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Review Article

Polyherbal For Wound Healing

# Polyherbal Formulation Approach: A Promising Wound Healing Strategy

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**Abstract:** Wound healing still remains as an unmet therapeutic challenge and a significant clinical and economic burden among medical society even with considerable research on advanced wound care globally. Myriads of wound healing products and strategies are currently available. Some of these products are synthetic chemical moieties like chlorhexidine, betadine etc with systemic side effects such as crystalluria, methaemoglobinaemia, renal impairment, ototoxicity and renal/thyroid dysfunctions. Even the advanced wound healing therapy such as bioengineered cellular wound therapies and stem cells therapies come with some disadvantages such as unaffordability to common man and difficulty in obtaining sufficient stem cells due to their low regenerative potential and ethical issues. The shortcomings associated with current available wound healing strategies necessitated the increasing interest in the use of medicinal plants in wound management. The growing interest towards medicinal plants primarily resulted as an outcome of scientific advancement in delineating the principles and molecular mechanisms behind the contribution of various phytoconstituents in them. Meticulous combination of several medicinal plants in the form of polyherbal formulations (PHFs) such as Ankaferd, herboheal and Ari's wound healing cream are reported to be safe and provide effective wound healing compared to the use of single plant. Several reviews on wound healing properties of individual plants and PHFs have been reported, but the multitargeted, synergistic and the complementary contributions of individual phytoconstituents in the PHFs at different complex stages of wound healing need to be given the desired attention. The aim of this review is to provide together a comprehensive report on the latent potentials of the positive herb-herb combination as a promising approach to wound healing. Our objective is to systematically collect research, and review articles from various open access research databases and study them to bring out the salient features of PHFs in relation to the effective wound management. A composite drug approach such as PHF is a better treatment option for improved wound management as many phytoconstituents in it can synergistically tackle multiple wound healing processes simultaneously. They are reported to be effective on multidrug resistant bacteria by inhibiting the complex quorum sensing regulated virulence factors' production and eventually causing their death. Transformations of active phytoconstituents that are reported to occur in PHFs may lead to formation of novel pharmacologically active compound/s, and nanoparticle/s. These transformations may lead to discovery of novel wound healing drug/s that researchers and pharmaceutical industries need to pay more attention to.

**Keywords:** Chronic Wound, Medicinal Plant, Phytoconstituent, Polyherbal Formulation, Wound Healing

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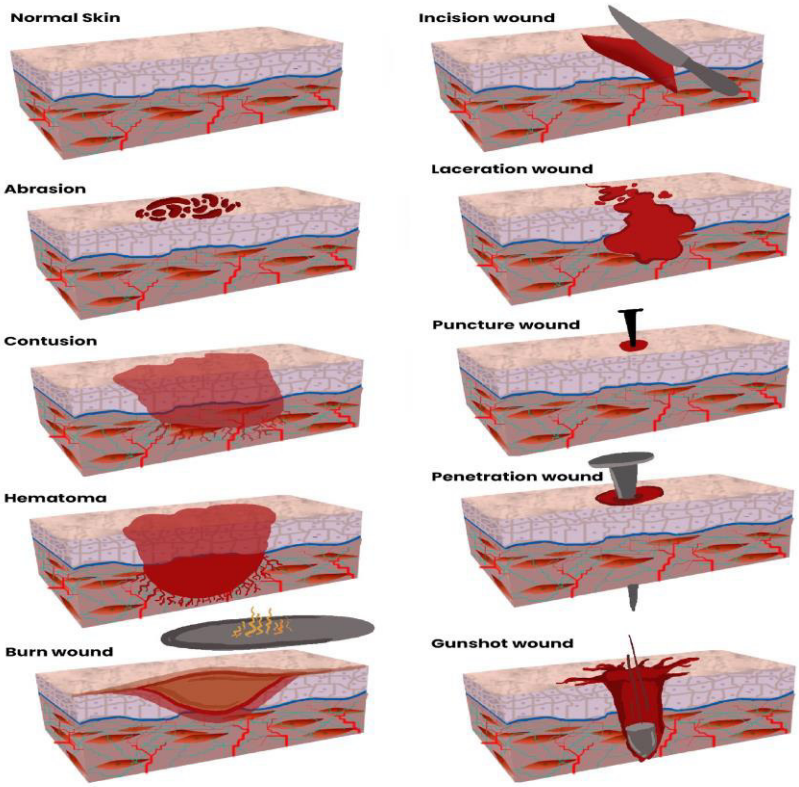
## 1. INTRODUCTION

Healing of wounds continues to exist as a taxing clinical problem despite scientific developments in the field with myriads of therapies currently available.[1,2] A global estimate of approximately 8.2 million people visiting hospitals suffers from various types of wounds with or without infections.[3,4] The most commonly used wound healing synthetic drugs such as silver sulfadiazine (SSD), mupirocin, mafenide, silver nitrate, nitrofurazone, chlorhexidine, and povidone-iodine,[5,6] have been reported to exhibit various adverse side effects such as allergic contact dermatitis,[7] local pain & ototoxicity,[8] formation of black scar & inadequate permeation to the wound bed,[9] and crystalluria or methaemoglobinaemia.[8] Prolonged use of mafenide or silver nitrate has been reported to contribute to systemic toxicity.[10,11] Iodine absorption from povidone-iodine topically applied on wounds enters into the systemic circulation and can lead to kidney and thyroid dysfunctions.[12] Shortcomings of the above mentioned wound treatments have pave way towards development of innovative approaches such as; negative pressure, biophysical, biological, bioengineered and stem cells wound therapies. Unfortunately, validation on the efficacy of majority of these advanced wound care strategies is deficient.[13] In addition, these advanced wound therapies come with some limitations pertaining to ethical and affordability aspects along with slow and low regenerative properties of stem cells.[14] Inadequacies of the current wound healing approach has made researchers turn back to nature to explore the potentials associated with medicinal plants in this aspect. This is because phytoconstituents in selected plant species are known to be effective in the treatment of different types of wounds with minimal or no side effects that are often associated with conventional wound healing agents.[15] In addition, scientific advancement have attempted defining the principles and molecular mechanisms of the contributions of multiple phytoconstituents in achieving faster and qualitative wound healing.[16] Using more than one medicinal plant in treatment of wound has been reported to significantly decrease the time of wound closure and increase the quality of healed wound compared to the use of individual plant.[17] Numerous processes and complex nature of the wound healing events have given a pointer to why single agent treatments and the use of individual plants for wound treatments has not produced the required result. The use of herb-herb combinations in the form of (PHFs) appear to have a lot of promising potentials in wound treatment and management due to the presence of diverse multitargeted groups of phytoconstituents in them.[15,18] Despite the fact that herb-herb combination (also referred to as PHF, polyherbal therapy or polyherbalism) has been in practice for thousands of years in Ayurveda and Chinese medicine, scientific validation of their detailed molecular mechanisms is lacking.[19] Carefully selected medicinal plants and their combination have been reported to show potential positive phytoconstituents' interaction such as synergism, reinforcement, potentiation, complementation and mutual enhancement/assistance. The plant selection process for combination must be accurately done to avoid incompatibility and counteraction of the phytoconstituents which can lead to undesirable outcomes.[17] This review aims to point out the salient features and latent potential benefits of positive herb-herb combination approach as an improved and effective wound management strategy compared to the current practices. It also highlights the synergistic contributions and multitargeted properties of PHFs in contrast to the one-drug-one target approach for wound management.

