sargassum ilicifolium* (SW12, SW13 and SW23) species was added 50 µl per well and incubated for further 24 h. A two-fold dilution series of ten dilutions were used and there were three (3) replicates (8 wells for each) in each concentration. Negative and positive control tests were also prepared using distilled water and ethanol (70%), respectively. 24 h later, the treatment solutions were removed from the wells which were washed again with PBS to remove any remaining traces. 10 µl of MTT (5 mg/ml in PBS) solution and 90 µl ascorbate free cell culture media, RPMI 1640 was added and incubated for another 4 h at 37 °C. Reaction was stopped by adding 100 µl Dimethyl Sulfoxide (DMSO, Spectrophotometric grade) and finally absorbance was measured using ELISA reader (Muitiskan Ex, German) at wave lengths: reference, 630 nm and test, 570 nm. *In-vitro* cytotoxic activity was measured by the MTT assay as previously described [20]. All experiments were carried out in triplicate.

2.5. Scratch wound healing assay

The scratch wound healing assay was used for studying cell migration and proliferation [21]. L929 cells (70–80% confluence, 24 h) were seeded into a 24-well tissue culture plate. Next, cell culture monolayers and co-cultures were scratched with a sterile 200 µl pipette tip across the center of the well. While scratching across the surface of the well, the long-axial of the tip was kept perpendicular to the bottom of the well. After scratching, the wells were gently washed twice with medium to remove the detached cells. 300 µl of growth medium was added to each well and photographed using a camera attached to inverted microscope to obtain the same field during the image acquisition; markings were created to use as reference points close to the scratch. The cell culture medium was replaced immediately with 150 µl fresh medium supplemented with 150 µl S.*ilicifolium* aqueous extracts, to the corresponding wells containing the scratched cell monolayer. The wound gap was photographed at 0 h, 12 h and 24 h' time period to check the wound healing efficiency of the S.*ilicifolium* extracts.

2.6. Microscopy and image analysis

Scratch wound closure was viewed using an inverted microscope and image captured with a digital camera (Nikon Coolpix 4500: Nikon, Tokyo, Japan). Images were analyzed by Carl Zeiss Microscopy GmbH software by monitoring the width of the scratch area at different time intervals (0, 12 and 24 h) to calculate wound closure.

2.7. Statistical analysis

Graph Pad Prism Version 4.03 for Windows (Graph Pad Software, San Diego, CA, USA) was used for all statistical analysis. Data are expressed as values: Mean ± SE of eight replicates and analyzed by One-way analysis of variance. *P < 0.05 when compared with control. Control is the untreated L929 cells. *a* = when compared with the control, *b* = when compared with the positive, (*) indicates statistically significant difference from respective group using ANOVA, followed by Tukey comparisons test ($p > 0.05$). (†) indicates statistically no significant difference from respective group using ANOVA, followed by Tukey comparisons test ($p > 0.05$).

