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Original Article

Evaluation of Wound Healing Activity of Polyherbal Gel Containing *Azadirachta indica, A. Juss*. and *Tridax procumbens* L. Extracts

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ABSTRACT

Background: *Azadirachta indica A. Juss.* and *Tridax procumbens* L. have been utilized for wound management and therapy since ancient times and offer huge potential. **Objectives:** To formulate polyherbal gel containing *Azadirachta indica A. Juss.* and *Tridax procumbens* L. and to evaluate it further in wound management. **Materials and Methods:** In this study, three polyherbal formulations of *Azadirachta indica A. Juss.* and *Tridax procumbens* L. with varied proportions of both plants were formulated and appearance, nature, grittiness, phase separation, pH, viscosity and extrudability of all prepared formulations were compared with control, standard (Betadine 5%) and individual formulations of these plants. Excision wound model was used to study wound healing action of prepared formulation. **Results:** Formulation with *Azadirachta indica A. Juss.* and *Tridax procumbence* L. extracts in equal proportion showed the best results in formulation parameters. The rate of wound contraction, tensile strength, epithelialization rate, and hydroxyproline content of the wound were used to determine the rate of wound healing. According to the results, there was an increase in wound contraction as well as an increase in wound strength for this formulation. Correlations between hydroxyproline expression and healing patterns were also found in histopathology, it was observed that the wounds treated with an equal proportion of plant extract and betadine minimized scar formation and improved fibroblast proliferation, angiogenesis, keratinization, and epithelialization. **Conclusion:** As per the different physical, histological, and biochemical parameters, hydroalcoholic extract of *Azadirachta indica A. Juss.* and *Tridax procumbens* L. in equal proportions showed good wound healing activity.

Keywords: Wound healing, Polyherbal Gel Formulation, *Azadirachta Indica A. Juss.*, *Tridax procumbens* L., *in vivo* evaluation of formulation.

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INTRODUCTION

Azadirachta indica A. Juss. (Neem) in Meliaceae family is well known plant for its antimicrobial, anti-inflammatory, cell proliferation and wound healing properties.[1-5] *Tridax procumbens* L. known as coat buttons or Tridax daisy from Asteraceae family is traditionally used for the treatment of wounds by tribal people.[6-10] Hydroalcoholic extract and herbal gels of Tridax daisy and Neem are explored by researchers for its wound healing properties with promising results.[1-10] Herbal medicated plasters containing the extracts of *Tridax procumbens* and *Azadirachta indica. J*were formulated and its wound Healing activity was studied previously.[11] These studies were lacking formulation details, stability studies and proper evidences of mechanism of action. Both plant extracts contain carbohydrates, tannins, flavonoids, alkaloids, glycosides and steroids. Chemical constituents in the leaves of both the plants have antimicrobial, antioxidant and anti-inflammatory properties. Most of the chemical components in both extracts have antioxidant properties which maintain nontoxic ROS levels in the wound tissues and fasten the wound healing process. Polyherbal formulation containing Neem and Tridax daisy may provide efficient wound healing with additional antimicrobial, antioxidant and anti-inflammatory benefits.[12-17] Gel is preferably used topical formulation because of its better stability as well as convenience in application and washing of wound area. Formulation of polyherbal gel with more effective wound heling activity and more shelf life and stability properties is a challenge for the research. This study emphasizes on formulation and evaluation of polyherbal gel of

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Azadirachta indica A. Juss. and *Tridax procumbens* in different proportions and its effectiveness in wound management. All pharmaceutical parameter of gel formulation and stability study of these formulations were evaluated and further applied it for wound healing study.

MATERIALS AND METHODS

Plant Materials *Azadirachta indica A. Juss.* and *Tridax procumbens* L. were collected in October 2021 from Jalgaon District in Maharashtra, Identification was done from Botanical Survey of India, Western regional center, 7-Koregaon road, Pune 411001. A voucher specimen has been deposited at the BSI Herbarium (BSI/WRC/lden.Cer. /2021/22102100071142) dated October 2021. Carbopol-934 was procured from Yarrow Chem Product Mumbai- 421201, Methyl Paraben and Propyl Paraben were procured from Research lab, Propylene glycol was procured from Dhalop chemicals, Glycerin was procured from Hexon laboratories Pvt. Ltd., Triethanolamine procured from Loba Chemie Pvt. Ltd., Ethanol and other solvents used for the study were HPLC grade, Animal were taken by permission of IAEC/CPCCSEA Project Approval Committee and their Proposal number is MCP/IAEC/009/2021.