## *2. PREVALENCE AND ECONOMIC BURDEN OF WOUNDS*

Wounds poses serious health problems that have overwhelming costs for patients, healthcare systems and societies.[20] Chronic/non-healing wounds in advanced nations are estimated to be involving around 1% to 2% of their general population which is comparable to the burden contributed by myocardial infarction.[21,22] Acute wound medicare expenditure per individual was estimated to be between USD 3,415 – USD 3,859 annually. The figure usually goes up when it involves diabetic ulcers costing approximately USD 50,000, arterial ulcers around USD 9,105 – USD 9,418 and pressure ulcers about USD 3,696 - USD 4,436.[4] The occurrence of chronic and acute wounds in India is around 4.5 and 10.5 individuals per 1000 people respectively.[23,24] The snowball effect in the prevalence of diabetes mellitus in India, with a current status of 51 million and an expected doubling by 2025, suggests a high-risk factor that will be responsible for an increase in the cost of treatment/management of diabetic foot ulcers.[4,25] The global marketplace for wound treatment is anticipated to hit around USD 15-22 billion by the year 2024.[4,26] Age related complications that interfere with wound healing, occurrence of multidrug resistant wound colonizing bacteria and other comorbid diseases are some of the crucial factors that make chronic wound management a major economic challenge.[20] An upsurge in prevalence of acute and chronic wounds globally has necessitated a pressing need for improved and promising substitute to curb this threat. PHF as an alternative in the treatment of wounds of diverse nature (Figure 1) is hopeful in this regard.[27]

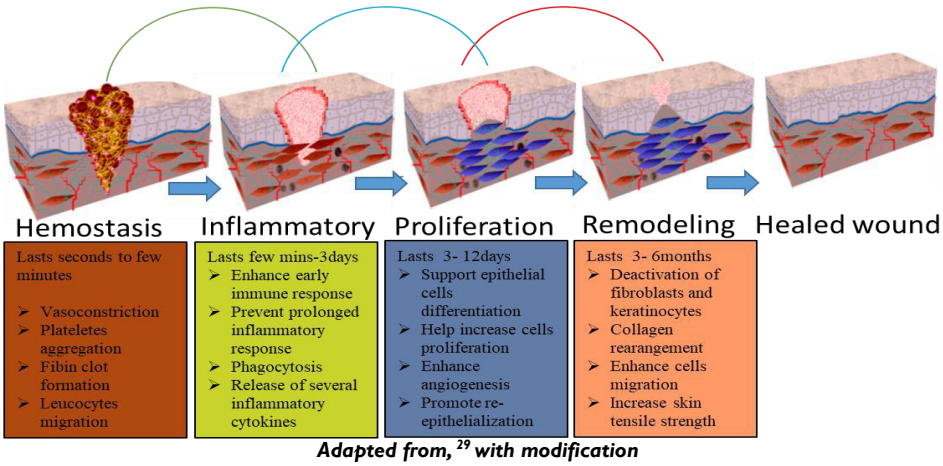



*Adapted from,[28] with modification*

**Fig 1. Schematic representation of various types of wounds**

## 3. MOLECULAR MECHANISMS ASSOCIATED WITH NORMAL WOUND HEALING PHASES

Immediately after injury, cells adjacent to the wound initiate coordinated sequence of molecular events aimed at restoring the injured tissue back to normal. These events are complex biological processes consisting of independent but overlapping phases (Figure 2).[29] Hemostasis is the first event that takes place minutes after injury. There is immediate activation of coagulation cascade to arrest loss of blood and other body fluids to allow commencement of tissue repair. In this phase, key events such as constriction of blood vessels, platelet clumping, degranulation, formation of fibrin clot, and neutrophils infiltration occur. Series of growth factors are also released by platelets to facilitate the wound healing process.[27,30] Inflammatory process now sets in to neutralize opportunistic bacterial and fungal infection that might have entered the wound. During this phase, there is also increase in pro-inflammatory cells such as neutrophils, monocytes, basophils, mast cells, and macrophages.[15] The next stage is proliferation phase which is characterized by increase in the number of cells implicated in wound repair process such as fibroblasts, keratinocytes and endothelial cells. These cells also migrate to the wound bed and initiate cascades of events that lead to wound closure and re-epithelialization of the lost tissue via activation of fibroblasts, keratinocytes, blood vessels formation, and secretion of collagen along with other constituents of extracellular matrix (ECM). These events are coordinated and controlled by various cytokines produced during the healing process.[15,31] Remodeling, the final phase of wound repair process is characterized by deactivation of activated fibroblasts and keratinocytes as well as down regulation of inflammatory response by triggering apoptotic pathways. Collagen remodeling, vascular maturation and increased tensile strength of the healed tissue take place at this level for enhancing the speed and quality of wound healing.[15,32]




*Adapted from, [29] with modification*

***Fig 2. Schematic representation of normal wound healing process. Note: - Curved lines indicate overlapping nature of wound phases while the blue arrows indicate progression of the wound healing process.***

## 4. RISK FACTORS AFFECTING WOUND HEALING PROCESS

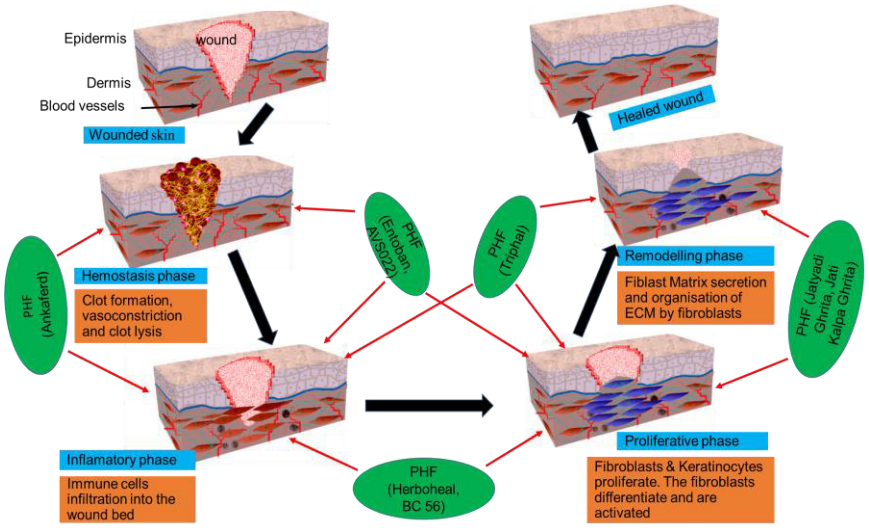
Diverse risk factors, a number of which can be managed/controlled and avoided (modifiable/avoidable), and a few that cannot be managed/controlled and avoided (non-modifiable/unavoidable) contribute immensely in delaying the process of wound healing.[33] The former includes factors like bacterial infection and biofilm formation, diabetes and related diseases, heavy alcohol consumption, selected medications, nutritional deficiency, repeated trauma, smoking, and stress.[27,32] The non-modifiable risk factors include advanced age, autoimmune diseases and inherited disorders such as pseudoxanthoma elasticum, Ehlers-Danlos syndrome and epidermolysis bullosa.[34] Bacterial infection is the most common risk factor that is responsible for majority of the impaired wound healing. This interrupts the progress of inflammatory and remodeling phases, causes host cell death, increases local inflammation response and prolongs acute inflammatory phase. The presence of necrotic tissue not only prevents the ingrowth of new tissue but also is a culture for bacterial proliferation, therefore, resulting in a vicious pathologic cycle.[35] These diverse risk factors coupled with the complex nature of wound healing process are the reasons why wound management is still a challenge that require serious attention.