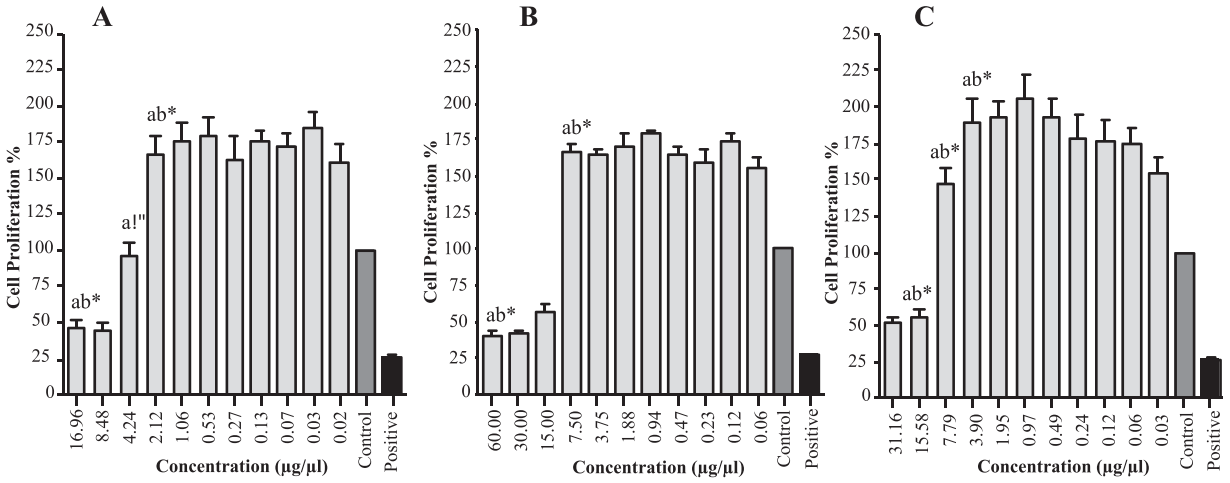

Fig. 1. The proliferation of mouse fibroblast cell line (L929). Control: received an equal amount of distilled water, Positive: received an equal amount of ethanol (70%), (A): received *S.ilicifolium* extracts (SW12, 16.96-0.02 µg/µl), (B): received *S.ilicifolium* extracts (SW13, 60.00-0.06 µg/µl), (C): received *S.ilicifolium* extracts (SW23, 31.16-0.03 µg/µl).

3. Results

3.1. Cells viability and IC50

The viability of L929 cells was increased by the treatment of aqueous S.*ilicifolium* extract (Fig. 1). Increase in extracts concentration led to a gradual decrease in cell viability as higher concentrations were found to be cytotoxic to L929 cells. The non-toxic concentration (0.03 mg/ml) of extract was used for further testing in scratch wound healing (cell migration) assay.

Fig. 1: The proliferation of mouse fibroblast cell line (L929). Control: received an equal amount of distilled water, Positive: received an equal amount of ethanol (70%), (A): received *S.ilicifolium* extracts (SW12, 16.96-0.02 µg/µl), (B): received *S.ilicifolium* extracts (SW13, 60.00-0.06 µg/µl), (C): received *S.ilicifolium* extracts (SW23, 31.16-0.03 µg/µl).

Fig. 2**: (I).** The Viability of mouse fibroblast cell line (L929) using *Sargassum ilicifolium* crude extracts. Control: received an equal amount of distilled water, Positive: received an equal amount of ethanol (70%), (A): received S.*ilicifolium* extracts (SW12, 16.96 µg/µl), (B): received S.*ilicifolium* extracts (SW13, 60.00 µg/µl), (C): received S.*ilicifolium* extracts (SW23, 31.16 µg/µl). **(II).** The IC50 of mouse fibroblast cell line (L929) using *Sargassum ilicifolium* extracts. A) *Sargassum ilicifolium*-SW12 extract (IC50 = 07.98 µg/µl), B) *Sargassum ilicifolium*-SW13 extract (IC50 = 28.73 µg/µl), C) *Sargassum ilicifolium*-SW23 extracts (IC50 = 28.12 µg/µl).

Table 1: Cell proliferation (%) values *vs*. concentrations and the respective *Sargassum ilicifolium* species. Control: received an equal amount of distilled water, Positive: received an equal amount of ethanol (70%), (A): received *S.ilicifolium* extracts (SW12, 16.96-0.02 µg/µl), (B): received *S.ilicifolium* extracts (SW13, 60.00-0.06 µg/µl), (C): received *S.ilicifolium* extracts (SW23, 31.16-0.03 µg/µl).

Table 2: The IC50 (µg/µl) of mouse fibroblast cell line (L929) using *Sargassum ilicifolium* extracts. Control is the untreated L929 cells.

3.2. Scratch wound healing assay

Fig. 3**: (I).** Proliferation of mouse fibroblast cell line (L929) treated with *Sargassum ilicifolium* extracts concentration 0.954 ± 0.043 µg/µl (Mean ± SE). **(II).** The scratch wound healing activity of mouse fibroblast cell line (L929). using *Sargassum ilicifolium* extracts. Control: received an equal amount of distilled water, Positive: received an equal amount of ethanol (70%), (A): received S.*ilicifolium* extracts (SW12, 1.06 µg/µl), (B): received S.*ilicifolium* extracts (SW13, 0.94 µg/µl), (C): received S.*ilicifolium* extracts (SW23, 0.97 µg/µl).

