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Review Article

Complimentary/Alternative Medicine Section

Phytotherapeutics for Wound Healing: A Systematic Review on Effectiveness of Indian Herbal Extracts in Rat Models

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ABSTRACT

Wound healing is a multifaceted process that encompasses a coordinated set of chemical and cellular events aimed at restoring the integrity of the skin and underlying tissues. Globally, many individuals turn to medicinal plants as an alternative therapy for this purpose. The present review focuses on evaluating the in-vivo wound healing activity of herbal plant extracts native to India, comparing them with povidone-iodine. Plant extracts show potential as promising wound healing agents due to their rich active compounds, wide availability and low risk of side-effects. Data were gathered from various sources, including scientific databases such as Google Scholar, PubMed and ScienceDirect. Keywords such as "wound healing," "animal studies," "herbal," "Wistar albino rats," "India," and "Ayurvedic" were used to identify relevant articles, which were then analysed. The present review compiles data from articles published over the past 14 years on the in-vivo wound healing potential of medicinal plants in rats, with povidone-iodine consistently used as a reference in these studies. The findings highlight the potential of medicinal plants to contribute to drug discovery as effective wound healers, which may lead to the development of new, safe drug molecules. Further research is required, including novel formulations and targeted drug delivery systems, alongside clinical trials, to scientifically validate these findings and transition them from the laboratory to industry for broader societal benefit.

Keywords: Haemostasis, Hydroxyproline, Wistar albino rats, Wound breaking strength

INTRODUCTION

The skin serves a vital role in human physiology, acting as a protective barrier and playing a critical part in preventing serious systemic disorders that can result from extensive damage [1,2]. Wounds (also known as *vrana* in Ayurveda) are injuries that compromise the skin or other body tissues. These can be classified as acute or chronic based on the healing time and other related factors. Wound healing is essential for restoring tissue integrity and involves four key stages: haemostasis, inflammation, proliferation and tissue remodelling.

During haemostasis, the body initiates cellular and molecular processes to repair damaged blood vessels and stop bleeding. The inflammation stage follows, characterised by the release of biochemical agents to prevent bacterial infection and clear away debris. While inflammation is crucial for cleaning the wound, prolonged inflammation can damage tissues, impede cell growth and result in chronic wounds. In the proliferative phase, new blood vessels form, granulation tissue develops, collagen is deposited and the wound begins to contract and epithelialise. Finally, tissue remodelling leads to the maturation of scar tissue and the formation of new epithelium. Wounds are a significant public health issue, affecting approximately 15.03 per 1,000 people and encompassing both acute and chronic wounds [3,4].

Sushruta, an ancient Indian surgeon, provided detailed guidelines for the care of *Sharirika vrana* (physical wounds) in his *Sutrasthana*, outlining seven major categories that expand into 60 different treatment forms [5]. The traditional Indian Ayurvedic system emphasises natural products to promote healthy living and prevent diseases. Known for its herbal formulations that address health imbalances, Ayurveda aims not only to restore health but also to prevent the recurrence of disease [6]. Herbal remedies are integral to various ancient medical systems, including those from Greece, China and Egypt. The World Health Organisation (WHO) estimates that nearly 80% of the world's population depends on natural herbal products for maintaining their health [7]. These natural remedies may be used individually or in combination as polyherbal formulations [8].

Many plant extracts and phytoconstituents possess potent healing properties, are readily available and have minimal adverse effects, making them promising alternatives for wound healing [9]. Documenting ethnomedicinal knowledge is crucial for discovering novel therapeutic molecules, as medicinal plants contain diverse biologically active compounds [10,11]. These compounds exhibit chemical complexity and are often difficult to synthesise. It is this complexity that contributes to their broad therapeutic activities [12,13]. Notably, around 25% of contemporary medications originate from plants, either directly or indirectly, underscoring the importance of plant-based treatments [14,15]. The therapeutic properties of between 35,000 and 70,000 medicinal plants have been established to date. Historically, ethnopharmacologically active herbs were a primary source of medicine [16]. In India, traditional medicines are commonly used for skin disorders [17]. The availability, affordability and low side-effect profile of herbal plants have increased the demand for these natural treatments for various skin diseases [18]. Even in areas where modern medical systems are easily accessible, alternative medicine systems such as Ayurveda or Unani are still preferred [19,20]. There is a pressing need for proper cataloguing and evaluation of herbal medications for wound healing. Despite the extensive use of herbal remedies in India, comprehensive analyses from multiple perspectives are still lacking and the clinical relevance of these traditional practices needs further exploration.