## 5. ROLE OF PLANTS IN WOUND HEALING

Despite remarkable advancement in the field of modern medicine, medicinal plants still play a significant role in the current wound management strategy.[36,37] *Bacopa monnieri* (incision and excision wound on albino rats),[38] *Acalypha indica* (incision wound on wistar rats),[39] *Calotropis gigantea* (incision and excision wound on albino rats),[40] *Aloe vera* (full thickness burn wound),[41,42] *Curcuma longa* (incision and excision wound on albino rats),[36] *Terminalia arjuna* (incision and excision wound on rats),[43] *Tridax procumbens*(applied on linen fabric for treating wound colonizing bacteria),[44] *Malva sylvestris* (diabetic wound),[45] *Glycyrrhiza glabra* (cutaneous wound on Sprague Dawley male rats),[46] and *Ficus religiosa* (excision wound),[47] are some medicinal plants known to bring about faster wound healing. Fast and quality wound healing is achieved when more than one plant extract is combined in an optimized ratio. For instance, *A. vera* which is a popular and widely reported plant for its anti-inflammatory and anti-bacterial properties was reported to exhibit a faster and qualitative wound healing in combination with other plants than when used alone in an *in vivo* experiment using albino rat excision wound model.[48] Similarly a PHF containing *C. longa* tested on burn wound model using wistar rat showed improved wound healing effect than using *C. longa* alone.[49] Ankaferd, a commercially available PHF containing *G. glabra* was reported to demonstrate significant hemostasis and re-epithelialization of the wound area in wistar albino rat excision wound.[50,51] These findings indicate that there is a kind of interactions that is happening among the combined plants which is responsible for the improved wound healing activity. These interactions could be synergistic, complementary, potentiation and mutual reinforcement/enhancement/assistance.[17]

## 6. POLYHERBAL FORMULATION CONCEPT

Polyherbal combinations/formulations have existed world-wide with long recorded history and proven effective in wound treatment, yet evidence of scientific validation of many of their therapeutic benefits are deficient.[19] There are two principles that guide formulation of herbal medicines. First method uses a single medicinal plant in a base and the second method involves use of multiple plants known to be effective in wound healing of which the latter is referred to as PHF. PHF approach makes use of combination of several medicinal plants or plant components to achieve extra therapeutic efficacy.[19,52] Different bases are used for its preparation depending on the intended method of the drug administration. For instance, PHF for oral administration can be made by mixing suitable quantity of the plant extracts with appropriate base such as gum acacia, water and tween-20.[53] Topical PHFs are usually prepared in the form of cream, gel or ointment. For topical application, the base is warmed or heated to liquid form to which the extracts are added, thoroughly mixed and allowed to solidify for application.[54] Currently there exist numerous polyherbal preparations. Table 1 is a compilation of various PHFs developed for the treatment of diverse types of wounds.[55] Synergistic contribution of PHFs during different phases of wound healing is presented in Figure 3.




*Adapted from,[28]and,[56] with modification*

**Note: - Various PHFs such as Ankaferd, Triphala, Herboheal, BC56, Jatyadi ghrita, Kalpa ghrita and Entoban (green) showing multi-targeted effects (Orange arrows) on different phases of wound healing to ensure faster wound closure and improved tissue remodeling.**

***Fig 3. Schematic representation of PHFs' possible synergistic role in wound healing.***

**Table 1. Summary of the researches on PHF**

| Plant candidates in PHFs | Findings | Study design | References |
|---|---|---|---|
| Aqueous extracts of *T. procumbens, Vitex negundo, Emblica officinalis Gaertn* | Increase in the proliferation of fibroblast and keratinocytes, enhanced angiogenesis, wound contraction and antioxidant parameters | *In vivo* study (Rat model) | 57 |
| Aqueous extracts of *Cassia auriculata, Mangifera indica, Ficus benghalensis, Cinnamomum tamala and Trichosanthes dioica* | Increased wound breaking strength, re-epithelization, antioxidant potential and wound contraction | *In vivo* study (wistar albino rat model) | 58 |
| Aqueous extracts of *Hippophae rhamnoides* Linn, *A. vera* Linn and 70% ethanolic extract of *C. longa* rhizome | Increased cellular proliferation and collagen synthesis in comparison with positive control treated with povidone–iodine ointment. The PHF promoted angiogenesis and tissue regeneration. Granulation tissue of the PHF-treated normal rats showed reduced immune cells infiltration compared to control animals | *In vivo* study (Diabetic rat model) | 59 |
| Aqueous extracts of *Malva sylvestris* and *Solanum nigrum* leaves, and oily extract of *Rosa damascena* petals | Exhibited *in vitro* antibacterial activity against *Staphylococcus aureus.* PHF enhanced the rate of re-epithelialization and neovascularization *in vivo.* Reduced immune cells infiltration and showed antioxidant and anti-inflammatory properties | *In vitro* study (antimicrobial activity) and *In vivo* study (Rat model) | 60 |
| Aqueous extracts of the roots of *Astragalus membranaceus* and *Rehmannia glutinosa* (in 2:1 ratio) (PHF:NF3) | Significantly reduced the wound area of rats when compared to water control group and enhanced anti-inflammatory, fibroblast-proliferating activities and angiogenesis | *In vivo* study (Diabetic rat model). | 61 |
| Aqueous extracts of whole plant of *Scutellariae radix*, *Lonicerae flos*, *Forsythiae fructus*, F*el ursi* and *Naemorhedi cornu* | Enhanced antibacterial and anti-biofilm effect against methicillin-resistant *S. aureus*. Inhibited virulence factors production in *Pseudomonas aeruginosa* | *In vitro* study (different *in vitro* models) | 62 |
| Hydroalcoholic extract of whole plants of *Clerodendrum serratum*, *Hedychium spicatum* and *Inula racemosa* | Showed enhanced free radical scavenging activity and anti-lipid peroxidation in different *in vitro* models | *In vitro* study (different *in vitro* models) | 63 |
| Chloroform and hydro-alcoholic extracts of *Centella asiatica* (whole plant), *C. longa* (rhizome), *F. benghalensis* (stem bark) | Showed faster wound contraction and re-epithelialization compared to control. Exhibit antioxidant activity | *In vivo* study (Rat model). | 64 |
| Combination of various parts of *Jasminum officinale, Azadirachta indica, Stereospermum suaveolens, Hemidesmus indicus, Pongamia pinnata, Vetiveria zizanioides, G. glabra, Rubia cordifolia, Symplocos racemosa, C. longa, B. aristata, Nelumbo nucifera, Woodfordia fruticosa* with copper sulphate, Bee's wax and cow ghee | Improved wound closure, enhanced re-epithelialization compared to the controls | *In vivo* study (wistar albino rat model) | 65 |
| Ethanolic extracts of *Angelica dahurica* and *Rheum officinale* | Antimicrobial, anti-inflammatory effects and significant wound contraction | *In vivo* study (Sprague-Dawley rat model) | 66 |
| Ethanolic extract of *Psoralea corylifolia* and *Achyranthes aspera* | Faster wound contraction, enhanced re-epithelialization and improved skin breaking strength | *In vivo* study (Rat model) | 67 |
| Ethanolic extract of *Zanthoxylum chalybeum* and *Warburgia ugandensis* (PHF: Jena) | Improved wound closure, significant reduction in the epithelialization time, less inflammation, more collagen fiber content and better tissue remodeling | *In vivo* study (wistar albino rat model) | 68 |
| Ethanolic extracts of *A. indica, C. longa, Allium sativum, Ocimum sanctum, Cinnamomum zeylanicum nees* and *Tamarindus indica* | Exhibit antimicrobial activities against *S. aureus, Bacillus subtilis, Aspergillus niger* and *Escherichia coli* Showed better zone of inhibition as compared to control | *In vitro* study (antimicrobial activity) | 69 |
| Ethanolic extracts of *Erythrina indica, Bergenia ciliata, Cissampelos pareira* and Carbomer 934P | Enhanced re-epithelialization and wound contraction was shown to be significantly better than standard betadine ointment group | *In vivo* study (Rat model) | 70 |
| Ethanolic extracts of *Justicia tranquebariensis, A. vera, and C. longa* | Faster re-epithelialization, wound contraction as well as anti-inflammatory and, antibacterial effect | *In vivo* study (Rat model) | 48 |
| Ethanolic extracts of *Rungia pectinata, R. cordifolia* Linn, and *Scoparia dulcis* | Faster wound contraction, enhanced re-epithelialization, increased hydroxyproline production and increased breaking strength of the incision wounds | *In vivo* study (wistar albino rat model) | 71 |
| Ethanolic extracts of *Terminalia arjuna* (bark), *C. asiatica* (leaves) and *C. longa* (rhizomes) | Significant wound contraction and enhanced antimicrobial activity | *In vivo* study (Albino rat model) | 72 |

