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Scoping Review: Evaluation of Sea Cucumber as a New Therapeutic Agent for Wound Healing Treatment in Clinical Trials and in vivo Studies

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ABSTRACT

Objectives: The development of biomaterials with the potential to hasten wound healing is a major concern in the biomedicine industry. Sea cucumbers, also known as bêche-de-mer or gamat, have long been used for food and folk medicine in Asian and Middle Eastern countries. Due to their pharmacological benefits, sea cucumbers have been explored for medical use, especially in dermatological formulas for wound healing treatment. The biological activities of sea cucumbers, such as their anti-inflammatory, anti-bacterial, antioxidant, and anti-coagulant properties, are imperatively facilitating the wound healing process owing to their abundance of bioactive compounds, such as phenolic peptide, glycosaminoglycan, saponin, collagen, and fucoidan chondroitin sulphates. However, there were still insufficient studies that systematically reviewed the existing literature on the evaluation of sea cucumber as a wound healing agent. Thus, this scoping review will encompass the wound healing potential of sea cucumber for in vivo and clinical trials. Materials and Methods: The searches were conducted using three main databases, which are PubMed, Science Direct, and Google Scholar, with papers released between 1970 and 2022. Twelve studies met the inclusion criteria in which these studies compromise in vivo and clinical trials that evaluate five different types of wounds, which are excision, incision, ulcer, diabetic, and burn wounds. Results and Conclusion: Findings from in vivo and clinical trials provide consistent evidence through macroscopic and microscopic observation; the studies prove that sea cucumber can enhance tissue repair and wound healing through regulation of inflammatory response, fibroblast proliferation and amplifying the angiogenesis process.

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INTRODUCTION

The introduction of complementary and alternative medicine to wound healing is changing mainstream medical practice. In recent years, a rising number of research papers on the relationship between wound healing treatment and diverse types of natural resources have been published (Figure 1). It includes the used of organic and inorganic materials such as the medicinal plant extracts (Centella asiatica, Aloe barbandensis, Bidens pilosa, Rauwolfia serpentine, Dudonae vicosa), honey, animals derived extract (chitosan, collagen, anabolic steroids, silkworm sericin, peptides, peptidoglycan) and recently, the sea cucumber. Sea cucumber is a marine invertebrate which is popularly used as a traditional medicine in Southeast Asian countries (Wen et al. 2018). It is one of the most popular and precious health products owing to its nutritional content and good taste (Zhang et al. 2010). In Malaysia, sea cucumber is reported to be an effective alternative treatment in wound healing management, especially in cuts and burns (Wen et al. 2010). It has more than one thousand species, with new species reported and described each year (Conand, 2006).

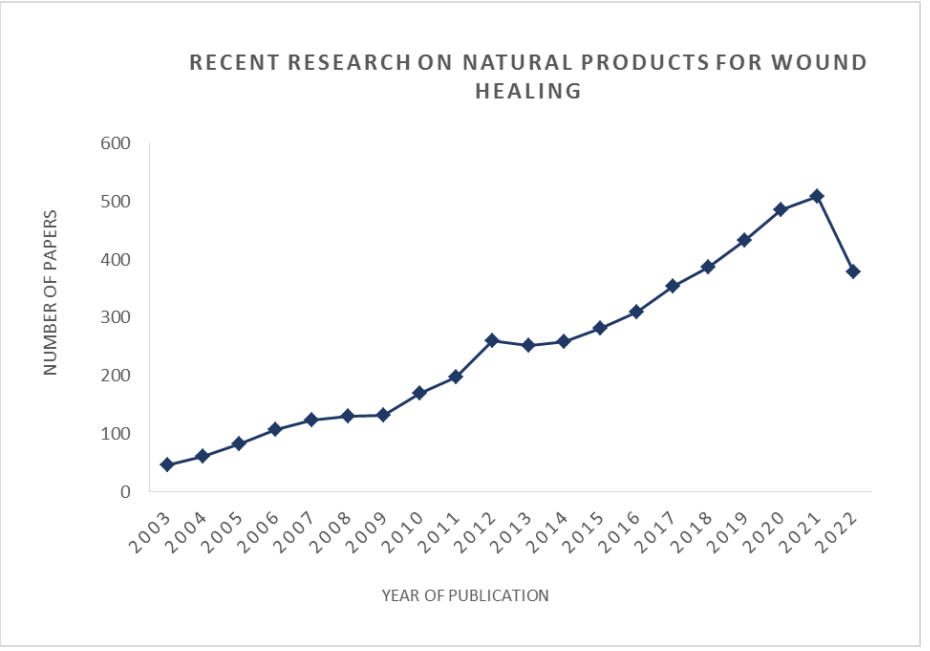

Fig. 1. Research study on the use of natural products for wound healing treatments from the year of 2003 to 2022 (source: PubMed, 2022)

Most of the biodiversity of the sea cucumber species is in the Asia-Pacific region (Pangestuti and Arifin, 2018). Among the most important commercial species includes the Isostichopus badionotus, Holothuria arenicola, Stichoppus horrens, and Actinophyga mauritiana (Pauzi et al., 2018; Damaiyanti et al., 2019; Arfani et al., 2020). Based on the previous study, the therapeutic effect of sea cucumbers is due to the presence of numerous bioactive compounds such as the glycosaminoglycan (GAG), Fucosylated chondroitin Sulphate (FCS), triterpene glycosides (saponin), cerebrosides, fucoidan, Frondanol A5, acid mucopolysaccharide, and phosphotidylcholines (Shi et al., 2022). These pharmacologically active compounds considerably help to treat a variety of medical conditions, particularly those related to wound healing, such as hyperglycemia, insulin resistance, diabetes, slowing the ageing process, increasing immune function, reducing inflammation, and promoting wound healing (Shi et al., 2016).

