International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2024; 12 (1): 99-104
ISSN:2320-7817(p) | 2320-964X(o)

Research Article Open Access

Evaluation of wound healing potential of polyherbal formulation in human subjects

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Manuscript details:
Received: 27.02.2024
Accepted: 24.03.2024
Published: 31.03.2024

Cite this article as:
Lalita Kushwah, Rupali Dutt, Manisha Singh and DK Sharma (2024) Evaluation of wound healing potential of polyherbal formulation in human subjects, *Int. J. of Life Sciences*, 12 (1): 99-104

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Tagetes erecta- flowers, *Tridax procumbens*- wholeplant, *Azadirachta indica*- leaves, *Ficus benghalensis*- bark, *Curcuma longa*- rhizomes and *Aloe vera*- leaves are popular all over the world for their medicinal values. The present study aimed to develop a polyherbal formulation and validate its wound-healing potential in human subjects. Selected plants parts were subjected to Soxhlet extraction individually. The phytochemical-based Formulation (ointment) was topically applied on the wounds of experimental human subjects. A significant (**p<0.01) rate of wound contraction was observed between the 10th day to the 50th day. However, the polyherbal formulation (5%) was found superior in human subjects, the wound was completely (100%) healed on 50th day followed by an evaluation of its biochemical parameters. Hydroxyproline (49.55%) and Hexosamine (41.74%) were also found significantly increase on 30th day. This study revealed that the 5% Polyherbal formulation holds immense therapeutic significance and can be extrapolated to diabetic wounds where delay in wound healing is a major issue.

Keywords: Polyherbal formulation, wound healing, hydroxyproline, hexosamine.

INTRODUCTION

Wounds are physical injuries that cause a break in the skin, leading to disruption of its normal anatomy and function (Chhabra *et al.,* 2017). The process of wound healing divided into four overlapping phases: homeostasis, inflammation, proliferation, and remodeling (Landen *et al.,* 2016). Wound management involves disinfection, debridement, and creating a moist environment to facilitate natural healing (Pazyar *et al.,* 2014). Herbal medicines have long been used for treating various ailments in traditional medicine (Biswas, 2004). Plants such as *Alchemilla genus, Curcuma genus, and those in the Composite families, as well as Azadirachta indica, Delonix elata, Ficus benghalensis, Calendula officinalis, Caesalpinia ferrea, Citrus tamurana*, and *Aloe vera* have been utilized in Ayurvedic and Chinese medicine to treat digestive tract, liver, skin, and wound disorders (Albahri *et al.,* 2023).

More than 70% of wound healing pharma products are of plants origin. Poly herbal formulations made by selected medicinal plants (Sembian *et al.,* 2015). Polyherbal formulations are better than the chemical wound healing formulations because the crude polyherbal formulations have various phyto-contituents which have anti-inflammatory, antimicrobial properties and antioxidant potential promotes the wound healing. Plant based wound therapy not only accelerates healing process but also safe (Talekar *et al.,* 2017). Hence Polyherbal formulations have high degree of potential in the treatment of wound and high efficacy.