Seaweeds aqueous extracts of *S.illicifolium* -SW12 (1.06 µg/µl), *S.illicifolium* -SW13 (0.94 µg/µl), and *S.illicifolium* -SW23 (0.97 µg/µl) were shown a higher rate cell proliferation and migration.

Table 3: The scratch wound healing activity (migration) of mouse fibroblast cell line (L929) using *Sargassum ilicifolium* extracts. Control: received an equal amount of distilled water, (A): received S.*ilicifolium* extracts (SW12, 1.06 µg/µl), (B): received S.*ilicifolium* extracts (SW13, 0.94 µg/µl), (C): received S.*ilicifolium* extracts (SW23, 0.97 µg/µl).

Fig. 4: L929 fibroblast cell observed after injury to the cell monolayer from the *in-vitro* scratch assay. Microscopic inspection (40x magnification) of immediately after scratching (0 h) and after 12 and

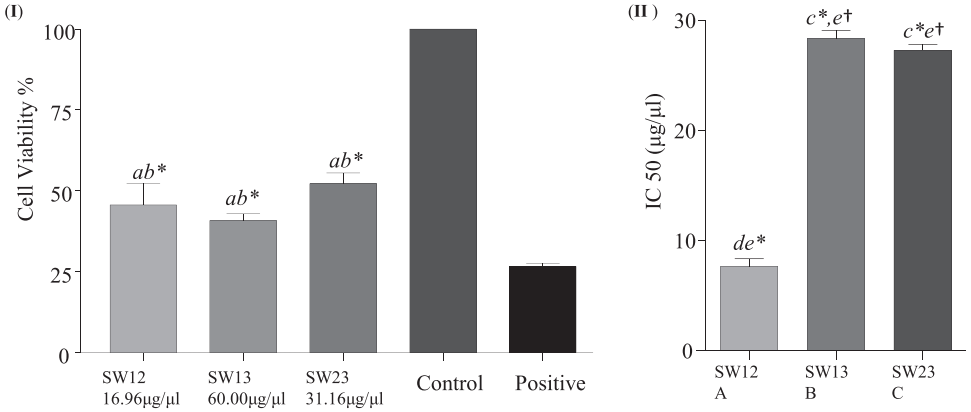

Fig. 2. (I). The Viability of mouse fibroblast cell line (L929) using *Sargassum ilicifolium* crude extracts. Control: received an equal amount of distilled water, Positive: received an equal amount of ethanol (70%), (A): received S.*ilicifolium* extracts (SW12, 16.96 µg/µl), (B): received S.*ilicifolium* extracts (SW13, 60.00 µg/µl), (C): received S.*ilicifolium* extracts (SW23, 31.16 µg/µl). **(II).** The IC50 of mouse fibroblast cell line (L929) using *Sargassum ilicifolium* extracts. A) *Sargassum ilicifolium*-SW12 extract (IC50 = 07.98 µg/µl), B) *Sargassum ilicifolium*-SW13 extract (IC50 = 28.73 µg/µl), C) *Sargassum ilicifolium*-SW23 extracts (IC50 = 28.12 µg/µl).

Table 1
Cell proliferation (%) values *vs*. concentrations and the respective *Sargassum ilicifolium* species.

Sargassum illicifolium SW-12 Kankasannthure		*Sargassum illicifolium* SW-13 Negombo		*Sargassum illicifolium* SW-23 Ahangama	
Concentration µg/µl	Cell proliferation	Concentration µg/µl	Cell proliferation	Concentration µg/µl	Cell proliferation
16.96	45.67 ± 6.675	60.00	40.86 ± 2.071	31.16	52.18 ± 3.373
8.48	43.81 ± 7.227	30.00	42.86 ± 1.673	15.58	55.31 ± 4.839
4.24	96.49 ± 9.146	15.00	56.20 ± 6.525	7.79	146.3 ± 11.09
2.12	165.9 ± 12.69	7.50	167.1 ± 4.462	3.90	189.2 ± 15.94
1.06	175.8 ± 13.09	3.75	164.5 ± 3.059	1.95	193.1 ± 11.30
0.53	179.1 ± 12.69	1.88	169.5 ± 10.23	0.97	205.4 ± 17.32
0.27	162.1 ± 17.84	0.94	178.8 ± 2.222	0.49	193.6 ± 11.62
0.13	174.8 ± 7.610	0.47	164.0 ± 5.979	0.24	178.4 ± 15.55
0.07	171.5 ± 10.17	0.23	159.1 ± 8.646	0.12	175.9 ± 14.21
0.03	184.2 ± 11.19	0.12	174.5 ± 5.118	0.06	173.7 ± 11.37
0.015	160.0 ± 13.40	0.06	156.2 ± 7.165	0.03	154.1 ± 11.69