Plant Collection and Treatment of Plant Materials

The leaves were collected, clean and dried in shade at 25°C, and coarse powder of leaves was prepared. The powder of leaves was macerated with 80% of ethanol in proportion of 1:8. The mixture was shaken after every 12 hr for 4 days, and filtered by using Whatman filter paper number one. Collected filtrate was evaporated on rotary evaporator under reduced pressure at 40°C temperature. Collected residue was dried properly and stored in airtight container at temperature of 4°C until further use. Yield of dark green color extract obtained from leaves of *Azadirachta indica A. Juss.* was 10.79% and slightly light green color extract of *Tridax procumbens* L. leaves was 12.85%.

Preparation of Gel

Carbopol 934 was accurately weighed and distributed in 10 mL of distilled water in a beaker. Beaker was kept aside for half an hour to allow the Carbopol to swell and then stirred for 30 min with a mechanical stirrer at 1200 rpm. 3 mL propylene glycol was placed into a separate beaker and combined with the required quantity of extract, and also the weighed amounts of propyl paraben and methyl paraben. After all of the Carbopol have been dispersed, 0.2 g extract and preservative solutions were added to the mixture, which was constantly stirred. Final volume was made up by adding the remaining distilled water to the formulations, the volume was increased to 20 mL, and triethanolamine was added drop by drop to the formulations to correct the skin pH (6.8-7) and 2 drops of Glycerin were added to get the smooth texture. Details of all formulations are mentioned in Table 1.

Physical evaluations

The gels were assessed for its organoleptic characteristics such as Appearance, Nature, Grittiness, Phase separation. Other physical tests were carried out such as applicability and washability of gel from skin surface.

pH Determination

A calibrated digital pH meter was used to determine the pH of the herbal gels. A total of 1 g of gel was dissolved in 100 mL of distilled water and kept it aside for 1 ht. The pH of each formulation was tested three times and the average results were calculated (Table 3).

Viscosity

For 30 min, herbal gel samples were kept at room temperature. The viscosity of the formulation was measured using a Brookfield viscometer. After attaching Spindle No. 7, the viscosity was measured at 200 rpm. The tests were carried out three times and recorded (Table 3).

Spreadability

A standard-sized glass slide was utilized, on it 0.5 g of gel was spread in a circle 1 cm in diameter on the glass slide, which was then covered with second same glass slide. A 125 g of weight was kept on upper slide to ensure that the gel formed a fine film over both slides. The weight was then withdrawn, as well as any excess gel at corner. The lower slides were fix on their location and upper slide was attached with weight of 20 g. The time was recorded to detach from one another. Formula used to calculate Spreadability is as follow.

$$S=M\times LT$$

Where:

S-Spreadability in grams/seconds.

M-Mass in grams.

L-Length of slide.

T-Time in seconds.

Extrudability

10 g of each formulation were precisely weighed and put into collapsible tubes that were tightly pushed on one side and clamped. After removing the cap to enable the gel to extrude, the gel was collected and weighed, and the gel percentage was determined.

Sensitivity

To the six individuals' specific amount of gel is applied to their forearms and left for 20 min. Any discomfort that developed after 20 min was documented.

Table 1: Ingredients of gel formulation.

Ingredients	S	C	F1	F2	F3	F4	F5
Azadirachta indica A. Juss.		0	200 mg	150 mg	100 mg	50 mg	0
Tridax procumbence L.		0	0	50 mg	100 mg	150 mg	200 mg
Carbopol 935		0.4 g	0.4 g	0.4 g	0.4 g	0.4 g	0.4 g
Propylene Glycol		3 g	3 g	3 g	3 g	3 g	3 g
Methyl Paraben		0.06 g	0.06 g	0.06 g	0.06 g	0.06 g	0.06 g
Propyl Paraben		0.12 g	0.12 g	0.12 g	0.12 g	0.12 g	0.12 g
Glycerin		6.4 g	6.4 g	6.4 g	6.4 g	6.4 g	6.4 g
Triethanolamine		Q. S	Q. S	Q. S	Q. S	Q. S	Q. S
Water		Q. S	Q. S	Q. S	Q. S	Q. S	Q. S

Table 2: Animal Grouping and their Dose.