Povidone-iodine is an effective and safe iodophore-based antiseptic formulation that has been popular for wound care for decades. It offers broad-spectrum antimicrobial activity, penetrates biofilms, reduces bacterial resistance and demonstrates anti-inflammatory effects with minimal cytotoxicity, with no reported negative impact on wound healing in clinical settings [21]. Evidence from clinical studies suggests that iodine does not have the harmful effects once believed to hinder wound healing in burns and chronic wounds and it continues to be a valuable antiseptic agent [22]. Povidone-iodine is among the limited number of antimicrobial agents employed in wound care [23,24]. Identifying a wound-healing agent with efficacy comparable to iodine could be highly beneficial. While there is extensive literature on various phytomedicines for wound healing, a clear comparison of effective herbal medicines against povidone-iodine has not been conducted. This review addresses this gap.

The present systematic review compiles and compares various phytotherapies tested on different rat models to investigate their wound-healing potential, using povidone-iodine as a comparator. The primary objective is to identify herbal formulations that have demonstrated significant preclinical efficacy in wound healing. Additionally, the review examines other key pharmacological aspects of these herbal medicines, such as their antimicrobial and anti-inflammatory effects. The aim is to analyse primary literature on wound healing, identify existing gaps and outline a comprehensive future research direction.

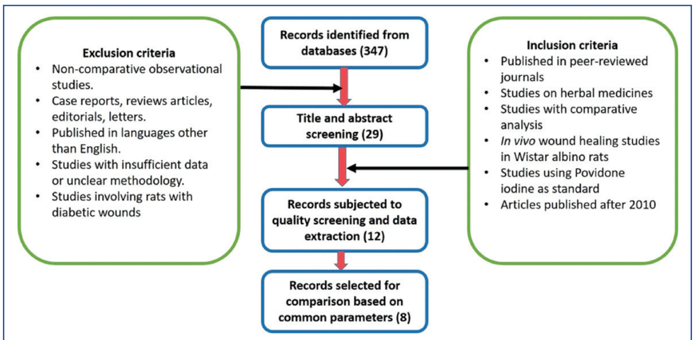
REVIEW OF LITERATURE

Research Methodology

Given the extensive body of literature on phytomedicines, a systematic review is an appropriate method for evaluating the current research on this subject. The growing interest in herbal formulations has attracted considerable attention from researchers, highlighting it as a significant and emerging field in alternative medicine. Consequently, the present systematic review provides a clear and organised summary of the relevant studies.

Sources: The review process began by searching three primary research databases: Google Scholar, PubMed and ScienceDirect, to gather relevant articles. Google Scholar, however, yielded mostly non specific results with the chosen keywords. Therefore, ScienceDirect was selected as the main source for collecting primary data for the review. The search strategy employed a mix of pertinent keywords and Medical Subject Headings (MeSH) terms. The selection of articles from the database was conducted using relevant key search terms including "wound healing," "animal studies," "herbal," "Wistar albino rats," "India," and "Ayurvedic," among others. The collected research papers were analysed and used for further evaluation.

Theme identification: The theme for the review was determined after a thorough analysis of the research papers obtained using the specified keywords. This analysis employed a research framework that initially involved screening the titles to identify key themes related to herbal formulations for wound treatment. Based on this process, the papers were categorised into various research themes. One prominent theme that emerged was in-vivo studies using rats. Further examination of the data focused on a particular sub-theme where povidone-iodine (Betadine) was used as a comparator [Table/Fig-1].