Tagetes erecta, also known as French marigold, is a medicinal and ornamental plant with significant therapeutic value in medicine. It belongs to the Asteraceae family and exhibits antibiotic, antimicrobial, antiparasitic, antiseptic, antispasmodic, and anti-inflammatory properties. *Tagetes erecta* is rich in alkaloids, phenolic compounds, terpenes, and salicylic acid (Devika, 2014). *Tridax procumbens*, also known as Ghamra, is a medicinal plant used for various disorders such as cuts, wounds, and burns. It shows various pharmacological activities including anti-inflammatory, antibacterial, and antioxidant activities. The plant contains chemical constituents like alkaloids, flavonoids, carotenoids, B-sitosterol, fumaric acid, luteolin, quercetin, and tannin. *Tridax procumbens* promotes wound healing (Ankita and Amita, 2012). *Azadirachta indica*, commonly known as Neem, contains active ingredients such as nimbidin and sodium nimbidate with anti-inflammatory, antifungal, and antibacterial properties that aid in the healing process. Neem also contains an excellent amount of amino acids, vitamins, and minerals that play an important role in wound healing processes in the proliferation phase and in the formation of collagen and angiogenesis (Naveen *et al.,* 2015). *Ficus benghalensis*, known as the Indian banyan tree, contains various components such as tannin, phenols, saponins, sugar, alkaloids, terpenoids, flavonoids, glycosides, proteins, separated amino acids, and steroids. *Ficus benghalensis* shows antimicrobial, antioxidant, anti-inflammatory, anti-ulcer, and wound healing activities (Ogunlowo *et al.,* 2013). *Aloe vera* is universally known as a wound-healing herb and is composed of various sugars such as glucose, mannose and cellulose, as well as various enzymes and vitamins and minerals (calcium, sodium, magnesium, zinc, and copper). *Aloe vera* possesses anti-inflammatory, antimicrobial, and antioxidant properties, all of which are beneficial for wound healing. Its benefits include reducing inflammation, synthesizing collagen, promoting skin regeneration, and increasing blood supply, oxygenation, and the production of hemoglobin in red blood cells. Curcumin is a polyphenol derived from turmeric and has been widely used for centuries in indigenous medicine for the treatment of inflammatory conditions. The active medicinal ingredients of *Curcuma longa*, curcuminoids, and curcumin have been beneficial in the treatment of various disorders, including skin diseases. Curcumin plays an important role in wound healing, skin regeneration, and has been shown to enhance wound healing (Rajesh, 2013). *Curcuma longa* also exhibits antimicrobial, antioxidant, anti-inflammatory, anti-ulcer, and wound healing activity.

The aim of this work was to evaluate of wound healing potentials of combination of extracts (polyherbal formulation) ointment containing phytochemicals on wounds in human subjects.

MATERIALS AND METHODS

The desired part(s) of herbal plants (*Tagetes erecta*- flowers, *Tridax procumbens*- wholeplant, *Azadirachta indica*- leaves and *Ficus benghalensis*- bark) were collected from Gwalior and were identified by the Coordinator of Institute of Ethanbiology, Jiwaji University Gwalior. The selected part of plants and herbs were shade dried, grounded. Extraction was prepared by Soxhlet method using 95% ethanol. 300gm of fresh *aloe vera* leaves were grounded. Solution was shocked in 100 ethanol for 24 hours on magnetic starrier. Then mixture was centrifuged at 10,000 rpm for 30min. The supernatant was evaporated at 45^{o} for 4 to 5 days. Curcumin of *Curcuma longa* was used commercially (Kushwah *et al.,* 2024).

5% w/w polyherbal formulation (ointment) was made by mixing equal amounts of all extracts with emulsifying wax, soft paraffin and liquid paraffin as tabulated below and applied topically on wounds in human subjects. Human wound subjects (n=10) were selected from Diabetes and Foot Care Center 41, Saraswati Nagar, Near Mahalgaon, City Centre, Gwalior (M.P.). The Human study was approved by the Communication of Decision of the Institutional Human Ethics Committee (IHEC) of the University No.

JU/IHEC/2019-A/10. Complete clinical history and physical examination of subjects was recorded. Firstly, wound clean with normal saline. Then 5% Formulation was topically applied daily (with help of clinic staff) on the wound and covered with bandage till completely healing. On 0th and 30th day collected the wound sample for estimations. Wound area measurements was done by using a measurement scale (length, width depth) in cm. Images were taken of wound area. Granulation tissue collected on 0th and 30th day were for biochemical parameters like hydroxyproline (Woessner, 1961) and Hexosamine (Kleson and Morgan, 1933) content estimation. Analysis of variance (ANOVA) was calculated to test significance between 0th and 30th day's values.