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Wound healing is a natural process, where tissue damage is repaired involving of four overlapping processes, namely haemostasis (blood clothing), inflammation, proliferation and tissue remodelling (Wilkinson & Hardman, 2020). This intricate process is carried out by various types of cells such as the fibroblasts, keratinocytes, inflammatory cells, and endothelial cells, and is influenced by several factors. Infection, age, sex hormone, stress, diabetes, alcoholism, medications, smoking, nutrition, oxygenation, and obesity is among the crucial factors that influence wound healing (Wilkinson & Hardman, 2020). The disruption of the skin barrier is due to the physical disturbance that leads to the abrasion of the skin surface and lead to complex wound healing process (haemostasis). The vital aspect in wound healing is to reduce the inflammation, prevent wound infection and speed up the wound healing.

Sea cucumbers are said to have the ability to accelerate wound healing (Damaiyanti et al. 2019), and as they feed on bottom sediment, they are believed to contain a prominent level of branched chain fatty acids that facilitate tissue healing and wound repair (Subramaniam et al. 2013). According to Masre et al. (2010), sulfated glycosaminoglycan can improve rat wound contraction by up to 60% and this study was supported by Damaiyanti et al. (2019), who found that the water extract of gold sea cucumber enhanced oral traumatic ulcers by increasing and speed up the wound closure. Besides, in a study on diabetic foot ulcers (Ogai et al. 2017), sea cucumber was demonstrated to be a potent antibacterial agent that produces unfavorable conditions for the growth of bacteria (acidic pH), rapidly clears wound infection, and facilitates the growth of new tissue for wound healing.

Even though humans from all over the world have used a variety of methods to heal wounds for generations, the development of modern wound healing treatments only began in the twentieth century. To the best of our knowledge, this is the first comprehensive review of the efficacy of sea cucumbers for wound healing treatment across multiple in vivo pre-clinical studies and human clinical trials. The current scoping review was conducted to analyse the most recent information available on the efficacy of sea cucumber-derived bioactive for human health and nutrition based on in-vivo and clinical studies between 2012-2021 (Liang et al., 2022). These bioactive compounds could be used for further development of new interventions in biomedicine industry for wound healing management in the future.

METHODS

Study inclusions

The research study is guided by Reporting standards for Systematic Evidence Syntheses (ROSES) review protocol. Reliable summaries of the evidence will be provided using this protocol to facilitate evidence-informed decision making in conservation and environmental management. The formulation of the research question for this study was based on PICo. PICo is a tool that focuses on the Patient or Population, Intervention, Comparison and Outcome of a usually quantitative articles. It is commonly used to identify components of clinical evidence for systematic review in evidence-based medicine and is endorsed by the Cochrane Collaboration (Methley et al., 2014). Based on these concepts, three main aspects included in the review which are sea cucumber (Population), sea cucumber as a wound healing agent (Intervention) and different types of wounds such as incision, excision, ulcer, diabetic and burn (Comparison) which guide the authors to formulate its main research question - How does sea cucumber act as a wound healing agent in a different type of wounds? (Outcome).

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Searching strategy

The search is conducted primarily through three prominent databases, namely PubMed, Science Direct, and Google Scholars, and the keyword is enriched in the search string using Boolean operators denoted by the terms AND, OR, and NOT (Table 1). Identification is the process of looking for synonyms, related terms, and variations for the study's major keywords, which are sea cucumber, wound, and heal. The goal is to improve the keywords used in database searches so that more relevant articles can be found. The searching process in these three databases has resulted in a total of 4555 articles.

Table 1. Search string

Database	**Search string**
PubMed	TITLE-ABS-KEY (("sea cucumber" OR holothuria OR holothurian) AND (wound OR injury) AND (heal OR cure OR remedy))
Science Direct	TITLE-ABS-KEY (("sea cucumber" OR holothuria OR holothurian) AND (wound OR injury) AND (heal OR cure OR remedy))
Google Scholar	"sea cucumber" OR holothuria OR holothurian AND wound OR injury AND heal OR cure OR remedy

This study screened the selected articles by using article selection criteria that can be automatically set on the sorting tools across the database. The selection criteria are shown in Table 2. This process has excluded 4513 articles as it does not fit the inclusion criteria. The remaining articles is manually monitored to ensure all the remaining articles are in line with the criteria. During the process, four duplicate articles from different databases were removed, and 26 articles were excluded because they did not explain sea cucumber as a wound healing agent, and some articles explained the mechanism of healing in the sea cucumber itself, which was irrelevant to the study. Overall, there were only twelve articles that were selected and included in the research study as shown in Figure 2.