Groups	No. of Animals	Dose / Drug
Group I (Control)	6	Placebo gel formulation.
Group II (Standard Betadine)	6	Betadine 5% w/w.
Group II (Gel Formulation)	6	*Azadirachta indica* 1% w/w.
Group IV (Gel Formulation)	6	*A. indica* 0.75%+*T. procumbens* 0.25%w/w.
Group V (Gel Formulation)	6	*A. indica* 0.50%+*T. procumbens* 0.50%w/w.
Group VI (Gel Formulation)	6	*A. indica* 0.25%+*T. Procumbens* 0.75%w/w.
Group VII (Gel Formulation)	6	*Tridax procumbens* 1% w/w.

Washability

A little amount of gel was applied to the skin surface and left to flow for 10 min under the force of running tap water. It was observed when the gel was fully withdrawn.

Stability test

The stability test was carried out in accordance with ICH recommendations. The gels were packaged in airtight containers and kept in stability chamber for three months at 40°C and 75% Relative Humidity (RH). After one month interval, the samples were removed and examined for pH, appearance, nature, grittiness, spreadability, viscosity, odour and extrudability.

Animal

Albino rats weighing 150-200 g were housed in cages (6 rats per cage), The climate was kept constant at 24±1°C, with a Relative Humidity (RH) of 40-60% and a 12/12 hr dark/light cycle. Water and food were provided daily. The study was conducted out with the Institutional Animal Ethics Committee's approval. (MCP/IAEC/09/2021).

Excision wound model

Excision wound was made 1-1.5 cm away from the vertebral column on either side and 5 cm away from the ears in the dorsal thoracic area. On the animals' shaved backs, a circular area of about 1 cm^2. was drawn using a marker. The designated region was entirely removed after the animal was mildly anaesthetized with diethyl ether with a medical sterile blades and scissor. Starting on the day of operation, the corresponding formulation therapy was provided topically to the animals in the respective groups until the full tissue regeneration. The parameters of percentage wound contraction; collagen estimate and duration of epithelialization were investigated.

Design of study

Animal were divided into seven groups each group containing six animals each, Group I considered as control and treated with placebo formulation, Group II as Standard and treated with betadine, Group III, IV, V, VI, VII treated with gel formulation with different proportions of active extract of *Azadirachta indica A. Juss.* and *Tridax Procumbens* L. The wound healing rate was assessed by tracing the wound on days 0, 4, 8, 12, 16, 20 post-wounding using a transparency sheet and permanent marker, the percentage of wound contraction calculated up to complete wound healing. During the whole investigation, no other medication was provided to the animals. Infected Animals were replaced with new ones.

Wound Healing activity of formulation

The continuous decrease in wound area was observed planimetrically by drawing the raw wound borders in mm^2 first on a sterilized transparent paper sheet without causing any harm to the wound region, and then on a sterilized transparency paper sheet. Every four days, Graph paper was used to measure the wound area. Based on the wound area of the first day, the wound

Table 3: Pharmaceutical evaluation parameter for gel formulation.

Parameter	Control	Standard	F1	F2	F3	F4	F5
Appearance	Transparent	Dark Red	Green	Green	Dark Green	Dark Green	Dark Green
Nature	Hm.	Hm.	Hm.	Hm.	Hm.	Hm.	Hm.
Grittiness	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Phase Separation	No	No	No	No	No	No	No
pH	6.5333± 0.0577	6.7333± 0.057735	6.5666± 0.05773	6.6±0.1	6.8333± 0.05773	6.6666± 0.5773	6.5666± 0.05773
Viscosity	8682.66± 1.5275	7022±1	11438±1	11460.33± 0.5773	11372.33± 56.8712	11231±1	11091.33± 1.5275
Spreadability	30.1±0.2645	30.9±0.5567	28.33±0.2081	27.73±0.7767	29.56±0.05773	28.5±0.1	27.33±1.5307
Extrudability	88.14±1.2869	93.77±0.8737	82.40±0.8737	85.24±0.7756	86.15±0.6445	86.25±0.7838	87.36±0.7408

Hm.=Homogeneous, *n*=3.