[Table/Fig-1]: Research methodology.

Data extraction and synthesis: After identifying the main theme, the papers categorised under the sub-themes were thoroughly reviewed. Each paper in these sub-themes was then subjected to a quality assessment. Ultimately, common parameters were identified and the data were collected for comparison.

RESULTS

The reviewed studies primarily examined the wound healing effects of different herbal extracts in Wistar albino rats and compared their efficacy to that of a 5% w/w povidone-iodine ointment. The herbs investigated included polyherbal formulations, *Woodfordia fruticosa* gold nanoparticles and single plant extracts such as *Bambusa vulgaris, Cestrum nocturnum, Eucalyptus citriodora, Delonix elata, Coleus aromaticus* and *Ficus benghalensis*. The extraction solvents varied widely, including dimethyl sulphoxide, water, ethyl acetate, ethanol and multiple solvents, indicating diverse extraction methods to maximise the efficacy of bioactive compounds. The studies were conducted across various locations in India (Rajasthan, Madhya Pradesh, Andhra Pradesh, Karnataka and Gujarat), involving sample sizes ranging from 24 to 36 rats [Table/Fig-2] [9,25-31].

S. No.	Study	Title	Year	Herb	Extract solvent	Location	Study population	Comparison
1	Dev SK et al., (2019) [9]	Antimicrobial, anti-inflammatory and wound healing activity of polyherbal formulation	2019	Polyherbal Drug (*Plumbago zeylanica*, *Datura stramonium*, *Argemone mexicana*)	Dimethyl sulphoxide	Udaipur, Rajasthan, India	30	5% w/w povidone iodine ointment
2	Raghuwanshi N et al., (2017) [25]	Synergistic effects of *Woodfordia fruticosa* gold nanoparticles in preventing microbial adhesion and accelerating wound healing in Wistar albino rats in-vivo	2017	*Woodfordia fruticosa* Nano-Gold Particles (WfAuNPs)	Water	Bhopal (23°25′N, 77°41′E) Madhya Pradesh, India	30	Povidone iodine 5% w/w ointment
3	Lodhi S et al., (2016) [26]	Preliminary investigation for wound healing and anti-inflammatory effects of *Bambusa vulgaris* leaves in rats	2016	*B. vulgaris* (Barberry)	Ethyl acetate (BVL-A) and aqueous (BVL-B) fractions of ethanol extract	Village Kukri Kheda, Near Jabalpur, Madhya Pradesh, India	36	5% w/w povidone iodine ointment
4	Nagar HK et al., (2016) [27]	Pharmacological Investigation of the Wound Healing Activity of *Cestrum nocturnum (L.)* Ointment in Wistar Albino Rats	2016	*Cestrum nocturnum (L.)* leaf extract	Ethanol	Govindpura, Bhopal, Madhya Pradesh, India	30	5% (w/w) povidone iodine
5	Velmurugan C et al., (2013) [28]	Wound healing potential of leaves of *Eucalyptus Citriodora* lin rats	2013	*Eucalyptus Citriodora* lin leaves	Ethyl acetate and ethanol extracts	Government hospital, Madanapalle, Chittoor District, Andhra Pradesh, India	24	5% (w/w) povidone iodine
6	Krishnappa P et al., (2016) [29]	Wound healing activity of Delonix elata stem bark extract and its isolated constituent quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside in rats	2016	Delonix elata stem bark extract and its isolated constituent quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside	Multiple solvents	Chitradurga, Karnataka, India	24	5% (m/m) povidone-iodine ointment

7	Jain AK et al., (2012) [30]	Wound healing activity of leaves and roots of aqueous extracts of Coleus aromaticus in rats	2012	Leaves and roots of Coleus aromaticus	Water	Barkatullah University, Bhopal (Madhya Pradesh), India	36	5% povidone-iodine ointment
8	Murti K et al., (2011) [31]	Healing promoting potentials of roots of *Ficus benghalensis L.* in albino rats	2011	Roots of *Ficus benghalensis L.*	Water, Ethanol	Modasa, Gujarat, India	NA	NA

[Table/Fig-2]: Details of the analysed studies [9,25-31].