Table 2. Inclusion and Exclusion criteria

Criteria	**Inclusion**	**Exclusion**
Document type	Article journal (empirical data)	Encyclopedia, magazines, article review, conference proceeding
Language	English and non-English	-
Timeline	1970 – 2022	<1970

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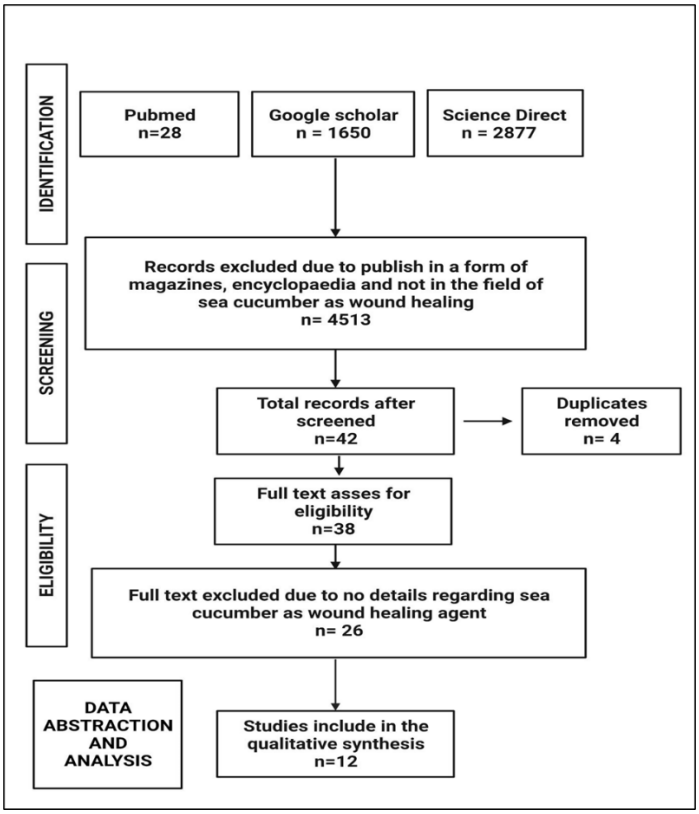

Fig. 2. Decision trail of included studies

Theme of Study

These articles were reviewed using the integrative review technique. Whittermore and Knafl (2005), reported that qualitative or mixed-method techniques are the best way to synthesize or analyse integrative data. The authors firstly do thematic analysis to generate themes. As the authors read all the related articles, a notable pattern is identified among the abstracted data of all reviewed articles. All the similar or related abstracted data are placed together, and two main groups were created. The researchers recheck all the articles again to ensure that all the main themes and sub-themes created are accurate enough. The main theme for the scoping review is in vivo and clinical study and the sub-themes are macroscopic and microscopic observation as shown in Table 3.

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No.	Studies	In vivo	Clinical studies	Type of wounds	Macroscopic			Microscopic			
					TH	CLR	DM	IF	ANG	CGN	VS
1.	(Li et al. 2018)	/		Diabetic	/			/	/	/	
2.	(Ollu et al. 2019)	/		Incisional	/	/	/	/	/		/
3.	(Subramaniam et al. 2013)	/		Excisional	/		/				
4.	(Wen et al. 2018)		/	Excisional	/		/				
5.	(Ogai et al. 2017)		/	Diabetic				/			
6.	(Damaiyanti et al. 2019)	/		Ulcer			/			/	
7.	(Fahmy et al. 2015)	/		Ulcer		/		/			
8.	(Pauzi, et al. 2018)	/		Burn				/	/		/
9.	(Zohdi et al. 2011)	/		Burn	/		/	/	/	/	/
10.	(Arfani et al. 2020)	/		Excisional	/	/	/	/		/	
11.	(Bazmakdar et al. 2021)	/		Excisional	/	/	/			/	
12.	(Mazliadiyana et al. 2017)	/		Excisional							

TH – Time healing, CLR – Color of wound, DM – Diameter of wound, IF – Inflammatory response, VS – Vascularization, ANG – Angiogenesis, CGN – collagen deposition

RESULTS

A summary of the study characteristics is shown in Table 2. In summary, 12 articles were included in the review. Five types of wounds have been remarked namely excision, incision ulcer, diabetic and burn wound. Oil, gel, serum, and sea cucumber extract were among the four different sea cucumber formulations topically applied to the wound, and two studies specifically isolate specific compounds of sea cucumber and administered intragastrically and intraperitoneally toward the subject. According to geographical origin, one study obtained its sea cucumber oil from Malaysia, and another research were not particularly mentioned.

Sea cucumber in wound healing – in vivo studies

Some parameters can determine the wound healing outcome which can be seen through microscopically and macroscopically. The degree of wound healing in various types of wounds is assessed using biochemical analysis.

