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REVIEW ARTICLE

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The Wound Healing Potential of *Lignosus rhinocerus* and Other Ethno-myco Wound Healing Agents

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ABSTRACT

Wound care has become increasingly important over the years. Various synthetic products for wound care treatment have been reported to cause toxic side effects and therefore natural products are in significant demand as they have minimal side effects. The presence of bioactive compounds in medicinal mushrooms contributes to various biological activities which assist in the early inflammatory phase, keratinocyte proliferation, and its migration enhancement which are pertinent to wound rehabilitation. *Lignosus rhinocerus* (tiger milk mushroom) can reduce the inflammation phase in wound healing by fighting off bacterial infection and modulating pro-inflammatory cytokines expression in the early stage to avoid prolonged inflammation and tissue damage. The antibacterial, immunomodulating, and anti-inflammatory activities exhibited by most macrofungi play a key role in enhancing wound healing. Several antibacterial and antifungal compounds sourced from traditional botanicals/-products may prevent further complications and reoccurrence of injury to a wounded site. Scientific studies are actively underway to ascertain the potential use of macrofungi as a wound healing agent.

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1. Introduction

Wound treatment and its therapeutic impediments have become a fundamental healthcare concern, presenting a significant economically challenging burden worldwide [1–5]. Based on the report published by Fior Markets [6], the global market for skin and wound care is expected to reach USD 25.98 billion by 2025. The growing demand for skin and wound care products in the market is factored by the increasing number of diabetes cases, the rising geriatric population, and increasing awareness for wound care and management. Meanwhile, the global market for complementary and alternative medicine is expected to reach a whopping USD 296.3 billion by 2027; with a CAGR of 19.9%, considering the increasing demand for alternative medicine across developing countries and the expansion of traditional medicine applications in Asia [7].

The practice of herbal medicine has begun in ancient times even when there was limited information on the disease(s), plants' identity, and the prescribed regimens. Much of the knowledge regarding the usage of herbal medicaments was collected merely based on user experience [8]. The usage of herbal medicine and its consumption is widely acceptable till this day and age across the world with many people seeking them as treatment and healthcare alternatives for a healthier life, higher efficiency with fewer side effects, and relatively low cost [9–11]. Since 1999, the demand for alternative therapies showed an increasing trend in the United States, Australia, and Germany [12] while herbal medicines are commonly utilized in Asian, African, and South American countries as part of their traditional medicaments [13]. Herbal medicaments from plants; aka phytomedicine, have a seemingly good potential for wound healing. About 70% of pharmaceutical products in the market for wound healing purposes contain extract constituents of plant parts as their base material, whilst the remaining percentage is based upon mineral compounds (20%) and from animal sources (10%) [14].

This review highlights the parameters for wound healing enhancement and details the scientific findings of macrofungi traditionally utilized for wound rehabilitation. It subsequently focuses on *Lignosus*

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rhinocerus, a prized medicinal mushroom from Southeast Asia; reporting on its phytochemicals and pharmacological properties which may be beneficial for wound rehabilitation. The prospects on the potential of *L. rhinocerus* as a wound healing agent are also deliberated as the conclusion to this review.

2. Parameters for wound healing enhancement

The wound healing mechanism is divided into four phases: (i) hemostasis, (ii) inflammation, (iii) proliferation, and (iv) remodeling. A series of complicated intercellular interactions involving a plethora of soluble factors, intercellular matrix components, immune cells, and signaling molecules occurs during these phases [15–17]. Depending on the wound severity, it may lead to significant morbidity and mortality rates. Therefore, continuous efforts have been carried out to enhance wound healing through a multiphase mechanism. Key factors such as the accelerated inflammation phase, stimulation of new blood vessels, and enhancement of wound closure contraction are crucial for the treatment of cutaneous wounds.

Figure 1 depicts the key parameters for wound healing improvement. Enhancement of one or more of these parameters will be beneficial for wound rehabilitation. Despite the advancement of synthetic wound care products which were reported to be clinically effective, traditional therapies based on natural products such as plants, honey, and fungi, are considered good alternatives. Traditional therapies may help to overcome the increasing demand for new wound treatment possibilities at a much-reduced cost in the market.

2.1. Antibacterial treatment strategy for infected wounds

Bacterial infection causes prolonged inflammation which leads to delayed wound healing [18]. Therefore, antibiotics are utilized to fight bacterial infections in the wound site and are commonly used as one of the standard treatments for wound care management [19]. Finding an effective treatment is necessary to reduce bacterial colonization and infection, which will subsequently reduce the duration of inflammation and improve the wound healing process [20].

2.2. Early induction of the inflammatory phase

Neutrophils and macrophages recruitment into the wound area is an essential event in the inflammatory phase for homeostasis maintenance, fighting pathogen invasion, and dead tissue removal. Previous studies have shown that the number of macrophages that migrate and infiltrate into the wound area is at maximum on day 3 post-wounding and remains until day 7. The number of neutrophils that migrated to the wound site was maximum at 12 h post-wounding and continuously declined until day 3. The inflammatory phase progression can be accelerated by early infiltration and elimination of neutrophils and macrophages, which prevent excessive inflammation that may lead to chronic wound(s) and scar formation [21–25].