Control: received an equal amount of distilled water, Positive: received an equal amount of ethanol (70%), (A): received *S.ilicifolium* extracts (SW12, 16.96-0.02 µg/µl), (B): received *S.ilicifolium* extracts (SW13, 60.00-0.06 µg/µl), (C): received *S.ilicifolium* extracts (SW23, 31.16-0.03 µg/µl).

Table 2
The IC50 (µg/µl) of mouse fibroblast cell line (L929) using *Sargassum ilicifolium* extracts.

Voucher no; and Location	Seaweeds Name	IC50 µg/µl
SW-12 Kankasannthure	*Sargassum illicifolium*	07.98 µg/µl^{bc*}
SW-13 Negombo	*Sargassum illicifolium*	28.73 µg/µl^{a*c†}
SW-23 Ahangama	*Sargassum illicifolium*	28.12 µg/µl^{a*b†}

24 h of wound healing. Control: received an equal amount of distilled water, (A): received S.*ilicifolium* extracts (SW12, 1.06 µg/µl), (B): received S.*ilicifolium* extracts (SW13, 0.94 µg/µl), (C): received S.*ilicifolium* extracts (SW23, 0.97 µg/µl).

The wound area has been outlined and pseudo-colored in red. Comparison of migration in different S.*ilicifolium* extracts and time in the stretch wound healing assay, migration was quantified under control conditions and in the presence treatment.

4. Discussion

The wounds are the major cause of morbidity and mortality in the world. The wound healing process of repair that follows skin injury is dynamic, complex, and well-organized [22] and inflammation response, cell proliferation, angiogenesis promotion and matrix deposition culminating with the reorganization of the injury [23]. It mainly depends on the repairing ability of the tissue, the type and extent of damage and general state of the health of the tissue. Wound to a great extent is considered as a major threat to humankind since there is a gap of availability of promising wound healing drugs. This is a compelling reason to search for new wound healing compounds from various natural sources like seaweeds. In the present study aqueous extraction was used to test the *in vitro* scratch wound healing activity. Therefore, aqueous extraction was used for the ease of preparation, cost-effectiveness and less side-effects. Before testing the effectiveness of seaweeds for their therapeutic effects, it isimportant to investigate the cytotoxicity, and we estimated the non-toxic concentrations of the doses that used be used for futher experiments. The scratch wound healing activity of Sri Lankan seaweeds has not been studied extensively so far. There were zero studies reported from Sri Lanka that have been conducted to evaluate scratch wound healing activity of seaweeds on live cells (eg; L929 cell line). In addition, the findings of this study will aid in the development of cheap wound healing therapeutics in the future with the maximum utilization of available natural resources. Therefore, the major objective of this study is to investigate scratch wound healing properties of *Sargassum ilicifolium* species against L929 cells. The proliferation and migration capabilities of fibroblasts exposed to estimated using the *in vitro* scratch wound assay, which measures the expansion of a cell population on surfaces [24]. *Sargassum* species one of the marine macro algae belonging to the class Phaeophyceae, is widely distributed in tropical and temperate oceans. It belongs to the marine family Sargassaceae and order Fucales. It is large, cost effectively important and ecologically dominant brown algae present in much of the tropics. In the last decades, seaweed metabolites presenting biological activities have been increasingly discovered. Such compounds have shown antibacterial, cytotoxic and stimulating cell migration, anticancer properties, effects on the immune response and