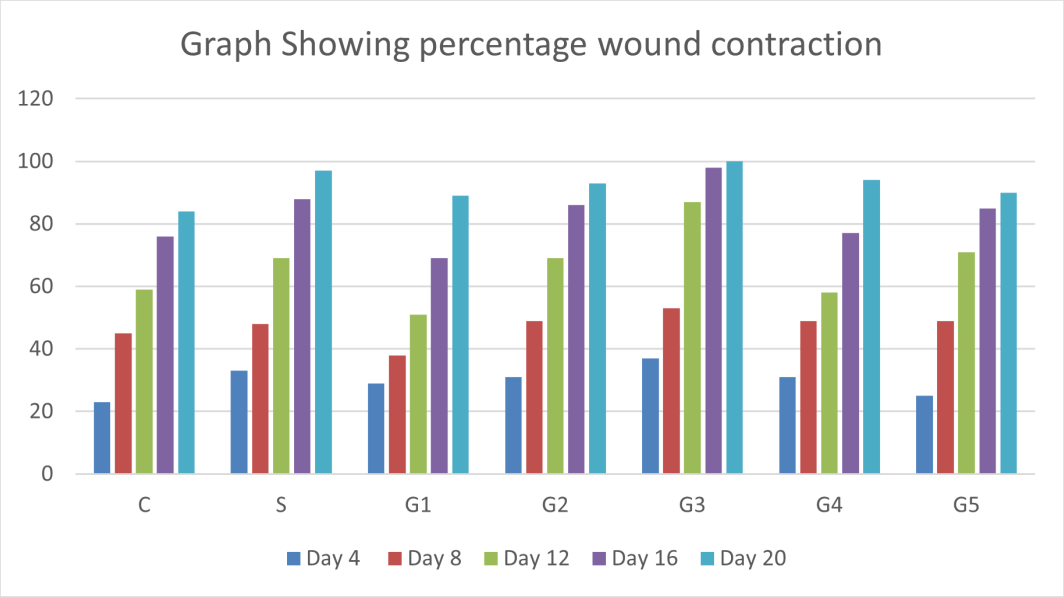

Figure 1: Wound healing group of all group in percentage wound contraction of rats on every four days.

contraction percentage was computed and the column graph was plotted to illustrate the healing activity of the drug in the wound.

Statistical Analysis

The results of the seven groups were compared using ANOVA test accompanied by Kruskal-Walli's test for comparison of wound areas, and ANOVA test accompanied by Tukey's test for comparison of the histopathological results. GraphPad InStat 3 software was used to run these statistical analyses. Each comparison was set at a significance level of $\alpha = 0.05$.

Hydroxyproline

One animal from each group was sacrificed by using an excess of diethyl ether inhalation on the 11th day after the wounds were created. To evaluate the amount of hydroxyproline in the wound tissues, Samples of injured tissue were collected. The tissue was dried in the oven for 12 hr at 70°C, and the weight was recorded. They were then hydrolysed in glass tubes for 24 hr at 120°C in 5N HCl. 1 mL of 0.01 M $CuSO_4$, 1 mL of 2.0 N NaOH, and 1 mL of 5 % H_2O_2 were added to the neutralised hydrolysate samples. The solution was well stirred for 8 min, and shook frequently. After that, the tubes were incubated at 70°C for 5 min. 5 mL 2N H_2SO_4 and 2.5 mL 5% p-dimethylaminobenzaldehyde were added after cooling. After a 20-min incubation period at 70°C, the samples were kept in a 20°C on water bath. Spectrophotometry was used to detect the absorbance at 540 nm.

Standard hydroxyproline sample was made for determination of unknown concentration of tissue sample. In standard four different concentration hydroxyproline were prepared such as 5 ppm, 10 ppm, 15 ppm, and 20 ppm. form absorbance value of standard solution, line of equation was calculated and concentration of unknown sample was determined.