2.3. Keratinocyte proliferation and migration enhancement

The second phase of wound healing is re-epithelialization, a process that involves keratinocyte

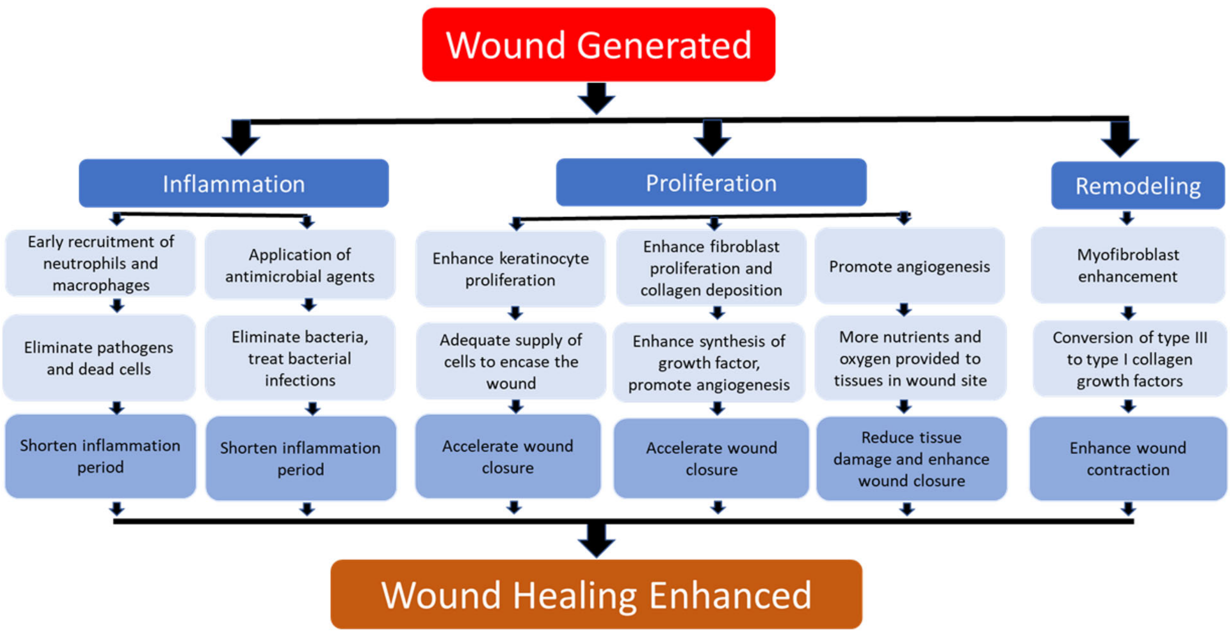

Figure 1. Graphical scheme of key parameters in enhancing wound healing.

proliferation and migration to ensure an adequate supply of cells to encase the wound. The process of re-epithelialization which eventually leads to wound closure is one of the parameters for consideration of a healed wound. This re-epithelialization process is flawed in chronic wounds. Growth factors, cytokines, integrins, keratins, matrix metalloproteinases, chemokines, and extracellular matrix (ECM) play key roles in the regulation of keratinocyte proliferation and modulating wound closure [26–28].

2.4. Fibroblast proliferation and collagen deposition enhancement

Another important process in the proliferative phase is collagen deposition, which plays an important role in wound healing. It involves fibroblast proliferation and migration [29]. Fibroblasts play a key role in new ECM formation and collagen deposition in the wound area [30,31]. There have been reports that transforming growth factor-beta (TGF-β) could enhance fibroblast proliferation and collagen deposition which will enhance the synthesis of growth factor and angiogenesis to the newly formed tissue [32,33].

2.5. Angiogenesis promotion

Angiogenesis is a crucial process during the proliferation phase to provide nutrients and oxygen to the newly formed tissue in the wound site [34]. Angiogenesis is flawed in chronic wounds. It leads to hypoxia, insufficient nutrient delivery, and causing further tissue damage [35,36]. Angiogenesis inhibition was reported in patients with diabetes as a high glucose condition leads to poor formation of new blood vessels [35,37]. It has been reported that induction and stabilization of hypoxia-induced factors will promote angiogenesis which can stimulate new vessels, enhance cell motility, and activate the transcription of vascular endothelial growth factor (VEGF) [38,39].

2.6. Collagen maturation and wound contraction

Both wound contraction and ECM maturation play important roles during the remodeling phase of wound healing [29,40,41]. The expression and differentiation of myofibroblasts are crucial for wound contraction [42]. Previous studies had reported that TGF-β stimulates fibroblast differentiation into myofibroblasts in wounds [43,44]. Therefore, wound healing progression can be accelerated by increasing TGF-β expression during the remodeling phase to increase the myofibroblast level and further enhance wound contraction [45].

3. The utilization of macrofungi for wound healing

Fungi are members of a large, diverse group of heterotrophic organisms which are frequently found living on dead, decaying wood, and other organic matter. They are eukaryotic, with a scope of internal membrane systems, and membrane-bound organelles, and possess a distinct cell wall that is made largely from polysaccharides and chitin [46]. The consumption of medicinal mushrooms has long been practiced and there is growing interest in the discovery of bioactive compounds with medicinal values from industries and the scientific communities alike [47]. This section provides an overview of the capacities of fungi for wound healing enhancement, both in modern science and traditional knowledge.