RESULTS AND DISCUSSION

The study involved human patients with wounds that were 10 days to 1 month old. A polyherbal formulation was applied daily until complete wound healing. The size of the wound was measured on the 10-days intervals. The study scientifically validated the wound-healing activity of these wounds in human subjects. Complete healing of the wound was observed on the 50th day through the topical application of the polyherbal formulation. A significantly (**p<0.01) rate of wound closure was observed between the 10th day and the 50th day. Images showed the complete healing process (Fig. 2 & 3).

Table 1: Human subjects were selected according to the criteria.

Variables				
Subjects N=10	**Min**	**Max**	**Mean**	**SE**
Age (Year)	26	70	44.50	1.16
BMI (Kg/m²)	17	28	23.33	0.46
Blood Glucose Level-Fasting (mg/dl)	81	115	99.83	1.75
Postprandial (PP) (mg/dl)	115	164	139.97	2.50
Area of Wound (cm³)	4	15	12.22	2.08
Age of Wound	10 Day	1 Month	-	

Table 2: Effect of polyherbal formulation on wound healing in human subjects (Wound Contraction shows in cm³ and %).

Wound Contraction (cm³ and %)						
Day	**0th**	**10th**	**20th**	**30th**	**40th**	**50th**
cm³	12.0±2.0	8.48±1.45	5.42±1.36	1.53±0.87	0.23±0.12	Healed**
%		29.33	54.81	87.22	98.06	100.00

The values are expressed as Mean ± SEM; *N=10*, **p<0.01; significantly difference from 0 day by Repeated Measures one-way ANOVA.

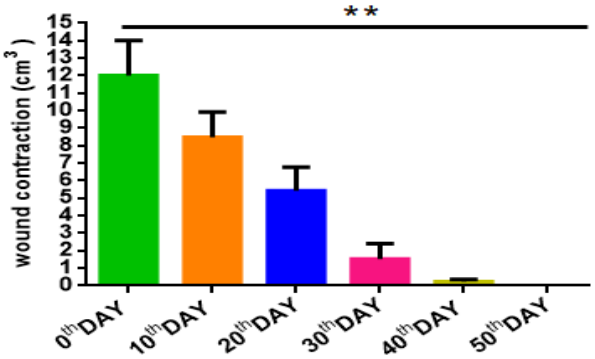

Fig.1: Effect of polyherbal formulation on Wound healing in human subjects (cm³).

Fig. 2: Images of wound healing therapy on left leg showed complete healing on 50th day.

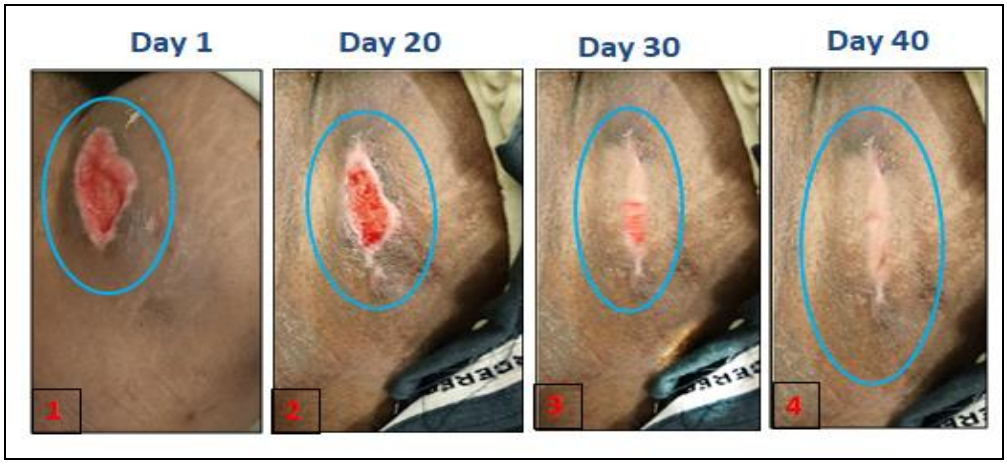

Fig 3: Images of wound healing therapy on left thigh showed complete healing on 40th day.