| | | | |
|---|---|---|---|
| Ethyl acetate and ethanolic extracts of *Momordica charantia, Pongamia glabra* and *Piper nigrum* | Enhanced wound contraction and re-epithelialization compared to the control | *In vivo* study (Diabetic rat model) | 73 |
| Honey, ghee, and aqueous extracts of *G. glabra* and *Nerium indicum* | Faster wound contraction, rapid re-epithelialization, collagen deposition and arrangement | *In vivo* study wistar albino rat model) | 74 |
| Hydro-alcoholic extracts of *Urtica dioica* and *Sambucus ebulus* | Anti-inflammatory, enhanced wound contraction, anti-microbial effect, neovascularization potential and proliferation of fibroblasts | *In vivo* study (wistar rat model) | 75 |
| Hydro-alcoholic extracts of *A. vera (whole plant), Eucalyptus globulus* (leaves), *Ficus infectoria* (bark), *F. religiosa* (bark), *Piper betle* (leaves) | Exhibited a strong antibacterial activity against isolated multi-drug resistant (MDR) *P. aeruginosa* and *S. aureus* from patients suffering from skin infections | *In vitro* study (antibacterial activity) | 76 |
| Hydroalcoholic extracts of *Piper nigrum* and *C. longa* formulation: Polyherbal ointment | *In vitro* study showed significant antimicrobial activity and excision wound model results supported enhanced wound closure by the PHF | *In vitro* study (antimicrobial activity) and *in vivo* study (Rat model) | 77 |
| Hydro-alcoholic extracts of *Psidium guajava* (leaves) *and F. religiosa* (bark) | Enhanced wound contraction and re-epithelialization. | *In vivo* study (Albino wistar rat model) | 78 |
| Hydro-ethanolic extracts of whole plants of *C. longa, Eclipta alba* and *T. procumbens* | Enhanced re-epithelialization and faster wound closure was significant comparable with control | *In vivo* study (wistar albino rat model) | 79 |
| Methanolic extract of henna (*Lawsonia inermis* Linn), pomegranate (*Punica granatum* Linn) and myrrh (*Commiphora myrrha*) | Showed strong antimicrobial activity against Gram +ve, Gram-ve bacteria and *Candida albicans* implicated in wound healing. Enhanced wound contraction, re-epithelialization and granulation tissue formation was observed in an *in vivo* study, relative to the control | *In vitro* study (antimicrobial activity) and *in vivo* study (Rat model) | 80 |
| Methanolic extract of *Martynia annua* and ethyl acetate extract of *Tephrosia purpurea* | Enhanced hydroxyproline and protein contents, granuloma tissue formation and organization. Increased fibroblast activation, collagen maturation, re-epithelialization and angiogenesis | *In vivo* study (wistar rat model) | 81 |
| Methanolic extracts of *C. longa, E. alba* and *T. procumbens* | Enhanced wound contraction and re-epithelization phases of healing and significantly increased breaking strength of the incision wound compared to the control | *In vivo* study (Rat model) | 79 |
| Methanolic extracts of *Plumbago zeylanica* Linn, *Datura stramonium* Linn and *Argemone mexicana* Linn | Enhanced antimicrobial activity on Gram +ve, Gram-ve bacteria with least Minimum Inhibitory concentration (MIC) value compared with the control. *In vivo* study showed enhanced anti-inflammatory activity, wound contraction and re-epithelialization | *In vitro* study (antimicrobial activity) and *in vivo* study (Rat model) | 82 |
| Mixture of superfine powder from; *Agrimonia pilosa*, *N. nucifera*, *Boswellia carterii*, and Pollen Typhae, named as (ANBP) | Anti-inflammatory. Enhanced re-epithelialization, wound closure, granulation tissue formation and angiogenesis compared to control | *In vivo* study (Streptpzotocin -induced diabetic C57BL/6 diabetic mice model) | 83,84 |
| Oil extracts of *R. cordifolia* (manjistha), *G. glabra* (licorice root) and *Symplocos racemosa* (lodhra) | Wound contraction activity of the PHF was remarkable which is comparable to that of povidone iodine ointment | *In vivo* study (Rat model). | 85 |
| Powdered mixture of *A. vera, C. myrrha* and *B. carterii* were added to hydrophilic gel | Enhanced re-epithelialization, neovascularization, anti-inflammatory and antimicrobial effects in the PHF treated group of rats | *In vivo* study (Rat model) | 86 |
| The carbopol 934-gel formulations containing different concentrations of ethanolic extracts of *Terminalia arjuna, C. asiatica and C. longa* | *In vitro* antimicrobial assay showed better activity against *B. subtilis* and *S. aureus*. *In vivo* excision wound model showed faster rate of wound contraction compared with controls | *In vitro* study (antimicrobial assay) and *In vivo* study (Rat model) | 72 |
| The carbopol 934-gel formulations containing varied concentrations [1%, 2% and 4% (G1, G2 and G3 respectively] of ethanolic extract of *Piper betle*, *C. longa*, *A. vera* and *Thymus vulgaris* | Topical administration of G3 gel reduces scar formation and promoted various phases of wound healing such as collagen synthesis, wound contraction and re-epithelialization | *In vivo* study (wistar albino rat model) | 87 |