3.1. Medicinal mushrooms for wound rehabilitation in the modern age

Recent research trend focuses on the identification of compound(s) responsible for wound healing processes outlined in the following section. This includes polysaccharides such as (1-3)-β-glucans, some secondary metabolites (terpenes, phenolics) as well as proteins and minerals (calcium, potassium, magnesium, iron, and zinc). The incorporation of their bioactive compound(s) into pharmaceutical/-nutraceutical formulation allows the development of products for commercialization. For instance, *Ganoderma lucidum* (Lingzhi or Reishi), *Sparassis crispa*, and *Tremella fuciformis* were currently developed as skin care cream products and are commercially available in stores. Table 1 shows the summary of the macrofungi studied for their wound healing activity.

3.1.1. Agaricus *spp.*

The lectin(s) from *Agaricus bisporus* (common name: White button mushroom) was reported to exhibit dose-dependent inhibition of the proliferation of fibroblast and retinal pigment epithelium (RPE) cells, and collagen lattice contraction [48–50]. These inhibition activities play an important role in controlling the scarring processes as a result of excessive myofibroblast expression and excessive collagen production. In a report by Lam et al. [51], the incision wound model was used and performed on ICR mice, with 0.5 mg of *A. bisporus* extract mixed with 100 IU of vitamin E that was applied twice daily for 6 days. Immunochemistry, TUNEL assay, and Western blotting techniques were adopted to reveal an increased expression of epidermal growth factor (EGF) and proliferative cell nuclear antigen (PCNA), but the expression of TGF-β was not significant and few

Table 1. Summary of macrofungi studied for wound healing agents.

Family	Species	Common name	Bioactive molecule	Experimental model	Reported activities	References
Agaricaceae	*Agaricus bisporus*	White button mushroom	Lectin	*In vitro*	• Fibroblast proliferation and lattice contraction inhibition	[48]
			Lectin	*In vitro*	• Inhibition of retinal pigment epithelium proliferation	[49]
			N.D.	*In vivo*	• Significant increase of epidermal growth factor, proliferative cell nuclear antigen, and collagen fiber • Limiting reaction of transforming growth factor β, immune factor CD4	[51]
	Agaricus blazei	Cogumelo do sol, Ji Song Rong, Himematsutake, Agarikusutake, Kawarihiratake.	Polysaccharide	*In vivo*	• Significant decrease in IL-1β production • May affect the acceleration of wound repair	[52]
Auriculariaceae	*Auricularia auricula*	Wood ear, Jew ear, Jelly ear fungus	Polysaccharide	*Ex vivo*	• Increasing epidermal migration, significant promotion of wound healing effect.	[54]
Ganodermaceae	*Ganoderma lucidum*	Lingzhi, Reishi	N.D.	*In vivo*	• Increasing hydroxyproline and hexosamine levels • Enhancement of re-epithelialization and fibroblastic deposition	[56]
			Immune-modulatory protein	*In vivo*	• Increasing fibrosis; inhibits NF-κB and caspase-3 expressions and reduces NF-κB nuclear translocation and apoptotic cell death	[62]
			Polysaccharide	*In vivo*	• Wound healing rate enhancement • Reduced the oxidative damage	[63]
			N.D.	*In vivo*	• Wound healing rate enhancement	[55]
	Ganoderma tsugae	Hemlock varnish shelf	Chitin	*In vivo*	• Accelerated wound healing and occurrence of acute inflammatory reaction; shortened the inflammatory period	[64]
			Chitin	*In vivo*	• Early proliferation and regeneration of collagen in both keratinocytes and scar tissue; accelerated wound healing	[65]
Hericiceae	*Hericium erinaceus*	Yamabushitake, Houtou, Bear's Head, Lion's Mane, Hog's Head Mushroom, White Beard, Old Man's Beard, Pom Pom, Monkey's Mushroom	N.D.	*In vivo*	• Regeneration of collagen with angiogenesis; acceleration of wound enclosure	[67]
			Polysaccharide	*In vivo*	• Accelerated recovery in rats and the sensory function of injured peripheral nerve • Facilitated recovery from traumatic nerve injury	[68,69]
Omphalotaceae	*Lentinus edodes*	Shiitake	N.D.	*In vitro*	• Antiprotozoal activity and mitogenic effect	[140]
			Polysaccharide	*In vivo*	• Increased activity of serum antioxidant enzymes and decreased serum mucosal IL-2 and tumor necrosis factor (TNF)-a levels in rats with oral ulceration	[70]
Hymenochaetaceae	*Phellinus gilvus*	Mustard yellow polypore	Polysaccharide	*In vivo*	• A potent macrophage stimulator; enhances macrophage cytotoxicity and phagocytic capacity	[72]
Sparassidaceae	*Sparassis crispa*	Cauliflower mushroom, Hanabiratake, Ggoksongee	Polysaccharide (β-glucan)	*In vivo*	• Promotes macrophages and fibroblasts migration, collagen regeneration, and epithelization	[74,75]
Tremellaceae	*Tremella fuciformis*	Silver ear fungus, white jelly fungus	Polysaccharide	*Ex vivo*	• Increased epidermal migration, significant promotion of wound healing effect	[54]
Polyporaceae	*Lignosus rhinocerus*	Tiger milk mushroom	β-glucan	*In vivo*	• Accelerated IEC-6 cell proliferation and migration • Enhancement of Cdc-42, Rac-1, RhoA, and Par-3 expressions	[138]

Abbreviation: N.D.: not determined.

immune factor CD4 T cells were observed. It was suggested that *A. bisporus* extract may enhance wound healing activity by stimulating the expression of inflammatory and immune cells in the inflammation phase, enhancing keratinocyte proliferation, and promoting angiogenesis.