The concentrations of hydroxyproline and hexosamine were measured in the granulation tissue on the 0th and 30th day, as shown in Figure 1 and Table 3. A significant (**p<0.01) increase of 49.55% in hydroxyproline and 41.74% in hexosamine was observed. On the 30th day, both hydroxyproline and hexosamine levels were found to be significantly increased, and these are important components of the extracellular matrix for healing.

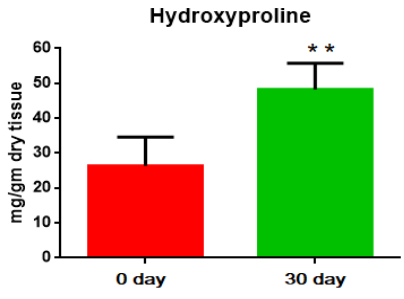

Fig. 4: Concentration of Hydroxyproline.

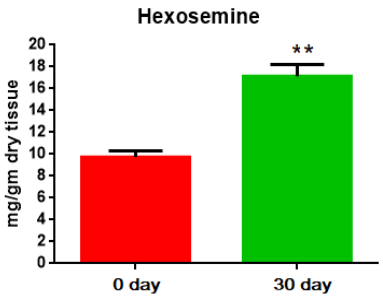

Fig. 5: Concentration of Hexosamine.

Table 3: Effect of polyherbal formulation on Hydroxyproline and Hexosamine concentration of granulation tissue on different days of wound healing.

Parameters	0th day	30th day	Difference (%)
Hydroxyproline (mg/g dry tissue)	26.31±8.24	48.24±7.52	(↑ 49.55%)
Hexosamine (mg/g dry tissue)	9.21±2.11	15.81±2.40	(↑ 41.74%)

The values are expressed as Mean ± SE; *N=10*, *p<0.05, **p<0.01; significantly difference from 0 day by paired t test.

The biochemical analysis revealed an increase in hydroxyproline content, indicating a boost in cellular proliferation and collagen synthesis. The elevated hexosamine content indicates the strengthening of collagen molecules through improved electrostatic and ionic interactions (Albaugh *et al.,* 2017). Collagen plays a crucial role in regulating the wound healing process. It aids in wound healing by attracting fibroblasts and promoting new collagen formation in the wound bed. (Mathew-Steiner *et al.,* 2021). Therefore, the enhanced synthesis of collagen and hexosamine in treated wounds contributes to the strength of the repaired tissue and the healing process.

Wounds are physical injuries to the skin, and a complex process is involved in the body's response to an injury to restore the function and integrity of damaged tissues (Guo and Dipietro, 2010). These selected plants have been found to have various pharmacologic properties and exhibited good wound-healing functions in various studies. Experimental plants have shown the presence of phenols, flavonoids, and tannins, which are responsible for anti-bacterial, anti-carcinogenic, anti-inflammatory, anti-coagulant, and antioxidant properties, as well as wound-healing properties.

Wound contraction is the process of healthy skin surrounding the wound covering the denuded area. This movement of the wound margin is believed to be due to the activity of myofibroblasts. The wound contraction is attributed to the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. During the maturation and remodeling phase, collagens are remodeled and cells that are no longer needed are removed by apoptosis (Chitturi *et al.,* 2015). The Formulation based on phytochemicals has a significant influence on one or more of the stages, resulting in faster wound closure.

The results, thus, clearly substantiate the beneficial effects of the topical application of phytochemical based polyherbal formulation in the acceleration of wound healing. 5% polyherbal formulation (ointment) enhanced the rate of wound contraction and decreased mean wound healing time.

CONCLUSION

Wound healing consists of an orderly progression of events that re-establish the integrity of the damaged tissue. The present study revealed the wound healing activity of phytochemical based ointment (5%) proves superior and achieves faster wound healing.