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Macroscopic findings

Reduction of wound area can be seen macroscopically and can serve as an early indicator of wound healing. The time course of healing usually ranges from 5 to 10 days or within 30 days and above that can be considered as a chronic wound (Velnar et al. 2009). Ollu et al. (2019) reported that incision wound healing toward incised mice by surgical blade through detailed macroscopic findings. Based on the result, there is no significant difference between betadine and ethanol black sea cucumber extract ($p>0.05$) on the wound diameter throughout 8 days which indicates that ethanol black sea cucumber and betadine have the same effectiveness toward enhancing the process of wound healing. Meanwhile, Subramaniam et al., (2013) reported the gross morphology outcome in excisional wound study by the sea cucumber, Stichopus horrens extract. In the study, percentage of wound contraction between normal saline, povidone-iodine and sea cucumber oil is compared. Stichopus horrens extract treated group showed significantly smaller wound size compared to normal saline treated group on day 4 ($p<0.01$) and there is no significance different between normal saline and povidone-iodine treated group. In addition, Stichopus horrens extract treated group showed better cosmetic appearance on the 18th days of treatment compared to others treated group.

One study reported gastric wound healing through macroscopic evaluation of the stomach and assess the oxidative stress marker after extract of sea cucumber administered orally (Fahmy et al. 2015). Based on the macroscopic evaluation of the stomach, it was noted that the ulcer index is significantly lower in the sea cucumber extract group. The pre-treatment group with Holothuria arenicola and ranitidine give better result with 80.56% ulcer inhibition compared to the post treatment and control groups.

Damaiyanti et al. (2019) published a paper on the effectiveness of sea cucumber in the healing process of oral traumatic ulcers. In their study, topical sea cucumber gel was used to determine the effectiveness of the healing process by observing the ulcer diameter. Based on the results, 80% of gold sea cucumber (Stichopus hermanii) gel give the most decreasing diameter of the ulcer compared to negative control group ($p<0.05$). Similar results find by Mazliadayana et al., (2017) where 0.5% aqueous extract Stichopus chloronotus emulsifying ointment shows higher wound reduction compared to the positive control groups.

One of the studies found out the wound healing process through wound contraction (Zohdi et al. 2011). Based on the results, there were no significant differences in wound contraction at days 7 and 14 of post-burn treatment between all the sample groups ($p > 0.05$). However, on day 21 to 28 post-burn, there was a significant difference in the rate of wound contraction where Gamat Hydrogel (Stichopus hermanii) reported to show better wound contraction (94.43%) compared to other treatment groups; control hydrogel (58.94%), Opsite film (60.06%) and untreated wound (56.72%) which at the later stage of wound repair ($p < 0.05$). Meanwhile, study by Wen et al. (2018) shows that there are no significant differences in epithelization rate between Duoderm Hydroactive gel (ConvaTec Malaysia Sdn. Bhd.) and Stichopus Hermanii extract starting day 10 onwards.

Studies done by Arfani et al. (2020) demonstrated that all formulation (cream, gel and serum) contributes to significant improvement in wound healing activities within 14 days of treatment compared to the untreated group. This study was supported by Sunmugam et al. (2021), where he reported that applying 0.1 g of sea cucumber extract (Stichopus horrens) based creams (15% w/w) to full thickness excision wounds of rats daily for 14 days significantly improved wound contraction. In addition, Arfani et al., (2020) discovered that 9% Actinophyga mauritiana ethanol extract (SCAE) serum treatment has a significantly better result with a wound size reduction of up to 0.55 ±0.32 cm, with the largest fibroblast distribution level (10.00 ±1.00) and the highest collagen dense level (10.67±0.00) when compared to cream and gel formulation.

Meanwhile, study done Bazmakdar et al. (2021) showed that there were statistically significant differences in wound contraction between all treatment groups of Holothuria parva extract (HPE) compared

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with negative control group ($p < 0.05$). Wound contraction in 5% HPE group has a significant difference with HPE 3% and HPE 1%.

Microscopic findings

Microscopic finding is particularly important in determining the progress of the wound healing process. It includes macrophages, neutrophils, fibroblast, collagen and elastin formation (Truong et al. 2019). Same as the gross morphology outcome, most of the studies included the histopathological analysis. One of the studies reported comprehensive results regarding microscopic findings where inflammatory response, angiogenesis, collagen deposition, oxidative stress and nutritional status were evaluated (Li et al. 2018). In the study, small molecule oligopeptides isolated from sea cucumber (SCCOPs) have been intragastrically administered resulting in increased vascularisation, collagen deposition and epithelialization at four days post-wounding.