Agaricus blazei (Common name: Ji song rong) polysaccharides were shown to increase the recovery rate of wounded skin caused by burns on rats in a dose-dependent manner. Oral administration of 50 and 100 mg/kg of the *A. blazei* polysaccharides showed 45.7 and 63.2% recovery rates, respectively. The polysaccharides were isolated from the fruiting bodies of *A. blazei* by repeated extraction with hot water, cold NaOH, and then hot NaOH. Compositional analysis indicated that *A. blazei* polysaccharides are (1-6)-β-glucans, with the presence of glucose (93.87%), mannose (3.54%), and arabinose (2.25%). The level of pro-inflammatory IL-1β was further increased; indicating the potential of *A. blazei* polysaccharides as an effective way to promote wound healing by enhancing immunity activity. The rate of wound contraction was increased due to rapid wound contraction, epithelialization, and collagenization [52]. In addition, IL-1β contributes to re-epithelialization, angiogenesis, and collagen synthesis [53].

3.1.2. Auricularia auricula

The polysaccharides of *A. auricula* (Common name: Wood ear) showed significant wound healing effects in the porcine skin *ex-vivo* wound healing model. The extracted polysaccharides can promote keratinocyte migration up to 40% (140.43%) at 10 mg/ml 48 h post-exposure in comparison to the negative control containing PBS (100%). The reported effect is comparable to EGF (154.2%); a key player in stimulating keratinocyte proliferation during epithelialization [54].

3.1.3. Ganoderma *spp.*

Water extract of *G. lucidum* (Common name: Lingzhi) mycelia was reported to shorten the recovery period of wound healing in an excision wound model. *G. lucidum* hot water extract stimulated early neutrophil recruitment into the wound area and on day 3 post-wounding, the wound was fully covered with a thin layer of epithelium, suggesting an enhancement in keratinocytes proliferation [55]. On the other hand, rats treated with *G. lucidum* proteins exhibited increased wound closure rate (Day $5 - 18.8$ mm^2, Day $8 - 5.9$ mm^2) as compared to treatment by povidone-iodine (Day $5 - 22.4$ mm^2, Day $8 - 8.2$ mm^2) in an excision wound model study. Hematoxylin-eosin stained sections of granulation tissues at day 8 showed well-organized thick epithelium and deposition of collagen into the tissues, suggesting the proliferation of keratinocytes and fibroblasts [56]. In another study, water extract of *G. lucidum* fruiting body was reported to possess an abundance of glutamic proteases of the peptidase G1 family. Glycoside hydrolases were also identified from *G. lucidum*, such as β-N-acetyl hexosaminidase, α-1,2-mannosidase, endo-β-1,3-glucanase, and β-1,3-glucanase [57]. Several studies have reported that glycoside hydrolases were able to disrupt the formation of fungal and bacterial biofilm. Biofilm-related infections were one of the main factors leading to chronic wound healing [58–60]. Protease, one of the key factors for tissue repair, plays a role in the influx of leukocytes, angiogenesis, and re-epithelialization in the wound healing process. It breaks down damaged extracellular matrix, especially matrix metalloproteinases, to enable the remodeling of the new tissue [61].

The *G. lucidum*-derived LZ-8 protein was applied to the anterior lesion created on Sprague-Dawley rats' liver. Histological analysis showed that wound size reduced and fibrosis increased in the LZ-8-treated liver tissues, compared to the untreated group [62]. In a separate study, 10% (w/w) of polysaccharides from the fruiting body of *G. lucidum* hot water extract showed excision wound healing improvement in diabetic rats with higher wound closure rate (Day 8 - 60%, Day 12 - 97%) as compared to a positive control (Day 8 - 55%, Day 12 - 70%). Histopathological analysis showed more formation of capillary vessels on day 6 post-wounding with fewer inflammatory cells, good epithelialization, and well-formed granulation tissue compared to the negative control. *G. lucidum* may have shortened the inflammatory phase, enhanced collagen formation, and re-epithelialization as well as angiogenesis promotion [63].

Ganoderma tsugae (Common name: Hemlock varnish shelf) has also been reported to enhance wound closure in an excision wound model. The treatment of *G. tsugae* chitin accelerated wound healing by stimulating the early recruitment of neutrophils and lymphocytes into the wound site within 24 h post-wounding and promoted early expression of PCNA and type I collagen. In addition, *G. tsugae* chitin promoted angiogenesis and the expression of trans-glutaminase (t-TGase), to the capillary vessels in the later phase of wound healing for sufficient blood supply and enhance of dermal matrix remodeling, respectively [64–66].

3.1.4. Hericium erinaceus

H. erinaceus (common name: Lion's mane) hot aqueous extract was topically applied on the wound in the dorsal of male Sprague-Dawley rats. The extract showed a higher rate of wound closure compared to negative control in an excision wound rat model. Histological analysis showed lower macrophage count, higher collagen content, and apparent angiogenesis in the treated group, leading to a conclusion that *H. erinaceus* aqueous extract promoted angiogenesis and collagen formation, and shortened the inflammation phase in the wound healing process [67]. In another study, the fruiting bodies of *H. erinaceus* demonstrated a neurogenerative role in the peripheral nervous system. An *in vivo* study done using rats with traumatic nerve injury treated with polysaccharides from *H. erinaceus* (at a dosage of 30 mg/kg body weight) showed accelerated recovery of the sensory function of injured peripheral nerves [68,69].