## 7. *PHFs AND THEIR IN VITRO, IN VIVO AND EX VIVO CONTRIBUTION IN WOUND HEALING*

There are many ways by which wound healing efficacy of PHFs can be carried out such as (a) using isolated cells in cell culture plates i.e. *in vitro* study,[88] (b) by using whole tissue or eggs from animal in tissue culture laboratory i.e. *ex vivo* study,[89] and, (c) by using whole animal for wound healing kinetics study i.e. *in vivo* study. The *in vitro* and *ex vivo* assays are convenient, relatively inexpensive, allow screening of multiple medicinal plants and provide rapid result compared to *in vivo* study. Experiments performed *in vitro* and *ex vivo*

cannot be used for valid final conclusion. On the other hand, *in vivo* assays give a more complete physiologic assessment of wound healing parameters compared to *in vitro* and *ex vivo* studies, however it consumes more time.[88]

### 7.1 *In vitro* studies

There are key events that start immediately after injury to initiate repair processes which include endothelial cell formation and activation, proliferation and migration of fibroblasts and keratinocytes, collagen secretion and contraction, re-epithelialization, formation of granulation tissue and remodeling of ECM.[88] All these processes are accomplished mainly by keratinocytes and fibroblasts which are predominant cells in epidermis and dermis respectively. The above-mentioned wound healing events form the basis for *in vitro* wound healing studies. Studies on the properties and behavior of these cells in tissue culture plates have provided insight on the wound healing capacity of many PHFs. For instance, a PHF containing extracts of *Combretum smeathmannii*, *Phyllanthus muellerianus* and *Pycnanthus angolensis* was reported to enhance proliferation of dermal fibroblasts and epidermal keratinocytes.[90] Another PHF containing six plants, Herboheal, was proven effective in treating wound infection compared to the use of individual plant extract via *in vitro* studies.[91] Yet another PHF containing extracts of *V. negundo* L., *E. officinalis Gaertn*, and *T. procumbens* L was tested for its *in vitro* wound healing efficacy using fibroblast (L929) and keratinocyte (HaCaT) cell lines. This formulation exhibited significant cell migration at the site of artificial wound (scratch assay) compared to the control.[57]

### 7.2 *In vivo studies*

This type of study makes use of small experimental animals mostly mammals as the model of choice. Some advantageous reasons for using these animals for wound healing studies is that they are relatively inexpensive, easily available, economical to keep and maintain, and can be modified into different genetic wound models.[92] This model allows exploration of pathophysiology of wound healing in real time.[93] Diabetic,[94] and caspase 8 knockout models are a few representatives that are explored as experimental animal wound models.[122] Furthermore, animals chosen for this have short gestation and incubation period, they give birth to multiple offspring at a time, have considerably short life span compared to humans, experiments can be performed through several generations and they also allow provision for experimentation where the results will be known only when the animals are dead. Wound created on these animals heal relatively fast compared to humans which allow repeated experiments to be completed within short period of time.[88] These animals are first anesthetized either by chemical anesthesia (ketamine hydrochloride),[95] or gaseous anesthesia (isoflurane),[96] before the wounding procedure. In an *in vivo* wound healing study, activity of topically applied oil extract containing *R. cordifolia, G. glabra* and *Symplocos racemosa* was reported to enhance wound contraction and re-epithelialization on healthy wistar albino rats.[85] Yet another experiment on wistar albino rats, Ankaferd blood stopper, a PHF containing five different plant extracts, demonstrated its contribution to hemostasis, faster wound contraction and re-epithelialization as well as anti-inflammatory property in the wound healing process. This was also attributed to the different interactions of the diverse phytoconstituents present in the PHF.[51]

### 7.3 *Ex vivo* studies

In *ex vivo* wound healing models, biopsies of different diameter from different parts of skin are excised. Usually a larger punch biopsy approximating to 6mm is taken where a central wound of full thickness having 3mm punch is created on the excised skin. The wounded skin is then placed with dermis down in tissue culture dish containing appropriate culture media. Wound treatment is carried out with drugs or medicinal plant extracts of interest until complete re-epithelialization occurs. Evaluation of healing parameters is done by measuring anti-scar and re-epithelialization capacity of the tested drug using hematoxylin and eosin (H&E) stained sections microscopically.[89,97,98] This model also allows assessment of the development, distribution and density of Chick Chorioallantoic Membrane (CAM) vessels in the medicinal plant treated eggs and the untreated control.[99]Wound healing efficacy of a PHF containing aqueous extracts of *Dalbergia odorifera*, *Epimedium sagittatum*, and *Trichosanthes kirilowii* was tested for its angiogenic efficacy using CAM assay. Results from this supported strong angiogenic activity associated with the PHF.[100] In another experiment, a PHF containing herbal extract of *V. negundo* L., *E. officinalis Gaertn*, and *T. procumbens* L. presented rapid and significant angiogenic activity compared to untreated control.[57] Variation in the number of newly formed CAM vessels in the eggs were evaluated using stereomicroscope at defined time intervals where the blood vessels were counted and calculated as percentage increase for each set of the experimental and control groups.[101]

## 8. ROLE OF PHFs IN VARIOUS ASPECTS OF WOUND HEALING PROCESS

Phases of wound healing are independent at the same time interlinked and occur simultaneously. It is therefore beneficial to garner knowledge on PHFs for their contribution in speedy and effective healing of wounds.[57] Following part of this review discusses studies that give information about the diverse role of PHFs in wound healing process.

### 8.1 Hemostatic managers

There are limited studies on PHFs that are used for managing blood flow when injury occurs. A topical hemostatic agent, Ankaferd Blood Stopper (ABS), is a standardized traditional polyherbal product from five plants namely, *Thymus vulgaris*, *G. glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica* that claims a historical utility for wound management in Turkish traditional medicine. This commercially available hemostat (Ankaferd Health Products Ltd, Turkey) is understood to stimulate the formation of an encapsulated protein network that provides focal points for erythrocyte aggregation via red blood cell - fibrinogen interactions. Hence it is said to be an effective treatment option for hemorrhage.[102]. ABS provides tissue oxygenation and facilitates physiological hemostatic process without affecting individual's coagulation factors. This makes it unique and more advantageous over other plant extracts with hemostatic property when used separately.[103,104]

### *8.2 Anti-oxidant and anti-inflammatory activity*

Antioxidants protect the body against damage caused by free radicals while inflammatory cytokines help in clearing the opportunistic bacteria responsible for chronic wound development. Medicinal plants with pronounced antioxidant properties are reported to scale back oxidative stress significantly. In a particular study, a PHF from plant extracts of "Karanj Beej oil, Jafi, Neem, Sariva sativa, *G. glabra*, *R. cordifolia*, and Patol patra" has been proven to offer a strong antioxidant and anti-microbial activity.[105]Antioxidant activity of a PHF containing *Elephantopus scaber* and *Clinacanthus nutans* was performed *in vitro* by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging antioxidant assay followed by *in vivo* wound healing activities using excision, incision and burn wound models.[17] Application of a PHF with antioxidant properties showed a twofold promotion of wound contraction and re-epithelialization compared to that of the individual plants.[106] PHF by name AVS022, comprising of five medicinal plant extracts (*Harrisonia perforata*, *Capparis micracantha*, *Clerodendrun indicum*, *Ficus racemosa* and *Themeda triandra*) has been reported to exhibit antioxidant activity via inhibition of reactive oxygen species (ROS) and contributed to faster wound contraction and healing.[17,107,108] Antioxidant, anti-inflammatory, and antimicrobial activity of another PHF containing methanolic extract of *Plumbago zeylanica Linn*, *Datura stramonium Linn* and *Argemone mexicana Linn* also contributed to faster wound contraction and healing compared to the effect by individual plant extracts.[109] Most antioxidants prevent the damage of oxidizable substrates like proteins, lipids, carbohydrates DNA, hence will have positive impact on wound healing.