Ollu et al. (2019) reported their histopathological analysis through the count of neutrophils, macrophages, lymphocytes, angiogenesis and fibroblast and compared it with three sample groups. Based on the results, there is a significant difference across the group. The neutrophils count in betadine and ethanol black sea cucumber are significantly lower compared to the control group using normal saline. The researchers relate the result with other studies that link the decrease of neutrophils with antiseptic properties. The total count of macrophages in that ethanol black sea cucumber group was also significantly higher compared to the two other groups. The researchers believed that bioactive compounds in the sea cucumber which are saponin and flavonoid can stimulate the macrophages and further increase the transforming growth factor (TGF) which can increase proliferation and migration of fibroblasts. Fibroblast cells are also significantly higher in the ethanol black sea cucumber group compared to other groups which will increase the collagen deposition which can hasten the wound healing process (Velnar et al. 2009).

In 2019, Damaiyanti et al. published a paper on the effectiveness of sea cucumber in the healing process of oral traumatic ulcers. In their study, topical sea cucumber gel was used to determine the effectiveness of the healing process by observing ulcer diameter and collagen type 1 expression. At the end of the study, ulcer diameters were decreased as collagen type I expression increased with the treatment by Sticopus hermanii extract. Microscopic evaluation through oxidative marker also can be seen where administration of sea cucumber enhances reduction in oxidative marker (Fahmy et al. 2015).

Ogai et al. (2017) reported that there was no significant difference in wound healing between the sea cucumber and honey groups. However, there are significant differences in TNF-a level between different duration in the sea cucumber group which may indicate that sea cucumber is effective as an anti-inflammatory agent for diabetic foot ulcer (DFU) compared with honey. However, the compound in sea cucumber that contributes to higher TNF-a levels in the sample is not mentioned.

For histological changes, there were increased blood structures with major vasodilation and less inflammation. A positive effect on fibroblast proliferation and angiogenesis process can also be seen in one of the studies (Pauzi et al. 2018). Arfani et al. (2020) showed that the untreated wound demonstrated the least collagen level compared to the formulated treatment. The serum formulation with 9% SCAE treatment gives the best results in collagen level distribution followed by gel and cream. Collagen dense level in 3% SCAE serum equivalent to collagen level in 6% SCAE gel and close to 9% SCAE cream treatment.

Meanwhile, study done by Bazmakdar et al. (2021) demonstrated the proliferation of keratinocytes was revealed on day 7 and completed in 14 days for the treatment groups, and 21 days for the untreated groups.1% HPE gel showed brilliant keratinocytes proliferation and decrease the inflammatory cells reactions.

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DISCUSSION

The scoping review provide some evidence for the efficacy of sea cucumber extract in wound healing management. The use of sea cucumber extract in wound care has traditionally been used in complementary and alternative medicine. This review summarised the evidence for the use of sea cucumber extract in the management of pre-clinical, in vivo (Table 4) and clinical trials (Table 5).

Ten studies (Fahmy et al., 2015; Zohdi et al., 2011; Subramaniam et al., 2013; Li et al., 2018; Mazliadiyana et al., 2017; Pauzi et al., 2018; Damaiyanti et al., 2019; Arfani et al., 2020; Ollu et al., 2019; Bazmakdar et al., 2021) examined the effect of sea cucumber extract on wound healing based on in vivo studies for burn, incision, excision, and diabetic wound, while two clinical studies looked at the impact of sea cucumber on the rate of epithelization in skin graft donor sites (Wen et al., 2018) and the impact of sea cucumber and honey dressing on wound healing in diabetic foot ulcers patients (Ogai et al., 2017).

A study conducted by Zohdi et al. (2011) demonstrated the importance of gamat hydrogel topical application in the treatment of burns and wounds. According to the research, Stichopus hermanii extract improved burn wound healing in mice with a better cosmetic appearance. This study was supported by Mazliadiyana et al. (2017), where sea cucumber treated groups displayed faster wound reduction than other groups starting on day six with 0.5% Stichopus chloronotus emulsifying ointment ($p<0.05$). The high fatty acid profile of the sea cucumber may describe the advanced re-epithelization by topical application of gamat hydrogel. Fatty acids have been shown to promote early angiogenesis, modulate cell metabolism and division, and regulate specific genes in dermal fibroblasts and endothelial cells in order to improve wound healing and promote tissue repair (Bordbar et al. 2011). In addition, the fatty acids in the sea cucumber may serve as an energy source for cellular division and epidermal regeneration. Besides, the fatty acid composition in sea cucumber, particularly eicosapentaenoic acid (EPA) has the ability to induce prostaglandin inhibition and blood clotting activity (Bordbar et al. 2011). Thus, these properties will help to speed up wound healing treatment and tissue recovery.

Meanwhile, a study done by Zohdi et al. (2011) showed that the golden sea cucumber (Stichopus hermanni) extract speeds up angiogenesis during the healing of traumatic ulcers in Wistar rats. This finding is correlated with research by Pauzi et al. (2018) where they found that the administration of sulfated GAG from Stichopus vastus influenced the invasive migration of fibroblast during burn wound healing and stimulated new vessel formation for restoration of blood flow to the site of injury (angiogenesis) compared to the control saline group. The presence of glycosaminoglycan (GAG) within the sea cucumber may have a vital impact on wound contraction. GAG plays a key role as an inflammatory modulator and participates in a variety of biological processes such as cell-matrix interactions, chemokine activation, and growth factor activation.