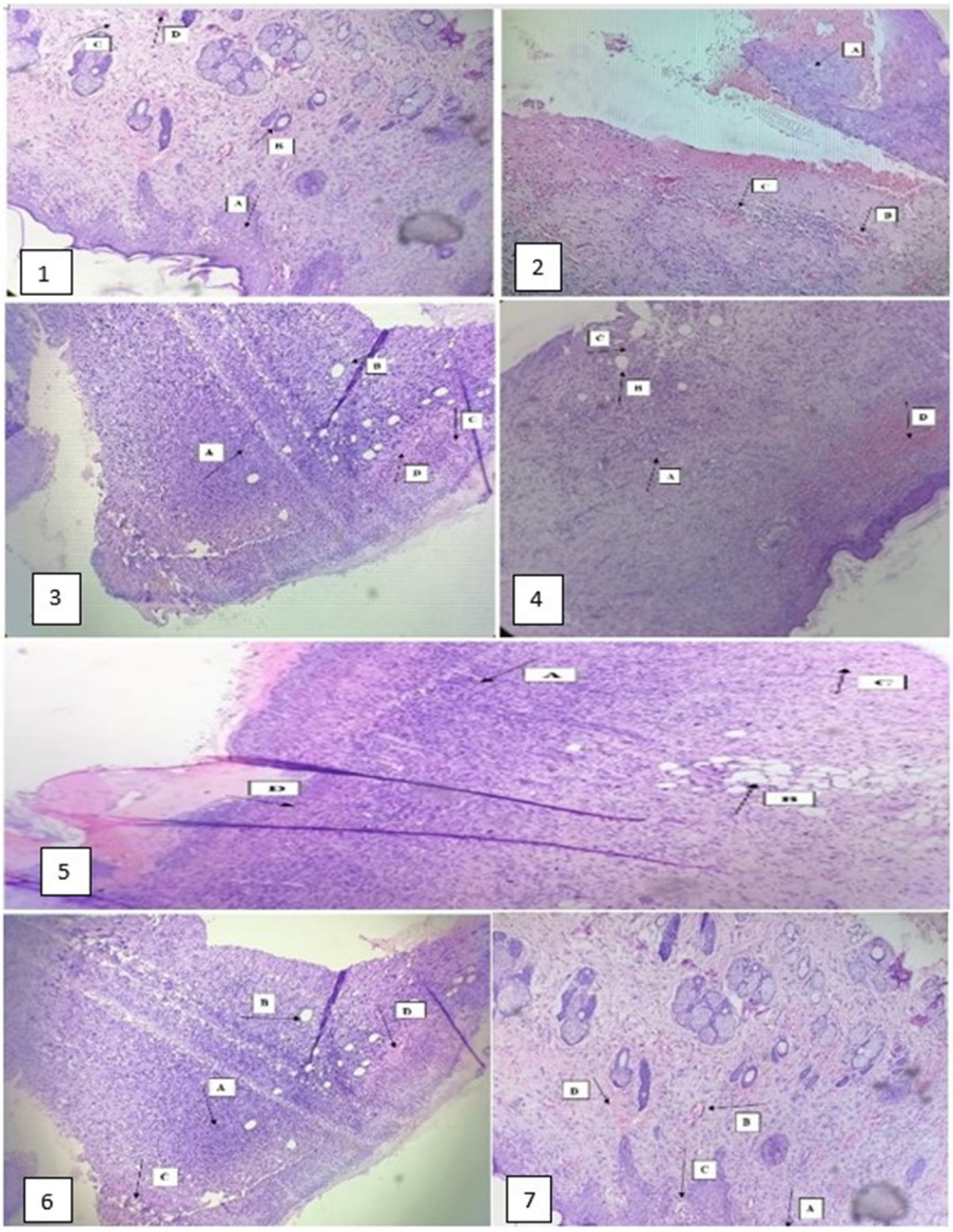

Figure 2: Histopath images of all seven group animals. A=Tissue Granulation, B=Neovascularisation, C= Fibroblast Proliferation, D=Collagen Deposition. 1-Control Group, 2-Standard Group, 3-*Azadirachta indica* 1% w/w, 4-*Azadirachta indica* 0.75%+*Tridax procumbens* 0.25%w/w, 5-*Azadirachta indica* 0.50%+*Tridax procumbens* 0.50% w/w, 6-*Azadirachta indica* 0.25%+*Tridax Procumbens* 0.75%w/w, 7-*Tridax procumbens* 1% w/w.