### 8.3 *Antimicrobial activity*

Bacterial pathogens associated with wound infections are mostly multidrug-resistant strains of gram-positive bacteria such as *S. aureus*, *B. subtilis, Streptococcus pyogenes* and gram-negative bacteria like *P. aeruginosa, Chromobacterium violaceum* and *Serratia marcescens*. Multidrug resistant bacteria are the reason why majority of wounds doesn't heal fast which make them one of the leading cause of high mortality and morbidity in patients suffering from chronic wound infection.[91] In a study, anti-infective potential of a wound-healing formula known as Herboheal (which has reached the level of commercialization) made up of the extracts of six plants; *A. indica*, *Acacia nilotica*, *Ocimum sanctum*, *Annona squamosa*, *C. longa* and *Ricinus communis* was tested against one of the most common wound-infecting bacteria *S. aureus*. Observations from this study suggest that this formulation could exert inhibitory effect on quorum sensing-regulated virulence factors, increase antibiotic susceptibility and inhibit biofilm formation.[91] Herboheal preparation was proven to be effective only in combination as against individual plant extracts.[110] Likewise, PHF containing ethanolic extracts of *Justicia tranquebariensis, A. vera* and *C. longa* exhibited significant antimicrobial activity on both gram negative and gram positive bacteria alongside faster wound healing.[48,77] Herbal formulation of extracts of *Piper nigrum* and *C. longa* was tested against three pathogenic bacterial strains viz. *S. aureus, B. subtilis* and *P. aeruginosa*. This herbal formulation exhibited significant antimicrobial and faster wound healing activity only in the formulation as against the application of individual plant extract.[77] Another PHF containing a mixture of medicinal plant extracts of Karanj Beej Oil, Jafi, Neem, Sariva sativa, *G. glabra*, *R. cordifolia*, and *Trichosanthes dioica* was evaluated against four pathogenic microorganisms by agar diffusion method. The results showed mild-to-moderate antimicrobial activity against *Escherichia coli*, *Klebsiella aerogenes*, *P. aeruginosa* and *Proteus vulgaris*.[108] The multiple component nature of PHFs make the bacteria less susceptible to the event of resistance and hence contributes to the improvement of wound condition besides faster healing.[14,91]

### *8.4 Anti-scar agents*

Scar is formed from excessive deposition of ECM during wound healing.[111] A raised, firm, irregular surface usually red or pink in color characterizes scar formation. It is either limited to the wound area or extends into the neighboring skin after the wound heals.[112,113]Various strategies are required to prevent scar formation along with quality of healed wounds. However, there are currently no effective treatments to achieve scarless wound healing in adult.[114] Recent research has employed the utilization of phytoconstituents to resolve this problem. The introduction of PHF wound care strategy appears to be further promising in this aspect.[111,115]It has been shown that the so-called anti-scar medicines prescribed by wound care professionals usually contain around 25% of active plant components. It has been reported that a PHF containing *Gelidium amansii* and *Scutellaria baicalensis georgi* gave evidence towards its promising contribution in the treatment of hypertrophic scars.[116,117] A study aimed at evolving an effective polyherbal ointment (PHO) containing oils of *Cordia obliqua willd*, *Dendrophthoe falcata(l.f) Ettingsh* and *Vigna radiata* Linn showed that it could contribute significantly to faster and scarless wound healing process by increasing wound contraction, controlled rate of collagen synthesis and high antioxidant activity.[115] Yet another study in which an ointment combination of *Tectona grandis*, *F. religiosa* and *Caesalpinia pulcherrima* was reported to promote faster wound closure, moderate collagen formation and increase breaking strength of the healed wounds with less scar formation.[118] A combination of the roots of *Astragalus membranaceus* and *Rehmannia glutinosa* (in 2:1 ratio) significantly reduced the wound area of foot ulcer in rat model, whereas no healing effect was observed when individual herb was applied.[61]

### *8.5. Angiogenic promoters*

The newly formed tissue during wound healing process must receive sufficient supply of nutrients and oxygen to be functional. Formation of new blood vessels (angiogenesis) plays a pivotal role in ECM formation and hence considered critical for faster and effective wound healing. [119] Various processes involved in angiogenesis require a mixture of multiple phytoconstituents which will target and facilitate these processes either individually or synergistically. Recent studies focusing on PHF in this context have shown effectiveness in increasing angiogenesis during wound healing.[57] PHF containing leaves of *V. negundo*, bark of *E. officinalis* and whole plant of *T. procumbens* was reported to exhibit a robust angiogenic potential in rat excision wound model. The increase in tertiary and quaternary blood vessels was found to be significant in PHF treated wounds. Angiogenic effect of this PHF was proven to be due to synergistic contribution of the components of plant extracts.[57]

## 9. MECHANISM OF ACTION OF PHFs ON WOUND TISSUE

The complex nature of wound healing processes which include hemostasis, promotion of local inflammation, removal of cellular debris and pathogens, proliferation of fibroblasts, keratinocytes and endothelial cells as well as their migration into the wound bed, secretion of ECM, angiogenesis and tissue remodeling require a multitargeted (polyvalent) drug such as PHF.[18,120] This is because PHFs have positive influence by acting synergistically and simultaneously on different processes of wound repair.[106,121] The multi-target properties of PHF concurrently modulate several signaling pathways responsible for effective wound healing. These signaling pathway mediators such as transforming growth factor-beta (TGFβ), focal adhesion kinases (FAK), phosphatidylinositol 3-kinases (PI3K), extracellular signal-regulated kinase (ERK), Smad2 and Smad7 are known to control various molecular processes that lead to appropriate and scar-less wound healing.[115,122] Interactions of the multiple phytoconstituents present in PHFs bring about transformations of some active phytoconstituents which lead to the formation of new pharmacologically active compounds that are not found in either of the individual plants.[121,123] Such phenomena can serve as an emerging option to discover novel drugs or a combination of drugs that need to be fully explored by pharmaceutical industries for improved and quality wound healing.[27] Colonization of wounds by bacteria is among the major reasons why wounds become chronic if not properly managed.[124] These bacteria develop resistance to a wide range of commonly prescribed antibiotics, leading to delayed healing of many infected wounds.[62] One of the effective options to tackle this issue is to reduce their pathogenicity making them susceptible to antibiotics. The multiple phytoconstituents in PHF have been reported to simultaneously inhibit the production of diverse virulence factors such as biofilm,[125] LasB elastase, pyocyanin and rhamnolipids.[126] Apart from inhibiting virulence factors' production, PHF has also been reported to exhibit significantly higher bactericidal property in comparison to the use of a single plant.[91] PHF can cause disruption of bacterial cell wall and cell membrane, modify important genetic makeup, cause mutation leading to cell damage and eventually death of the bacteria.[127] The quorum sensing inhibitory and antibacterial properties of PHFs make them better treatment option in combating wound colonizing bacteria.[128,129] Accumulated data reveals that some of the polyherbal combinations and ointment-based formulations developed for wound healing (Table 2) have reached the level of commercialization while a few of them have gone to various levels of clinical trials.[17,130]