Table 4. Pre-clinical and in-vivo animal model studies design

No.	Author, year and country	Gamat species	Wound type	Total (n)	Animal sample model	Administration	Treatment groups, intervention, dosage	Finding/ Outcome
1.	Fahmy et al. (2015)	*Holothuria arenicola*	Gastric ulcer (GU)	64	Wistar rats	Oral intake	The study was divided into four treatment groups.	Ulceration inhibition (%)

No.	Author, year and country	Gamat species	Wound type	Total (n)	Animal sample model	Administration	Treatment groups, intervention, dosage	Finding/ Outcome
	Egypt	(HaE)					• 1st group (8rats/group) Administered distilled water orally. • 2nd group (8 rats/group)- single oral dose indomethacin (150mg/kg) • 3rd group (Pre-treatment groups)-24 rats/group were treated with HaE or Ranitidine before exposed to GU. • 4th group (Post treatment group) -24 rats/group were exposed to GU before treated with HaE/Ranitidine	Pre-treatment groups 1st group - 72.5% 2nd group - 53.4% 3rd group - 80.56% Post-treatment groups 1st group - 51.66% 2nd group - 62.41% 3rd group - 67.78% Results of ulceration inhibition (%) indicates that pre-treatment with HaE and/or ranitidine significantly decreases the ulcer index compared to post treatment groups ($p<0.05$).
2.	Zohdi et al. (2011) Malaysia	*Stichopus hermanni*	Deep partial thickness burn wound	96	Sprague Dawley rats	Topical wound dressing	Treatment groups were divided into four with 6 rats/group. • 1st group - dressed with Gamat hydrogel • 2nd group - dressed with control hydrogel • 3rd group - dressed with Opsite film hydrogel • 4th group - negative control	Topical application treatment was studied for 28 days. At day 21 post burns, wound treated with Gamat hydrogel showed 82.31% contraction compared to control hydrogel (58.94%), Opsite film hydrogel (60.06%) and untreated wound (56.72%). At day 28 post burn, the contraction increased as below: Gamat hydrogel - 94.43% Control hydrogel - 72.25% Opsite film - 80.54%

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No.	Author, year and country	Gamat species	Wound type	Total (n)	Animal sample model	Administration	Treatment groups, intervention, dosage	Finding/ Outcome
								Negative control - 76.86% Topical application of Gamat hydrogel showed an increase of wound healing rate with better gross appearance compared to control group.
3.	Subramaniam et al. (2013) India	*Stichopus horrens*	Excision wound	18	Wistar albino rats	Topically applied twice daily	Treatment group divided into three with 6 rats/group. ● 1st group - Normal saline (control group) ● 2nd group - Povidone Iodine ● 3rd group - Gamat processed oil (Minyak Gamat Plus)	The study was done for 21 days. The percentage of wound contraction was expressed as Mean ± SEM on day 21. 1st group - 99.07 ± 0.42 2nd group - 96.9 ± 1.43 3rd group - 99.0 ± 0.48 No significant changes of wound contraction among the group ($p > 0.05$)
4.	Li et al. (2018) China	Small molecule oligopeptides isolated from sea cucumber (SCCOPs)	Diabetic wound (incision wound)	90	db/db male mice	Oral intake	The study was divided into 6 different groups, 3 control groups with 3 treatment groups. ● 1st group - normal control (NC) ● 2nd group - model control (MC) ● 3rd group - whey protein (WP) group (0.50g/kg) ● 4th group - SCCOPs-L dose group (0.25g/kg) ● 5th group - SCCOPs-M dose group (0.50g/kg)	On day 14, the macroscopic images of wound in SCCOPs treated groups closed more tightly, and the surface of the wound become smoother, compared to NC and MC groups ($p<0.05$).

No.	Author, year and country	Gamat species	Wound type	Total (n)	Animal sample model	Administration	Treatment groups, intervention, dosage	Finding/ Outcome
							• 6th group - SCCOPs-H dose group (1.00g/kg)	
5.	Mazliadiyana et al. (2017) Malaysia	*Stichopus chloronotus* (SC)	6mm diameter full thickness wound made by punch biopsy. (excision wound)	12	Sprague Dawley rats	Topically applied daily	Experiments were divided into 3 control groups and 3 treatment groups of aqueous extract emulsifying ointment (SC) • Normal control group (NC) - non-treated animals • Positive control group (PC) - animals treated with Flavine. • Negative control group (NC) - animal treated with Cetomacrogol emulsifying ointment. • 1st treatment group - 0.1 % SC • 2nd treatment group - 0.5 % SC • 3rd treatment group - 1.0%	The study was run for 10 days. Results demonstrated that 0.5% of aqueous extract *Stichopus chloronotus* emulsifying ointment has higher wound reduction and macroscopic observation compared to other groups starting at day 6 wound creation.
6.	Pauzi et al. (2018) Malaysia	Total sulfated glycosaminoglycan (GAG) *Stichopus vastus*	Full thickness burn injury	12	Male Sprague Dawley rats	Injected intraperitoneally daily for 5 days	Studies were divided into 2 groups. • Group A - rats injected with total sulfated GAG 5mg/kg. • Group B - rats were injected with Phosphate Buffer Saline (PBS).	Microscopic evaluation showed reduced neutrophils infiltration with enhanced proliferation of fibroblast and angiogenesis ($p < 0.05$) in the sulfated GAG treated group.