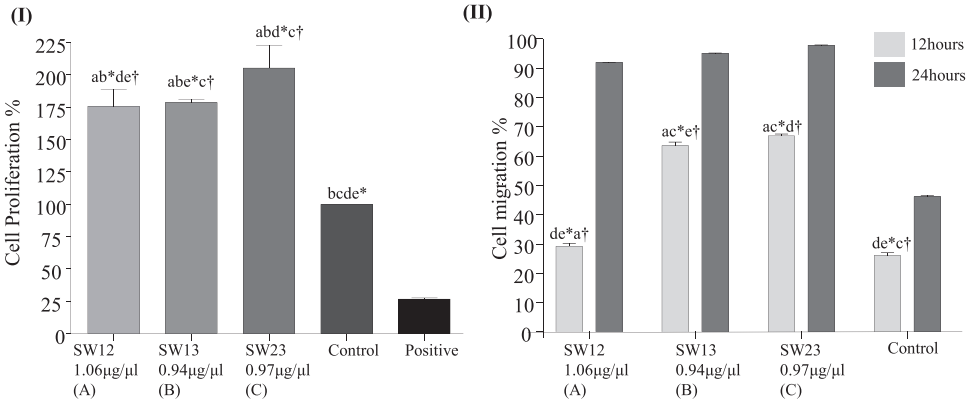

Fig. 3. (I). Proliferation of mouse fibroblast cell line (L929) treated with *Sargassum ilicifolium* extracts concentration 0.954 ± 0.043 µg/µl (Mean ± SE). **(II).** The scratch wound healing activity of mouse fibroblast cell line (L929). using *Sargassum ilicifolium* extracts.

Table 3
The scratch wound healing activity (migration) of mouse fibroblast cell line (L929) using *Sargassum ilicifolium* extracts. Control: received an equal amount of distilled water, (A): received S.*ilicifolium* extracts (SW12, 1.06 μg/μl), (B): received S.*ilicifolium* extracts (SW13, 0.94 μg/μl), (C): received S.*ilicifolium* extracts (SW23, 0.97 μg/μl).

Species name; and treatment	Concentration μg/μl (0.954 ± 0.04 μg/μl)	12hours	24 hours
SW-12; *Sargassum ilicifolium*	1.06	$29.29 \pm 0.88^{a\dagger cd*}$	$91.95 \pm 0.17^{acd*}$
SW-13; *Sargassum ilicifolium*	0.94	$63.64 \pm 1.16^{ab*d\dagger}$	95.11 ± 0.06
SW-23; *Sargassum ilicifolium*	0.97	$66.90 \pm 0.59^{ab*}$	97.83 ± 0.05
Control; Distilled water		$26.12 \pm 0.96^{b\dagger cd*}$	$46.11 \pm 0.54^{bcd*}$

anti-inflammatory activity [25]. MTT assay provides several desirable properties of living, metabolically active cells for assaying cell viability and proliferation and the absorbance value getting from the colorimetric assay is directly proportional to the live cell number [20]. The extracts of *S.illicifolium* (SW12, SW13, SW23) did not show any toxic effect on L929 cell line when using the low concentration of extracts. Crude extracts of *S.ilicifolium* (SW12;16.96 μg/μl, SW13;60.00 μg/μl, SW23;31.16 μg/μl) had cytotoxic effect against L929 cell line when compared with the control. The Viability of mouse fibroblast cell line (L929) treated with *S.illicifolium* -SW12 extract (16.96-0.02 μg/μl), *S.illicifolium* -SW13 extract (60.00-0.06 μg/μl), *S.illicifolium*-SW23 extracts (31.16-0.03 μg/μl) and the control were evaluated over the course of 24 h. The aqueous extract of *S.illicifolium* extracts diluted concentration has been induced promising cell proliferative or/and migration activity against L929 cell line. Among samples of *S.illicifolium* (SW23) were collected from Ahangama algae bed exhibited the highest cell proliferation activity against L929 cell line of significant different ($p < 0.05$) with other *S.illicifolium* samples collected from Kankasannthure (SW12) and Negambo (SW13) algae beds respectively. Cell migration and proliferation are the provable in skin regeneration and wound healing [26]. Among the concentrations evaluated with higher proliferation and/or migration in comparison to the control. The extract of *S.illicifolium* did not present cytotoxicity against normal L929 mouse fibroblast cell line with the lowest concentration. Studies performed with *S.illicifolium* from the Sri Lanka demonstrated an IC50 that aqueous extract of *S.illicifolium* -SW12 extract (IC50 = 07.98 μg/μl), *S.illicifolium* -SW13 extract (IC50 = 28.73 μg/μl), *S.illicifolium* -SW23 extracts (IC50 = 28.12 μg/μl) were evaluated over the course of 24 h on L929 cells.