The selected studies evaluated the effectiveness of herbal extracts in promoting wound healing, comparing their results to those of povidone-iodine, using the excision wound model in rats. Wound size reduction percentages were assessed at different time points, ranging from 11 to 20 days. The herbal extracts demonstrated varying effectiveness, with the test formulations achieving wound size reductions comparable to or better than those of povidone-iodine in several studies. For instance, Krishnappa P et al., and Murti K et al., reported 100% wound size reduction with their test extracts on day 16, which was superior to that of povidone-iodine [29,31]. Conversely, Raghuwanshi N et al., and Velmurugan C et al., showed relatively lower wound size reductions compared to povidone-iodine by day 12 and day 11, respectively [25,28] [Table/Fig-3] [9,25-31].

S. No.	Study	% reduction in wound size over baseline (Mean±SEM)		Day of assessment	Model
		Betadine	Test		
1	Dev SK et al., (2019) [9]	88.5	81.83	20	Excision wound model
2	Raghuwanshi N et al., (2017) [25]	96.01±0.21	93.80±0.15	12	Excision wound model
3	Lodhi S et al., (2016) [26]	100.35±3.94	100.27±4.27	18	Excision wound model
4	Nagar HK et al., (2016) [27]	94.3±0.43	93.58±0.76	16	Excision wound model
5	Velmurugan C et al., (2013) [28]	89.5	87.4	11	Excision wound model
6	Krishnappa P et al., (2016) [29]	96.7	100	16	Excision wound model
7	Jain AK et al., (2012) [30]	100	99.427	16	Excision wound model
8	Murti K et al., (2011) [31]	92.80±0.23	100.00±0.00	16	Excision wound model

[Table/Fig-3]: Wound healing comparison [9,25-31].

The studies compared the epithelialisation period (the time required for wound closure and the formation of new epithelial tissue) between povidone-iodine and various herbal extracts in rats with excision wound models. The epithelialisation period varied across studies, with some test formulations demonstrating shorter or comparable periods to povidone-iodine. For example, Murti K et al., reported a shorter epithelialisation period for their test extract (13.33±0.42 days) compared to povidone-iodine (16.00±0.26 days) [31]. Similarly, Krishnappa P et al., showed a slightly shorter period for their test extract (17±0.25 days) than for povidone-iodine (18±0.29 days) [29]. In contrast, Dev SK et al., and Raghuwanshi N et al., found a slightly longer epithelialisation period for their test formulations compared to povidone-iodine (23 days and 15.50±0.22 days; and 22 days and 14.83±0.30 days, respectively) [9,25]. Other studies, such as Lodhi S et al., and Nagar HK et al., demonstrated similar or marginal differences between povidone-iodine and the test extracts [26,27]. Overall, the results highlight that while some herbal extracts demonstrated improved or equivalent epithelialisation periods, others were slightly less effective compared to povidone-iodine in promoting wound closure in rats [Table/Fig-4] [9,25-31].

S. No.	Study	Epithelialisation period in days (Mean±SEM)		Model
		Betadine	Test	
1	Dev SK et al., (2019) [9]	22	23	Excision wound model
2	Raghuwanshi N et al., (2017) [25]	14.83±0.30	15.50±0.22	Excision wound model
3	Lodhi S et al., (2016) [26]	18	18	Excision wound model
4	Nagar HK et al., (2016) [27]	17.33±0.4	17.66±0.7	Excision wound model
5	Velmurugan C et al., (2013) [28]	2.94±0.51	3.63±0.75	Excision wound model
6	Krishnappa P et al., (2016) [29]	18±0.29	17±0.25	Excision wound model
7	Jain AK et al., (2012) [30]	12.5±0.34	13.666±0.33	Excision wound model
8	Murti K et al., (2011) [31]	16.00±0.26	13.33±0.42	Excision wound model

[Table/Fig-4]: Epithelisation period comparison [9,25-31].