3.1.5. Lentinus edodes

Polysaccharides from *L. edodes* (Common name: Shiitake) significantly enhanced oral ulcer healing rate by up to 73% as compared to the untreated group. ELISA demonstrated increased IL-1β and TNF-α expressions, which are important for re-epithelialization, angiogenesis, and collagen synthesis activities in wound healing enhancement [70,71].

3.1.6. Phellinus gilvus

Diabetic wounds are difficult to heal mainly due to the inhibition of angiogenesis caused by high glucose conditions. Impairment of angiogenesis leads to chronic hypoxia, decreased entrance of growth factors, and later further tissue damage [35,37]. Application of polysaccharides from *P. gilvus* (Common name: Mustard yellow) fruiting bodies aqueous extract on the dorsal side of the wound promoted dermal wound healing in both normal and streptozotocin-induced diabetic rats. The wounds of rats treated with *P. gilvus* showed a significant increase in re-epithelialization rate [72,73].

3.1.7. Sparassis crispa

Oral administration of *S. crispa* (Common name: Cauliflower mushroom) powder at 1000 mg/kg body weight was reported to accelerate wound closure in an excision wound diabetic rat model. Histology analyses revealed that *S. crispa* stimulated early recruitment of neutrophils and macrophages into the wound site, allowing early elimination of the cells and acceleration of the inflammation phase. *S. crispa* also enhanced re-epithelialization, fibroblast proliferation, and collagen deposition which promoted growth factor synthesis and angiogenesis to the newly formed tissue [74,75].

3.1.8. Tremella fuciformis

Wound treated with *T. fuciformis* (Common name: Silver ear fungus) polysaccharides (0.1 mg/kg) from fruiting bodies aqueous extract showed wound healing promotion effect by increasing keratinocyte migration rate up to 25% in an *ex-vivo* porcine skin wound healing model [54]. *T. fuciformis* may enhance re-epithelialization and wound closure during the proliferation phase of the wound healing mechanism.

3.2. Ethnobotanical usage of mushroom preparations for wound rehabilitation

In addition to scientific research on the wound healing activity of fungi, there are a few mushrooms that are traditionally utilized for wound treatment. These macrofungi were reported in the literature for traditional wound treatment practices. Table 2 summarizes the macrofungi utilized for traditional wound treatment.

3.2.1. Lignosus rhinocerus

The sclerotium of wild *L. rhinocerus* (Common name: Tiger Milk Mushroom) has been used by Aborigines as a traditional medicine to treat wounds, asthma, fever, breast cancer, stomach cancer, and food poisoning [76,77]. The *L. rhinocerus* powder also was mixed with Chinese rice wine and applied topically for treating lumps, sores, and boils [78].

3.2.2. Handkea utriformis

H. utriformis (Common name: Puffball) has been used in traditional practices for the treatment of wounds but lacks scientific reports. The fruiting bodies of *H. utriformis* are used in traditional medicine for surgical and burn wound dressings [79]. When the mature fruitbody of *H. utriformis* bursts or is impacted, clouds of brown dust-like spores are emitted and the spore powder is useful to stop bleeding. The practice can be found in the rural state of Europe, North America, and India. A review on "Puffball Usage among North American Indians" has reported that the Indian group from the Missouri River region utilized the puffball as a hemostat. The dried puffball was pulverized and applied to the wound to stop bleeding [80]. An extensive survey among the Baiga and Bharia tribes in Madhya Pradesh, central India state, have been showing that the giant puffball was utilized to stop bleeding for healing wounds [81]. An early report in 1860, reported on the usage of puffball as an anesthetic, like chloroform, for burnt treatment [82].

Table 2. Summary of macrofungi utilized as traditional wound treatment.

Family	Species	Common name	Mushroom part	Application	Remarks	References
Polyporaceae	*Lignosus rhinocerus*	• Tiger milk mushroom (Wild type)	Fruiting body, tuber (aka sclerotium)	Wound dressing	• In Malaysia, the wild type of *L. rhinocerus* is used by aborigines to treat wounds. • Wild *L. rhinocerus* powder is mixed with Chinese rice wine and applied topically for treating lumps, sores, and boils.	[76,78,110,141]
Lycoperdaceae	*Handkea utriformis*	• Puffball	Fruiting body	Wound dressing	• Utilized in India, Europe, and North America as traditional medicine for surgical and burn treatment. • The powder spore is useful to stop bleeding.	[80,81,142]
Ascomycota	*Morchella esculenta*	• Yellow morel • Kann Gitch • Batt Gitch	Fruiting body	Wound dressing	• In India and Pakistan, *M. esculenta* is highly utilized at the community and end-user levels. • The powder of *M. esculenta* fruiting body is applied over the wound, leading to faster healing and acting as an antiseptic.	[84,85]
Polyporaceae	*Fomes fomentarius*	• Hoof fungus • Tinder Fungus	Fruiting body	Wound dressing	• Classified by The Greek physician Hippocrates as a potent anti-inflammatory and used for cauterizing wounds. • In European, West Siberian, and Indian folk medicine, *F. fomentarius* was pounded with water until it is soft and externally applied to wounds to stop the bleeding. • In German and Austria, *F. fomentarius* was called a wound sponge or surgical sponge and was widely used as a styptic by farmers, surgeons, and dentists up to the 19th century. *F. fomentarius* was sold in pharmacies in the form of styptic bandages.	[86,87] [88,89] [90,91]
Agariaceae	*Calvatia gigantea*	• Giant puffball	Fruiting body, spores	Wound dressing	• Traditionally used by American Indians, Nigerian and German folks. Due to its hemostatic properties, *C. gigantea has* been used as a wound dressing and applied to the umbilicus of newborn infants, as well as in the treatment of inflammation. • In Serbia, the wound should be sprinkled with *C. gigantea* spores to stop bleeding.	[92,93,143] [94]
Fomitopsidaceae	*Fomitopsis betulina*	• Birch polypore	Fruiting body	Wound dressing, tea	• It has been practiced by the folk medicine of Russia, Poland, and other Baltic countries, primarily as an antiparasitic and antimicrobial agent and to stop wound bleeding. • For these purposes, it is used orally as tea or snuffed as a powder or ash.	[90,93,95]
Lycoperdaceae	*Lycoperdon pusillum*	• Anthua • Jatia rutka	Fruiting body	Wound dressing	• Used traditionally by tribal tribes in Northern and Central India for controlling bleeding and wound care. • In Nigeria, *Lycoperdon pusillum* was used to treat abrasions, sores, and deep cuts. • *Lycoperdon spp.* was utilized as a hemostatic agent by the American Indian, where the soft, central portion of dried, immature mushroom was powdered and covered into the wound to stop bleeding.	[96] [143] [80]