No.	Author, year and country	Gamat species	Wound type	Total (n)	Animal sample model	Administration	Treatment groups, intervention, dosage	Finding/ Outcome
7.	Damaiyanti et al. (2019) Indonesia	*Stichopus hermanii* (Golden sea cucumber)	Oral traumatic ulcer	20	Wistar rats	Topical application of gel daily	Studies were divided into four different groups: ● Negative control groups ● 20% golden sea cucumber extract ● 40% golden sea cucumber extract 80% golden sea cucumber extract	80% of golden sea cucumber extract is the most optimal concentration in reducing ulcer diameter on the Wistar rat's traumatic ulcer healing process.
8.	Arfani et al. (2020) Indonesia	*Actinophyga mauritiana*	Excision wound	30	*Mus musculus* (mice)	Topical application	The study was divided into 10 treatment groups with 3 rats/group. ● 1st group: untreated ● 2nd group: 3% SCAE cream ● 3rd group: 6% SCAE cream ● 4th group: 9% SCAE cream ● 5th group: 3% SCAE gel ● 6th group: 6% SCAE gel ● 7th group: 9% SCAE gel ● 8th group: 3% SCAE serum ● 9th group: 6% SCAE serum 10th group: 9% SCAE serum	Wound healing rate in serum formulation is better with 9% SCAE formulation with wound reduced up to 0.55 ± 0.32 cm.
9.	Bazmakdar et al. (2021) Iran	*Holothuria parva*	Excisional wound	35	Wistar male rat	Topical application	Study was divided into five different treatment groups: ● Group 1: 1% *Holothuria parva* extract gel.	1% of *Holothuria parva* extract gel demonstrated better wound healing compared to other

No.	Author, year and country	Gamat species	Wound type	Total (n)	Animal sample model	Administration	Treatment groups, intervention, dosage	Finding/ Outcome
							• Group 2: 3% *Holothuria parva* extract gel. • Group 3: 5% *Holothuria parva* extract gel. • Group 4: positive control group (phenytoin cream) Group 5: negative group (HPMC gel base)	treatment groups ($p<0.05$).
10.	Ollu et al. (2019) Indonesia	*Holothuria leucospilota*	Incisional wound	15	*Mus musculus (mice)*	Topical application	The study was divided into 3 different treatments group. • Group 1: Aquades • Group 2: Betadine Group 3: Ethanol black sea cucumber extract (*Holothuria leucospilota*)	The ethanol black sea cucumber extract of *Holothuria leucospilota* improved wound healing performance with better wound reduction and wound healing.
11.	Wen et al. (2018) Malaysia	25 patients were recruited with a median age of 48 years old. Male - 20 patients Female - 20 patients	Skin Graft Donor Site (excision wound)	*Stichopus hermanii*	Duoderm hydroactive gel by Convatec Malaysia Sdn. Bhd. Chosen due to availability in the hospital environment	Topical application Dressing was changed on day 10,14 and 21 for inspection	Epithelization was almost similar for both wound surfaces on day 10, 14 and 21 with $p > 0.01$. Day 10: 79.08 ± 25.12% Gamat 79.32 ± 23.35% Duoderm Day 14: 96.8 ± 4.62% Gamat 98.0 ± 4.33% Duoderm	

No.	Author, year and country	Gamat species	Wound type	Total (n)	Animal sample model	Administration	Treatment groups, intervention, dosage	Finding/ Outcome
							Day 21: 100.0 % Gamat 99.9 ± 0.40 % Duoderm	
12.	Ogai et al. (2017) Indonesia	42 patients with each 21 patients treated with sea cucumber and honey.	Diabetic foot ulcer (DFU) (incision wound)	*Stichopus hermanii*	Honey *(Apis dorsata)*	Topically applied in gel form	No significant differences were seen in DFU wound healing between sea cucumber and honey ($p > 0.01$) within 12 days treatment. Sea cucumbers were safer than honey dressing for DFUs in a clinical setting.	

Chemokines activation plays a key role in orchestrating the sequence of wound healing (Ridiandries et al. 2018). It involves the regulations of angiogenesis and the recruitment of inflammatory cells that secrete cytokines and growth factors to promote wound healing. It involves the regulation of angiogenesis and the recruitment of inflammatory cells that secrete cytokines and growth factors. There are a variety of growth factors in the extracellular matrix, such as the transforming growth factor-beta (TGF-B) and vascular endothelial growth factor (VEGF), which both stimulate cell division and improve tissue repair (Serra et al. 2017). As GAG is part of proteoglycan, it can regulate TGF-B and induce preparation of collagen fibrils of type I and type III, thus increasing wound healing and clinically decreasing the ulcer diameter (Zohdi et al. 2011). Meanwhile, Subramaniam et al. (2013) discovered that Stichopus horrens extract has remarkable therapeutic properties in the initial phase of wound healing and studies done by Wen et al. (2018) showed that there is no significance difference between topical application of sea cucumber extract and commercialized Duoderm (ConvaTec Malaysia Sdn. Bhd.) ($p > 0.05$) in the rate of epithelization.