The wound breaking strength was also assessed for both incision and excision wound models. The assessments were performed between 9 to 11 days post-treatment. Results varied, with some herbal extracts showing comparable or superior wound breaking strength. For instance, Krishnappa P et al., reported a higher breaking strength for the test extract (710.5±10.5 g) compared to povidone-iodine (694.5±5.5 g) [29]. In contrast, Dev SK et al., and Raghuwanshi N et al., found lower breaking strength in their test groups (450 g and 234.91±1.95 g, respectively) compared to povidone-iodine (650 g and 242.86±4.73 g, respectively) [9,25]. Studies by Lodhi S et al., showed similar strengths for both (939.4±22.74 g vs. 942.7±23.52 g) [26]. However, Murti K et al., demonstrated a notably higher strength for their test extract (502.3±2.26 g) than for povidone-iodine (429.7±3.21 g) [Table/Fig-5] [9,25-31].

S. No.	Study	Wound breaking strength in g (Mean±SEM)		Day of assessment	Model
		Betadine	Test		
1	Dev SK et al., (2019) [9]	650	450	10	Incision wound model
2	Raghuwanshi N et al., (2017) [25]	242.86±4.73	234.91±1.95	10	Incision wound model
3	Lodhi S et al., (2016) [26]	942.7±23.52	939.4±22.74	9	Incision wound model
4	Nagar HK et al., (2016) [27]	210.76±6.65	201.83±4.98	10	Incision wound model
5	Velmurugan C et al., (2013) [28]	464.16±33.32	436.6±70.12	10	Incision wound model
6	Krishnappa P et al., (2016) [29]	694.5±5.5	710.5±10.5	10	Incision wound model (tensile strength)
7	Jain AK et al., (2012) [30]	617.166±13.0	603.583±4.8	11	Excision wound model (tensile strength)
8	Murti K et al., (2011) [31]	429.7±3.21	502.3±2.26	10	Incision wound model

[Table/Fig-5]: Wound breaking strength comparison [9,25-31].

The studies were compared for hydroxyproline content, a marker of collagen content and wound healing quality, using different wound models. Hydroxyproline levels varied across studies, with some herbal extracts showing similar or higher levels than Betadine. Murti K et al., reported a higher content for their test extract (56.49±0.57 mg/mL) compared to povidone-iodine (40.80±0.25 mg/mL) in the dead space wound model [31]. Conversely, Dev SK et al., and Raghuwanshi N et al., found slightly lower hydroxyproline levels in their test groups (42 µg/100 mg tissue and 41.63±0.75 µg/100 mg tissue, respectively) compared to povidone-iodine (45 µg/100 mg tissue and 44.65±0.75 µg/100 mg tissue, respectively) in the excision wound model [9,25]. Studies by Krishnappa P et al., showed almost identical levels between the test and povidone-iodine [Table/Fig-6] [9,25-31].

S. No.	Study	(Mean±SEM)		Model
		Betadine	Test	
1	Dev SK et al., (2019) [9]	45 (µg/100 mg tissue)	42 (µg/100 mg tissue)	Excision wound model
2	Raghuwanshi N et al., (2017) [25]	44.65±0.75 (µg/100 mg tissue)	41.63±0.75 (µg/100 mg tissue)	Excision wound model
3	Lodhi S et al., (2016) [26]	75.14±2.89 (mg/g tissues)	78.63±3.28 (mg/g tissues)	Incision wound model
4	Nagar HK et al., (2016) [27]	34.50±0.84 (µg/100 mg tissues)	32.16±0.65 (µg/100 mg tissues)	Incision wound model
5	Velmurugan C et al., (2013) [28]	1159.33±2.39 µg	1983.66±3 µg	Incision wound model
6	Krishnappa P et al., (2016) [29]	4.76±0.07 (mg/100 mg dry tissue)	4.77±0.03 (mg/100 mg dry tissue)	Dead space wound model
7	Jain AK et al., (2012) [30]	71.513±5.0 (mg/g)	54.098±2.4 (mg/g)	Excision wound model
8	Murti K et al., (2011) [31]	40.80±0.25 (mg/mL)	56.49±0.57 (mg/mL)	Dead space wound model

[Table/Fig-6]: Hydroxyproline content comparison [9,25-31].