Abbreviation: N.D., not determined.

3.2.3. Morchella esculenta

M. esculenta (common name: Yellow morel) has been used in Chinese medicine for thousands of years [83]. It is also highly utilized by the various tribal group from Kupwara district (Kashmir, India) and Neelum Valley (Azad Jammu and Kashmir, Pakistan). The fruiting body of *M. esculenta* was pulverized to powder for wound application to speed up healing and acts as an antiseptic [84,85].

3.2.4. Fomes fomentarius

F. fomentarius (common name: Hoof fungus) was classified by The Greek physician Hippocrates circa 450 BCE as a potent anti-inflammatory agent for cauterizing wounds [86,87]. In European, West Siberian, and Indian folk medicine, *F. fomentarius* fruit body was made as part of the bandage material. It was pounded with water until it soften and externally applied to wounds to stop the bleeding [88,89]. In German and Austria, *F. fomentarius* was called a wound sponge or surgical sponge. It is widely used as a styptic by farmers, surgeons, and dentists up to the nineteenth century [90,91] (Figure 2).

3.2.5. Calvatia gigantea

C. gigantea (common name: giant puffball) has been reported to be traditionally used by American Indians in the Missouri River region and the Balkan peninsula in Europe. Due to its hemostatic properties, *C. gigantea* spores were traditionally powdered and applied to the wound as a dressing to stop bleeding, as well as in the treatment of inflammation. The Indians also reported applying the powder to the umbilicus of newborn infants [92–94].

3.2.6. Fomitopsis betulina

F. betulina (common name: birch polypore) is a wood-rotting medicinal and edible mushroom when at a young stage. It has been practiced by the folk medicine of Russia, Poland, and other Baltic countries, primarily as an antiparasitic and antimicrobial agent and to stop wound bleeding [90,95]. Interestingly, *F. betulina* fruiting bodies were crushed to powder and used as snuff in Austria, Northern America, and Siberia as pain relievers [93]. Snuffing the *F. betulina* powder may or may not contribute to wound healing, but it may aid in pain management for old folks during the wound healing process.

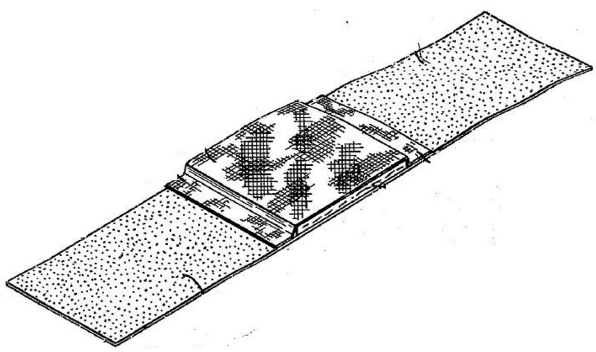

Figure 2. The isometric view of styptic bandage, invented in 1963 to facilitate the stanching of bleeding [144].

3.2.7. Lycoperdon pusillum

L. pusillum has been used traditionally by the tribal tribes in Northern and Central India (Local name: Anthua, Jatia Rutka) for controlling bleeding and wound care [96]. In Nigeria, *L. pusillum* was used by the Aboriginals to treat abrasions, sores, deep cuts, hemorrhages, and urinary infections [97]. The species of Lycoperdon were utilized as hemostatic agents by the American Indian, where the soft, central portion of dried, immature mushroom was powdered and covered into the wound to stop bleeding [80]. This practice also can be found in Central India by the Baiga tribe [96].