**Table 2. List of commercially available polyherbal products indicated in wound healing**

| Name of the product | Mechanism of action | Manufacturers | Plant members in the PHFs | References |
|---|---|---|---|---|
| Ankaferd | Hemostatic, enhance cell proliferation, differentiation and angiogenesis. | Ankaferd Health Products Limited, Istanbul, Turkey. | *Thymus vulgaris* (dried grass extract), *G. glabra* (dried leaf extract), *Vitis vinifera* (dried leaf extract), *Alpinia officinarum* (dried leaf extract) and *Urtica dioica* (dried root extract). | 50 |
| Amarantha Wound Healing Cream | Increases tensile strength of skin and collagen deposition. | Ari Healthcare Private Limited. Pune, Maharashtra. | Jatyadi oil, *F. religiosa*, *F. benghalensis*, *Centella asiatica*, *Shorea robusta*, *G. glabra*, *A. indica*, *P. glabra*, Yashad bhasma | 49 |
| Ari's wound healing cream | Enhance collagen deposition and tensile strength of skin. | Ari Healthcare Private Limited. Pune, Maharashtra. | *G. glabra*, *F. infectoria*, S. *rubusta*, *C. longa*, *B. aristata*, *R. cordifolia*, A. *indica*, *P. glabra*, Yashad bhasma | 49 |
| BC 56 Wound-Healing Cream | Antiseptic, anti-bacterial and enhance wound contraction. | L'amar healthcare products Pvt. Mumbai, Maharashtra. | *S. rubusta*, *A. indica*, *P. pinnata*, *Cassia tora*, *F. infectoria*, *G. glabra*, *Celastrus paniculatus* and Jasad bhasma. | 131 |
| Herboheal | Anti-bacterial, inhibit biofilm formation and quorum sensing related pigment reduction. | Sristi Innovations. Ahmedabad, Gujarat. | *A. indica* (Neem Leaves), *Acacia nilotica* (Bark), *Ocimum sanctum* Linn. (Leaves), *Annona squamosa* Linn. (Leaves), C. *longa* Linn. (Rhizome) and *Ricinus communis* L. (Seed oil) and Bee wax. | 91 |
| Himalaya Herbals Antiseptic Cream | Anti-bacterial, anti-inflammatory agent and antifungal. | Himalaya Wellness. Bengaluru, Karnataka. | *Barbados Aloe* (*A. vera*, kumari) Almond (*Prunus amygdalus*, vatada) Five-leaved Chaste Tree (*V. negundo*, nirgundi) Indian Madder (*R. cordifolia*, manjistha) Zinc Calx (Yashad bhasma) Sodium Biborate (tankana) | 132 |
| Jatyadi Ghrita | Anti-inflammatory, enhance re-epithelialization, angiogenesis, granulation | Arya Vaidya Sala, Kottakkal. | Purana Ghritam, Puranakera Tailam, *Myristica fragrans houtt*, *A. indica A. Juss*, *Trichosanthes dioica* Roxb leaf, *Picrorhiza kurroa* root, *B. aristata* | 133 |

| | | | | |
|---|---|---|---|---|
| | tissue maturation and remodeling | | stem, *C. longa* Linn, *Hemidesmus indicus R.*, *R. cordifolia* Linn, *Terminalia chebula* Retz, blue vitriol, *G. glabra* Linn, *P. pinnata* pierre, beeswax. | |
| Magnactive Premium Ayurvedic Herbal Antiseptic Cream | Anti-bacterial, anti-inflammatory, minimizes skin elasticity loss. | Green cure Private Limited. Arihant Nagar, West Punjabi Bagh, New Delhi, Delhi. | Tea tree leaf oil, Magnolia and Zincum. | 134 |
| Pentaphyte P5® | Antimicrobial, inhibit quorum sensing regulated pigment production. | Dr. Palep's Medical Research Foundation Private Limited. Mumbai, Maharashtra. | Bark extracts of *F. benghalensis*, *F. religiosa*, Ficus *racemosa*, *Ficus lacor* and *Albizia lebbeck*. | 135 |
| Ruuh heal cream | Antiseptic, anti-bacterial, enhance wound contraction, cell proliferation and collagen deposition. | Forest gold products private limited. Mumbai, Maharashtra. | Sal (*S. rubusta*), Neem (*A. indica*), Bhindur (*F. infectoria*) and Karanj (*P. pinnata*). | 136 |
| Triphala | Anti-bacterial, enhance wound contraction, collagen secretion, antioxidant properties. Reduction of matrix metalloproteinase (MMP) expression. | The Indian medical practitioners cooperative pharmacy and store limited (IMPCOPS), Chennai, Tamil Nadu, India | Methanolic extracts of *T. chebula*, *Terminalia bellirica* and *E. officinalis.* | 137 |
| Septilin | Anti-bacterial, anti-inflammatory, enhance wound contraction. | The Himalaya Drug Company Makali, Tumkur Road, Bangalore 562123. | Commiphora wightii, Conch shell calx, *Tinospora cordifolia*, *R. cordifolia*, *E. officinalis*, *moringa pterygosperma* and *G. glabra*. | 138,139 |
| Hepatogard | Increase the breaking strength and hydroxyproline content of granulation tissue | Raptakos brett & Co Ltd | Crude powders of *P. kurroa*, *Andrographis paniculata*, *Phyllanthus niruri*, *Boerhavia diffusa*, *A. indica*, *E. officinalis*, *T. chebula*, *Terminalia belerica*, *E. alba* , *Zingiber officinale* and *Piper longum* | 140 |
| Chandanadi Yamak | Enhance wound contraction, angiogenesis, antimicrobial and re-epithelialization | Go-Vigyan Anusha Kendra, Nagpur | *Pterocarpus santalinus*, *F. benghalensis*, *N. nucifera*, *Cyanodon dactylon*, *R. cordifolia*, *Woodfordia fructicosa*, *G. glabra*, Sesame oil, Cow's milk and Cow's ghee | 141 |