DISCUSSION

Povidone-iodine's effectiveness in wound healing is well-documented, attributed to its wide antibacterial spectrum, ability to combat biofilms, absence of cross-resistance, low cytotoxicity and generally good tolerance. It is bactericidal, fungicidal and virucidal within 1 to 5 minutes, making it a preferred disinfectant for minor wounds on skin and mucous membranes. It is also widely used in surgeries to avoid Surgical Site Infections (SSIs). However, concerns about cytotoxicity and incompatibility with silver dressings limit its use in chronic wounds [3]. The studies reviewed here demonstrated that various herbal extracts have wound healing potential comparable to or better than that of povidone-iodine. Approximately 25% of the reviewed studies showed that herbal extracts outperformed povidone-iodine in terms of wound area reduction, while the rest displayed comparable efficacy. This suggests that these extracts could potentially overcome some of the limitations associated with povidone-iodine, warranting further exploration to fully exploit their wound healing potential.

Epithelialisation, which involves the regeneration of epithelial cells over the surface of the wound, is closely linked to accelerated wound contraction. This phase can be assessed through histological studies and wound closure rate measurements, often beginning within a few days post-injury in rats [32,33]. In 25% of the reviewed studies, the epithelialisation period was shorter with herbal extracts compared to povidone-iodine, indicating faster wound closure.

Wound healing strength, a key parameter in wound healing assessment, measures the maximum load that healed tissue can withstand before breaking. This strength, along with hydroxyproline content, provides insight into the collagen content and quality of the newly formed tissue. A higher hydroxyproline content indicates greater collagen deposition, which is crucial for tissue strength and durability [34]. Previous studies, such as those investigating *Terminalia chebula* and *Vitis vinifera*, have reported accelerated wound healing, increased wound contraction, higher hydroxyproline levels and reduced epithelialisation times [35,36]. Similar findings were observed in the present review, where extracts with superior wound healing potential compared to povidone-iodine demonstrated greater wound healing strength, higher hydroxyproline content and shorter epithelialisation periods.

Various experimental wound models, including excision, incision, burn wound and dead space wound models, have been used to evaluate the effectiveness of herbal formulations, with the excision model being the most common [37,38]. However, comparing studies is challenging due to variability in experimental set-ups, wound models, measurement techniques, treatment dosages, biological variability, assessment timing, control group management and histological interpretations. These differences can lead to inconsistent results and impact the reliability of data. Accurate control groups and expertise in histological analysis are crucial to minimise biases and ensure valid comparisons.

Despite these challenges, a comprehensive assessment of the wound healing potentials of different treatments is crucial for identifying the most effective options for drug development. Such an analysis can help streamline the drug development process by guiding the selection of promising candidates for further research and clinical trials, ultimately leading to more effective and targeted wound healing therapies.

CONCLUSION(S)

Medicinal plants offer a rich source of natural bioactives with considerable therapeutic potential. Herbal formulations, which utilise these plant-derived compounds, provide numerous benefits and have been the subject of extensive research demonstrating their effectiveness. They are known to activate several physiological processes that promote and accelerate wound healing. Despite this promise, a systematic approach is required to fully leverage the potential of herbal formulations. Recent literature indicates that herbal preparations might be more effective than povidone-iodine for wound healing, as they can activate various healing pathways. However, the majority of the evidence comes from in-vivo studies, which provide only a partial understanding of their efficacy and safety. Therefore, it is crucial to advance herbal formulations through rigorous clinical trials to validate their effectiveness and safety in diverse populations. Addressing existing challenges, such as standardising production processes and scaling up production, will be essential for the development of more effective herbal wound healing products. By overcoming these obstacles, it will be possible to create herbal treatments with enhanced efficacy and fewer side-effects, offering a valuable alternative to conventional wound care options.

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