The results of the current study revealed that the cytotoxic effects is dose-dependent [27]. It has been shown on mouse fibroblast cell line (L929) treated with the highest concentration of *S.illicifolium* aqueous extracts. The highest proliferation of mouse fibroblast cell line (L929) was treated with *S.illicifolium* concentration 0.954 ± 0.043 μg/μl (Mean ± SE). Cell proliferation was *S.illicifolium* -SW23 extracts (0.97 μg/μl, 205.4 ± 17.32%) higher than compare with extracts of *S.illicifolium,* SW12 (1.06 μg/μl, 175.8 ± 13.09%), *S.illicifolium,* SW13 (0.94 μg/μl, 105. 178.8 ± 2.22%) and control (100%) (mean ± SE, *N* = 08). This could be due to an effect of the seaweed extracts as *S.illicifolium* which may enhance the activity of some stage in the wound healing process. Scratch wound healing assay is one of the earliest developed methods to study directional cell migration *in vitro* [28] and it has been widely used for complex process that requires various coordinated cellular activities in wound healing. Quantification of migration is the most problematic part of *in vitro* wound healing assay [29]. Mouse fibroblast cells (L929) migration is an important process in the proliferation of cells and the repair of damaged tissue from seaweed extraction treated. Cell migration is a complex process that requires various coordinated cellular activities. For our investigation, cell proliferation and migration has been shown *S.illicifolium* extracts. Cell proliferation and migration is the most important for research and discovery in biology and medicine. Several studies have reported that seaweed has a great potential of anti-inflammatory, anti-microbial and *in vitro* wound healing properties of brown seaweeds *Padina gymnospora* [30]. This study demonstrated that the future studies to determine essential active compound(s) which could be extracted from *S.illicifolium* and purified components are required to appreciate the complete mechanism of wound healing activity of aqueous extract of *S.illicifolium.* Therefore, isolation of principle compounds and development of novel wound healing drugs is important for possible future therapeutic applications.

5. Conclusion

According to the results, high amount of *S.illicifolium* aqueous extracts concentration has shown the cytotoxic effect against L929 cell line when compared with the control. The starch wound healing assay represent the cell migration. Extracts of *S.illicifolium* has properties that promotes accelerated wound healing activity compared with the control. It is possible to relate the favorable effect of *in vitro* wound healing of the *S.illicifolium* extracts to repair processes. In addition, the cell proliferation confers a synergistic therapeutic advantage to *S.illicifolium*. However, further investigations should be conducted to elucidate the mechanisms of action of the *S.illicifolium* extracts. Determining active constituents in the extract would be an economical and safe option for wound healing approaches in the clinic.

Ethical approval

The authors declare that no experiments were performed on humans or animals in this study.

Consent for publication

We certify this manuscript has not been published elsewhere and is not submitted to another Journal. All authors have approved the manuscript and agreed with submission to journal of Wound Medicine.

Availability of data and material

The datasets and materials are contained during the in this study.

Competing interests

The author(s) declare that they have no competing interests.