Inflammatory response is one of the crucial phases in wound healing. In normal setting, inflammatory response will take up to 3-4 days, but for diabetic patients, inflammation is a double edge sword where the subsequent phases heightened and prolonged (Wood et al. 2014). Both platelet and leukocytes release pro-inflammatory cytokines that provide chemotactic gradient for additional leukocytes to enhance the inflammatory process. These includes the IL-6,.IL-8, TNF-α, CCL2 and CPR. However, the inflammatory response is limited by anti-inflammatory cytokines, IL-10 and this significantly reduced the inflammation response and promote wound healing (Wise et al. 2014). Study done by Li et al. (2018) reported that small molecule oligopeptides from sea cucumber (SCCOPs) were found to reduce inflammatory response in diabetic mice and hasten the wound healing process. The results of the published study showed that the treatments with SCCOPs significantly reduced the level of IL-6, IL-8, TNF-α, CCL2, CRP and elevated IL-10 concentration in serum of diabetic mice with smoother wound surface compared to normal control (NC) and model control (MC) groups. The study was supported by Pauzi et al. (2018) where treated group with sea cucumber has higher IL-10 level and lower proinflammatory response (IL-12) compared to control group on day 6 and day 12, but there is no significance difference ($p > 0.05$) on day 1. The study showed

that the sea cucumber extract has the ability to regulate the inflammatory response, increase fibroblast proliferation, promote wound contraction thus enhance the wound healing treatment.

Another study performed by Ogai et al. (2017) on diabetic foot ulcer showed the potential of the sea cucumber (Stichopus hermanii) extract compared to the honey (Apis dorsata) dressing. Based on the experiment studied, it shows that the sea cucumber extract is a good anti-inflammatory agent compared to Apis dorsata. Honey contains flavonoid that works as anti-inflammatory agent, but sea cucumber has a unique bioactive compound known as saponin that plays role as anti-inflammatory agent in the wound healing treatment. Saponin prevent the lipopolysaccharide-induce production of TNF-α by blocking the transcription factor nuclear kappa-light chain enhancer of activated B cell (NF-κB) that regulated the transcription of many gene associated with inflammation (El Barky et al. 2017). In addition, as the major fatty acid in the sea cucumber are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), they produce resolvin and protectins which are the anti-inflammatory mediators (Calder 2010). Besides, DFU can be affected by external factors such as infection through bacteria colonization (biofilm). Up to 60% of chronic wounds are microscopically identified to be colonized by biofilm (Percival et al. 2015). Additionally, this film has the ability to build a physical barrier that hinders cellular movement as well as the penetration of antibiotics and antibodies (Van Koppen and Hartman 2015). It is believed that free fatty acid (FFA) within sea cucumber could inhibit and kill bacteria by creating unfavorable condition for microbial growth such as, acidic pH (Ogai et al. 2017). Thus, the Stichopus hermanii extract reduced the inflammatory respond and promoted wound healing in diabetic foot ulcer (DFU) patients. In addition, when compared to sea cucumber extract and honey dressing in DFU wounds, there were no significant differences in terms of wound healing. In regards of safety, sea cucumber extract was safer compared to honey. This may indicate that sea cucumber extract can be used as an alternative wound dressing in the clinical setting (Ogai et al. 2017) and lead to potential development in biomedicine industry in the future.

LIMITATIONS

A methodological limitation of the included studies is the difference in the composition of the sea cucumber extract. In addition, although most of animal models in the paper presented are rodents, there is difference in the genus and sex used in the studies and most have small sample sizes. There were also variations in the study population and type of wound across the studies. As with the in vivo animal model studies, it is not possible to pool the data due to heterogenicity in the study design.

CONCLUSIONS

There is evidence for the effectiveness of sea cucumber extract used for wound healing treatment. All studies included show a positive outcome on several types of wounds in rodents and humans, including excisional, incisional, ulcer, diabetic, and burn wounds. The data presented in this review supports continued study of the use of sea cucumber extract for wound healing management. Due to the limited number of clinical trials and their methodological differences, there is a need for large prospective well-designed randomized control trials to establish the efficacy of sea cucumber extract on wound healing treatment.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest with respect to the authorship or publication of this article.

AUTHORS' CONTRIBUTIONS

Nurshazwani Azmi: Investigation, Methodology and Writing – original draft. Widya Lestari: Project administration, Funding acquisition, Conceptualization, Supervision and Writing – review & editing. Khazlan Afiq Khazan: Investigation, Methodology and Writing – original draft. Nurzafirah Mazlan: Resources and Writing – review & editing. Shaiqah Mohd Rus: Writing – review & editing. Muhammad Salahuddin Haris: Conceptualization, Methodology, Validation, Supervision and Writing – review & editing.

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