REFERENCES

Albahri G, Badran A, Hijazi A, Daou A, Baydoun E, Nasser M & Merah O (2023). The Therapeutic Wound Healing Bioactivities of Various Medicinal Plants. *Life (Basel, Switzerland)*, *13*, 317.

Albaugh VL, Mukherjee K & Barbul A (2017). Proline Precursors and Collagen Synthesis: Biochemical Challenges of Nutrient Supplementation and Wound Healing. *The Journal of nutrition*, 147, 2011–2017.

Ankita J, & Amita J (2012). *Tridax pracumbens* (L.): A weed with immense medicinal importance: a review. *Internation Journal of Pharma and Bio Sciences,* 3, 6275-6299.

Biswas TK, Maity LN & Mukherjee B (2004). Wound healing potential of Pterocarpus santalinus linn: a pharmacological evaluation. *The international journal of lower extremity wounds*, *3*, 143–150.

Chhabra S, Chhabra N, Kaur A & Gupta N (2017). Wound Healing Concepts in Clinical Practice of OMFS. *Journal of maxillofacial and oral surgery*, *16*, 403–423.

Chitturi RT, Balasubramaniam AM, Parameswar RA, Kesavan G, Haris KT & Mohideen K (2015). The role of myofibroblasts in wound healing, contraction and its clinical implications in cleft palate repair. *Journal of international oral health, 7*, 75–80.

Devika R & Justin K (2014). Screening And Evaluation Of Bioactive Components Of *Tagetes Erecta* By Gc Ms Analysis. *Asian Journal of Pharmaceutical and clinical research*, 2, 204-212.

Guo S & Dipietro LA (2010). Factors affecting wound healing. *Journal of dental research*, 89, 219–229.

Kushwah L, Dutt R. & Sharma DK (2024). Comparative GC-MS analysis of phytochemical compounds in the ethanolic extracts of *Tagetes erecta* and *Tridax procumbens*. *International Journal of Biological Innovations*, 6, 01-08.

Landén NX, Li D & Ståhle M (2016). Transition from inflammation to proliferation: a critical step during wound healing. *Cellular and molecular life sciences, 73*, 3861–3885.

Mathew-Steiner SS, Roy S & Sen CK (2021). Collagen in Wound Healing. *Bioengineering (Basel, Switzerland)*, *8*, 63.

Naveen K, Chundran R & Husen I (2015). Effect of Neem Leaves Extract (*Azadirachta Indica*) on Wound Healing. The journal of Althea Medical, 2, 208-215.

Ogunlowo OP, Arimah BD & Adebayo MA (2013). Phytochemicals analysis and comparison of in-vitro

antimicrovial activity of the leaf, stems and root bark of *ficus benghalensis*. *Journal of Pharmacy,* 4, 33-38.

Pazyar N, Yaghoobi R, Rafiee E, Mehrabian A, & Feily A (2014). Skin wound healing and phytomedicine: a review. *Skin pharmacology and physiology*, 27, 303–310.

Rajesh L. Thangapazham SS and Radha K (2013). Maheshwari. Phytochemicals in wound healing. *The journal of advance in wound care,* 5, 56-62

Sembian S, Kalidass S, Jeevan R, Durai S, Femina W, Pemila E & Chitraselvi R (2015) Evaluation of wound healing activity of *Acacia caesia* in rats. *Wound Med.* 4, 105-110.

Talekar YP, Apte KG, Paygude SV, Tondare PR & Parab PB (2017). Studies on wound healing potential of polyherbal formulation using in vitro and in vivo assays. *J Ayurveda Integr Med*, 8, 73-81.

WOESSNER JFJR (1961). The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Archives of biochemistry and biophysics*, *93*, 440–447.

Acknowledgement

I would like to convey my heartfelt thanks to my guide Late. Prof. GBKS Prasad, School of studies in Biochemistry, Jiwaji University Gwalior.

Conflict of interest: The authors declare that they have no conflict of interest.

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Peer review information
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