4. Tiger milk mushroom

Lignosus rhinocerus (Cooke) Ryvarden, locally known as tiger milk mushroom, "cendawan susu rimau," "*betes kismas,*" "*hurulingzhi,*" and "*hijiritake*" inhabits the soil in the tropical region [98–101]. It can only be found in the Asia-Pacific region encompassing South China, Southeast Asia, and Oceania [102–104]. This mushroom is difficult to find in the wild as it grows in solitary. The collection of tiger milk mushrooms is rare and expensive due to the limited number of harvests from the wild. The decline of this mushroom caused by deforestation, overharvesting, and lack of know-how to cultivate has led this species to the brink of extinction [105]. An enormous effort to domesticate the mushroom was performed circa 2009 where successful cultivation of *L. rhinocerus* (TM02®) using a specially formulated culture medium consisting of rice, water, and other food-based materials was reported by LiGNO™ Biotech Sdn. Bhd., a Malaysian SME using their in-house proprietary method. In brief, the inoculated medium is incubated in a controlled culture room for up to six months for sclerotia formation before harvesting. Commercial production of *L. rhinocerus* TM02® led to an increased opportunity for research on the various purported medicinal properties of the mushroom [106–109].

The sclerotium is the part of the mushroom with medicinal value and it has been used as a traditional medicine to treat wounds, asthma, fever, breast cancer, stomach cancer, and food poisoning [76,77,110]. To date, *L. rhinocerus* has been reported to show therapeutic activity such as immunomodulatory, neurite growth promotion, antiproliferative, antiviral, anti-inflammatory, antimicrobial, and antioxidant activities [102,111–118]. Although *L. rhinocerus* had been reported as a traditional medicine for wound treatment, there is a lack of scientific evidence that supported such usage. In the next subsection, the biological activities of *L. rhinocerus* and how they may help in enhancing wound healing activity are discussed.

4.1. Pharmacological relevance of L. rhinocerus to wound healing

Due to the declining numbers of wild type *L. rhinocerus*, some institutes or companies were making the initiative on developing techniques for the cultivation of *L. rhinocerus*, such as LiGNO™ Biotech Sdn. Bhd. (cultivar TM02®), Hong Kong Polytechnic University, and Sanming Mycological Institute [108,115,119] to avoid this valuable mushroom from being extinct. Various in-depth scientific studies were then called for to elucidate the bioactivities of these cultivars. Table 3 summarizes the pharmacological relevance of the wild and cultivated types of *L. rhinocerus* to wounds.

L. rhinocerus was reported to immunomodulate by increasing cytokines (IL-5, IL-6, and MIP-2) expression in RAW 264.7 cells conceivably through the NF-κB/MAPK signaling pathways [115,116,119]. Both signaling pathways have been implicated in corneal epithelial wound healing, scratch injury, and cutaneous wound healing [120–125]. MIP-2 dominates the early part of the inflammation phase by regulating the migration of granulocytes including neutrophils and stem cells [126,127]. Meanwhile, pro-inflammatory IL-5 and IL-6 cytokines are released to promote inflammation by inducing immune cells to the wound site and concurrently activating growth factors that contribute to angiogenesis and collagen synthesis [128–131]. Increased release of IL-5, IL-6, and MIP-2 caused by *L. rhinocerus* will accelerate the inflammatory phase by early infiltration and elimination of neutrophils and macrophages.

Previous studies also demonstrated the anti-inflammatory activity of *L. rhinocerus* by its inhibition of TNF-α production in LPS-induced macrophages and reduced levels of IgE, Th2 cytokines, and eosinophil count [117,118]. Eosinophils play a beneficial role as pro-inflammatory cells in fighting pathogens through the release of cytokines and chemokines [132]. However, overregulation of pro-inflammatory cytokines caused by infections, allergic reactions, and autoimmune disorder(s) may lead to prolonged inflammation and tissue damage [133–135]. By regulating the expression of the pro-inflammatory cytokines, *L. rhinocerus* may assist in reducing the period of the inflammatory phase during wound healing.

Without proper treatment, generated wounds have a high possibility to be colonized by bacteria. The proliferation of colonized bacteria may lead to infection and delay wound healing [18,136]. Treatment of the wound site with an antimicrobial agent(s) may reduce the prolonged inflammation phase in wound healing. An antimicrobial assay was conducted on petroleum ether, chloroform, methanol, and water extracts of *L. rhinocerus*. The extracts showed active antimicrobial activity at 30 mg/ml [114]. *L. rhinocerus* may be utilized for wound care management by curbing bacterial infections, however, further study is warranted.

Although lacking, scientific research has reported the potential usage of *L. rhinocerus* for wound rehabilitation. Ahmad et al. [137] demonstrated the presence of protease and fibrinolytic activities in LR-1 of wild type *L. rhinocerus* sclerotium in which a clear zone on skim milk agar and fibrin plates was observed. The findings from this study may indicate the beneficial role of *L. rhinocerus* in new tissue formation and wound closure enhancement during the breakdown of damaged ECM proteins and foreign material. Administration of *L. rhinocerus* β-glucans at 100 µg/ml was also reported to hasten mucosal wound healing by accelerating IEC-6 cell proliferation and migration. The expression of Cdc-42, Rac-1, and Rho-A was found to be enhanced in the study [138]. All in all, *L. rhinocerus* β-glucans may augment the epithelial restitution process during the wound healing mechanism [139].

4.2. Future perspective of L. rhinocerus as wound healing promoting agent

L. rhinocerus has been utilized for many years due to its medicinal benefits. With its successful domestication and research advances, numerous biopharmacological activities and bioactive compounds have been reported. Yet, although *L. rhinocerus* had been reported as a traditional medicine for wound treatment, there is a lack of scientific basis to support the claim. *L. rhinocerus* is hypothesized to reduce the inflammation phase during wound healing by inhibiting microbial infection and increasing cytokines expression at the early stage of inflammation whereas its immunomodulating activity helps in regulating the pro-inflammatory cytokines to avoid prolonged inflammation and tissue damage. However, scientific studies are much needed to prove the hypothesis.