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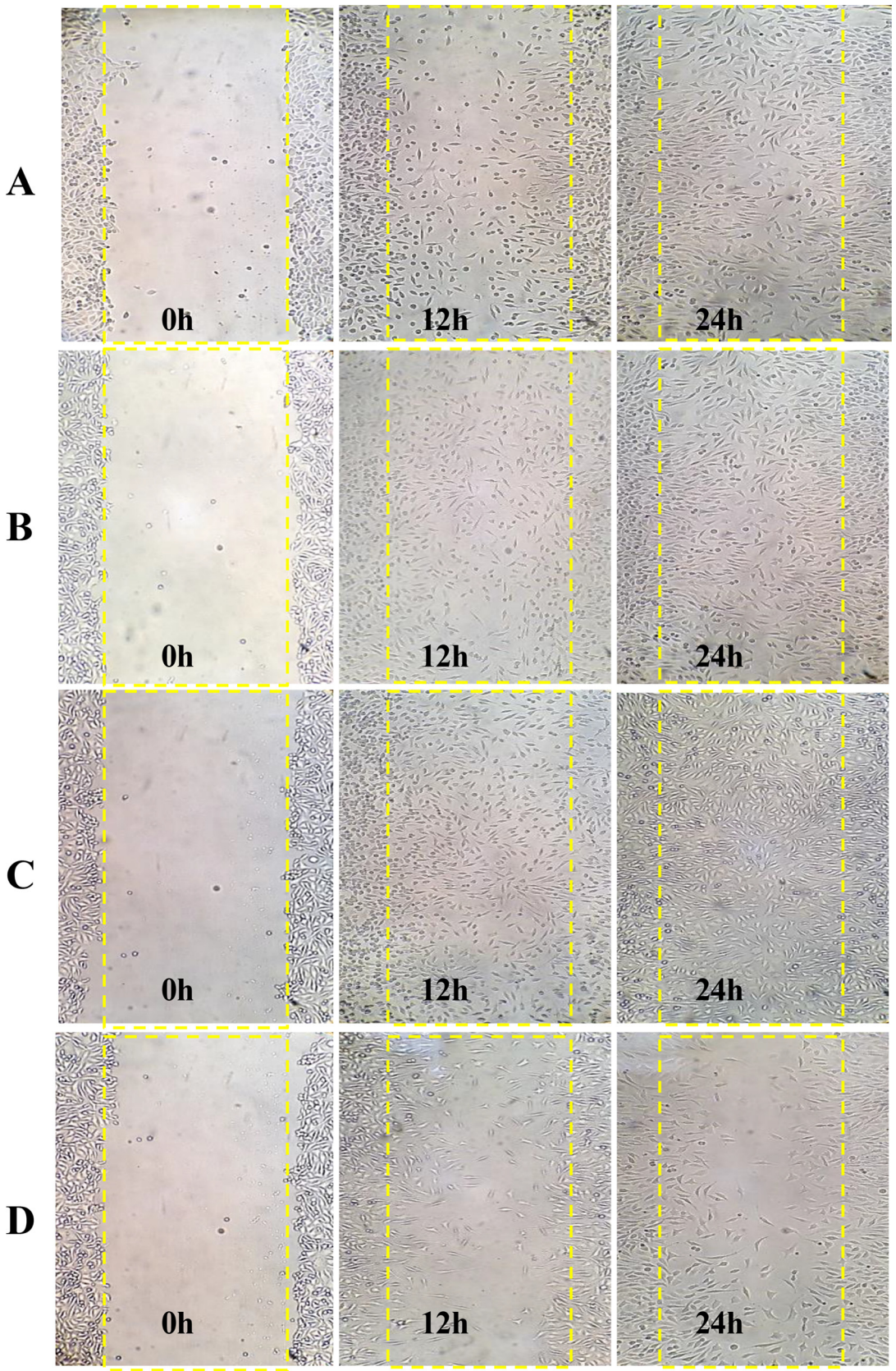

Fig. 4. L929 fibroblast cell observed after injury to the cell monolayer from the *in-vitro* scratch assay. Microscopic inspection (40x magnification) of immediately after scratching (0 h) and after 12 and 24 h of wound healing.

Authors' contributions

AD and RPVJ conceived the study, concept, and design and conducted most of the laboratory experiments; analyzed and interpreted experimental results. RPVJ, SK, RRMKK and AP contributed to the proposal for study design and supervision of the study, interpretation of data, drafting of the proposal and article. VW contributed with critical revision of the manuscript. TH supported carrying out laboratory experiments. All authors read and approved the final manuscript.

Ethical clearance

The authors declare that no experiments were performed on humans or animals in this study.

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