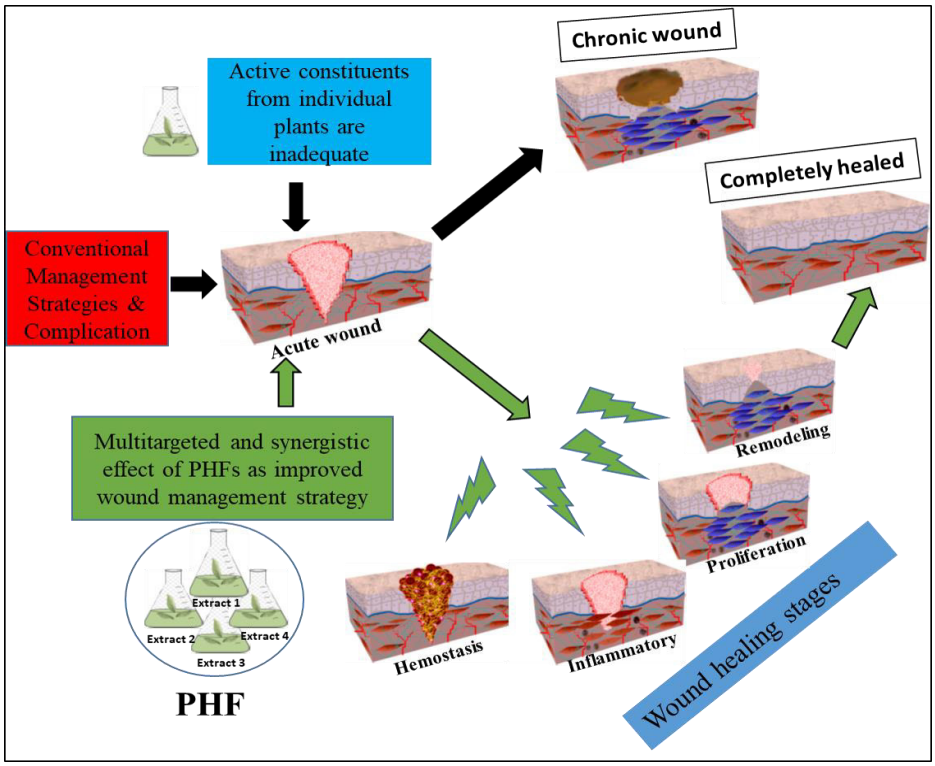
## 10. TOPICAL WOUND DRESSINGS

Currently around 80% of wound management strategies use some form of closure products/dressings such as; sutures, staples, fabric bandages, surgical dressing and tapes.[142] These products are used to prevent infection/absorb exudates/stop bleeding or keep wound moist and at the same time serve as delivery agent for herbal formulation into the wound area to speed up the healing process.[143,144] Some of the dressings used for topical herbal drug delivery include gauze, films, fibers, hydrocolloids, hydrogels, foams, hydrofibers, cryogels, scaffolds etc.[32] Choice between the inexhaustible availability of dressings and topical products for effective wound treatment depends on the type of wound, degree of tissue damage and to a large extent clinicians' experience. Not many of these available synthetic wound care products have strong data to completely support their efficacy in promoting wound repair process because of their associated side effects.[5,6,95,145,146]. However reports on the application of dressings containing PHFs for wound healing activity are scarce.[32]

## 11. ADVANTAGES OF PHFs OVER MONOHERBAL AND CONVENTIONAL WOUND THERAPY

Unlike synthetic drugs, topical application of PHF for wound treatment has generally not displayed any side effects such as skin irritation, toxicity, erythema, eschar, edema during acute dermal toxicity and skin irritation test in animal burn wound model.[122] Topical wound healing drugs can easily get into the systemic circulation because of the loss of epidermal layer in full-thickness wounds causing different kinds of dysfunctions. Studies investigating the adverse effects of herbal wound healing products on systemic functions after penetration through the skin are scarce.[12] PHFs explored so far present a lot of advantages over many current conventional wound healing drugs. Multiple target response at various phases of wound healing (Figure 4), no or fewer side effects, minimal scar formation, effectiveness at low and safety at high doses (wide therapeutic range) are to name a few of its merits along with the convenience it offers to patients as a single drug.[18,19] These advantages have prompted scientists and wound care professionals to consider alternative medicinal practices,

particularly in the area of PHF for the management and treatment of wounds.[122]




***Adapted from,*** [28] ***with modification***

**Note: - Black arrows show the path of wound healing contribution of individual plant extracts and conventional wound strategies while green arrows show the path of wound healing contribution of PHFs**

***Fig 4. Schematic representation of the advantage of PHF over monoherbal and conventional wound management strategies.***

## 12 DISCUSSION

To increase the efficiency of PHFs in wound healing, it is essential to fully understand the detailed molecular mechanism of their complex interactions.[16,147] It is important to invest more focus on the pharmacodynamic and pharmacokinetic synergy aspects of potential PHFs since therapeutic efficacy of crude form of PHFs is stronger than pure bioactive phytoconstituents.[121,148] Any side effects that might arise due to the herb-herb interaction during their compounding should be clearly studied and reduced to a minimum level that meets safety standards. Following are a few reasons set forth by researchers to support improved wound healing efficacy of PHFs compared to single plant based treatments: (a) despite the fact that individual plant has several phytoconstituents, the active ones might be insufficient to bring about the timely and desired therapeutic effect, (b) plant extracts in crude form give improved therapeutic effect than isolated pure phytoconstituents and (c) harmonized phytoconstituents in PHF work together to enhance the absorption and permeation of the active constituents into the site of action.[18,121] Table 1 and 2 of this review present a summary of selected studies on PHFs used for wound healing and the commercially available PHFs respectively. The medicinal plants combined in these PHFs have demonstrated their individual pharmacological efficacy towards wound treatment and other related diseases. Identifying bioactive component(s) that is giving the desired wound healing efficacy in this complex mixture is currently a daunting task.[149] It is interesting to note that plants such *A. vera*, *Centella asiatica*, *C. longa*, *G. glabra*, *R. cordifolia*, *A. indica*, *E. officinalis*, *F. benghalensis*, *F. infectoria*, *P. pinnata*, *Shorea rubusta*, and *T. procumbens* are frequently the choice in the preparation of the listed PHFs. Their varied individual therapeutic potentials appear to be the basis for selection towards PHF preparation. For instance, when *C. longa* (with anti-oxidant and anti-inflammatory activities), *A. vera* (with strong anti-bacterial property), and *A. indica* (with anti-bacterial and anti-inflammatory activities) were combined, it resulted in enhanced wound healing due to the compounding of these diverse individual therapeutic effects. Most of these plants were extracted using organic solvents such as methanol, ethanol, ethyl acetate, and chloroform, while in some situations even oils from these plants and hydro-alcoholic extracts were used. Based on the fact that there is high content of hydrophilic primary and secondary metabolites in medicinal plants,[150] making similar formulations with aqueous derived extracts may contribute to beneficial prospects towards wound healing efficacy more than the mostly used organic extract components.

## 13. CONCLUSION

Phytoconstituents in PHF provide the desired wound healing effect only when meticulously chosen and put together. This is achieved through a series of optimization processes. Selecting the right medicinal plants with required active phytoconstituents and combining them in an optimized ratio is key towards the preparation of an effective PHF for wound treatment. A thorough molecular study on the contribution of PHFs in the area of proteomic and at genomic levels using specific markers complemented with clinical trials will go a

long way in providing relevant solutions to the current wound healing complications.

## 14. AUTHORS CONTRIBUTION STATEMENT

Bindhu Omana Sukumaran conceptualized and designed the study. Habibu Tanimu prepared, reviewed and edited the original draft. Nadasha Koonath Vijayan reviewed edited and provided valuable inputs towards designing the manuscript. All authors critically reviewed the manuscript, contributed to its revision, and approved the final version submitted.

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## 16. CONFLICT OF INTEREST

Conflict of interest declared none

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