Table 3. Summary of pharmacological relevance of *Lignosus rhinocerus* to wound healing.

Origin	Mushroom part	Pharmacological activity	Extraction method	Bioactive/ major components	Experimental models	Findings	References
Cultivated (TM02®)	Sclerotium	Immunomodulatory activity	Cold water	Polysaccharides	*In vitro* • Nitric oxide production • Detection of cytokine profiles • Determination of cytokines	• The fraction of *L. rhinocerus* TM02® may immune-modulate RAW264 cells. • Possible involvement 420 in the NF-κB and MAPK signaling pathway	[120]
		Anti-inflammatory activity	Hot water	N. D.	*In vivo* • Sensitization, airway challenge, and treatment	• *L. rhinocerus* TM02® extracts showed effectiveness in reducing asthma-related at 500 mg/kg (IgE level, Th2 cytokines, eosinophil count, eosinophil infiltration in the lungs)	[121]
			Cold water, hot water, methanol	HMW of CWE-protein components	*In vivo* • Carrageenan-induced paw edema assay • Cotton pellet-induced granuloma assay	• CWE of *L. rhinocerus* TM02® at 200 mg/kg showed potent paw edema inhibition, followed by HWE and ME. • HMW and MMW fractions of CWE showed a strong inhibitory effect on TNF-α production in LPS-induced macrophages	[122]
		Wound healing activity	Hot water extraction	B-glucan	*In vitro* • Compositional analysis • Scratch wound assay • Cell migration assay • Golgi-orientation assay • Lamellipodium formation assay	• *L. rhinocerus* TM02® β-glucans at 100 µg/ml accelerating IEC-6 cell proliferation and migration. • The expression of Cdc-42, Rac-1, and Rho-A was found to be enhanced.	[76]
Cultivated (Hong Kong Polytechnic University)	Sclerotium	Immunomodulatory activity	Hot water, water	Polysaccharides	*In vivo* • Immuno-suppressive mice model	• Showed immuno-regulation activity by reversing the immuno-suppression in Cy-treated mice.	[119]
Cultivated (Sanming Mycological Institute)	Sclerotium	Immunomodulatory activity	Hot water	Polysaccharides, protein	*In vitro* • Pinocytosis uptake • Reactive oxygen species generation • Nitric oxide production • TNF-α release • Expression of Dectin-1, CR3, and TLR2 • NF-κB Signal Pathway Determination	• HWE at 25 µg/mL showed up-regulation of pinocytosis; an increase in the production of reactive oxygen species and nitric oxide; and an increase in TNF-α production. • Treatment of RAW 264.7 cells with 25 µg/mL of *L. rhinocerus* and lipopolysaccharides could increase phosphorylation of IKBα in a time-dependent manner, which could trigger the NF-κB signal pathway	[123]
Wild	Sclerotium	Thrombolytic activity	Water	N. D.	*In vitro* • Protease bioassay • Fibrinolytic activity	• Showed protease and fibrinolytic activities in LR-1 of wild type *L. rhinocerus* sclerotium in which a clear zone on skim milk agar and fibrin plates was observed.	[141]
	N. D.	Antibacterial activity	Petroleum ether, chloroform, methanol, and water	Alkaloids, flavonoids, carbohydrates, proteins	*In vitro* • Disc-diffusion assay	• The petroleum ether, chloroform, methanol, and water extracts of *L. rhinocerus* showed active antimicrobial activity at 30 mg/ml	[118]

5. Conclusions

Various synthetic products have been described as wound care treatments, but some authors suggest that these products can cause toxic side effects, even at doses required to achieve good results. In recent years, various research has been conducted on natural products; including macrofungi, to develop novel pharmaceutics that target wounds and much effort has been invested to investigate its associated healing biology. The presence of these bioactive compound(s) may contribute to various biological activities which facilitate the early inflammatory phase, keratinocyte proliferation, as well as cell migration enhancement which are important mechanisms for wound healing. There are also a few fungi that are traditionally utilized for wound treatment but lack scientific reports. Interestingly, the utilization of macrofungi in traditional wound care was mostly for hemostatic purposes, which is to stop the bleeding. Now, the advancement of current wound care research and development enables researchers to develop products that was specific to enhance the wound healing process, especially on antimicrobial action and promote growth factor. As such, more research is required in this area to divulge supplementary scientific data to support their ethnobotanical uses. For instance, the mechanism(s) by which macrofungi may influence wound healing in bacterial and fungal infections can be investigated *via* microbial internalization (*via* bacterial challenge assay) and immunocytochemistry. This enables a deeper clinical understanding of wounds and their pathophysiology which will be significant to biomedical innovations and clinical trials.

Author contributions

Hui-Yeng Y. Yap: Conceptualization, Writing—Review & Editing, Supervision, Funding acquisition. Mohammad Farhan Ariffeen Rosli: Writing—Original Draft. Soon-Hao Tan: Writing—Review & Editing, Supervision. Boon-Hong Kong: Writing—Review & Editing, Supervision. Shin-Yee Fung: Writing—Review & Editing, Supervision.

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