Histopathological studies

Histopathological examinations were conducted on regenerated tissue that had previously been harvested and fixed in 10% buffered formalin. After a day or two in buffered formalin, the tissue was removed, dehydrated in increasing grades of alcohol, cleaned in chloroform, embedded in paraffin with a tissue processor, and cut with a rotary microtone into slices of 3 to 5 µm in thickness. To aid the staining technique, the slice was dewaxed with xylene and the xylene was removed with descending grades of alcohol.

Staining procedure

Haematoxylin was used to stain the dewaxed portion for 5 to 8 min. For 2 to 3 min, it was washed thoroughly under tap water. It was then checked under a microscope to ensure that there was enough staining. 0.5 to 1.0% hydrochloric acid in 70% alcohol for a few seconds was used to remove extra discoloration. After reacting with the acid, blue stain of haematoxylin was turned red. Washing with running tap water for at least 5 min restored the blue colour and prevented decolorization. Then it was stained for 1 to 3 min with 1% aqueous eosin. For transparency,

Table 4: Stability test formulation F3 after three months.

Three-Month Stability Study			
Parameter	1 Month	2 Month	3 Month
Appearance	Dark Green	Dark Green	Dark Green
Nature	Homogeneous	Homogeneous	Homogeneous
Phase Separation	Nil	Nil	Nil
pH	6.86±0.0546	6.81±0.0584	6.80±0.0524
Viscosity	11535±1.48	11566±1.46	11577±1.39
Spreadability	29.09±0.26	28.72±0.31	28.11±0.33
Extrudability	86.10±0.23	85.70±0.19	84.86±0.21

Table 5: Absorbance and Concentration value for Hydroxyproline.

Sample	Absorbance at 540 nm	Concentration (ppm)
Control	0.0722	3.858209
Standard	0.3489	24.50746
F1	0.1004	5.962687
F2	0.1133	6.925373
F3	0.435	30.93284
F4	0.2201	14.89552
F5	0.1632	10.64925

it was dehydrated in alcohol and then cleaned in xylene. The piece was set in a synthetic resin media. Histopathological alterations in the regenerated tissue were identified for cell inflammation, epithelisation, collagen, fibroblast, and neovascularization during the wound contraction or healing phase in both the test and control groups. These characteristics were evaluated qualitatively and microphotographed at a magnification of "400X."

RESULTS

Pharmaceutical evaluation parameter

The pharmaceutical evaluation parameters of gel formulations were determined and the results are outlined in Table 3. These parameters include appearance, nature, grittiness, and phase separation, pH, viscosity, spreadability, and extrudability.

Stability test

The temperature had no effect on the stability of formulations, which retained their integrity and physical properties. The pH ranged from 6 to 7. All formulation parameter value are within range. Results of stability tests are given in Table 4.

n=3.

By observing three-month stability study of formulation, there is no considerable change in stability of formulation throughout study, this formulation was stable for three months at 40°C and 75% RH. Very minute changes in value of evaluation parameter were observed.

Wound Healing activity of formulation

Formulation F5 Containing equal proportion of *Azadirachta indica A. Juss.* and *Tridax procumbens* L. showed better result for wound healing as compared to other formulation. Rat wound which are treated with F5 formulation epithelialized fast in 21 days only as compared to standard, control and other formulations. Percentage of wound healing of all formulation were determined on 4th day interval and results are given in Figure 1.

Statistical measurement of Wound Areas

The animals were kept in the same quadrupedal position throughout each measurement using a digital calliper to measure wound width and height. Appropriate measurements were taken and the area of each wound was determined based on the measurements. Comparison of the wound area between the seven groups was made at the 1st, 4th, 8th, 12th, 16th and 20th postoperative days. No statistically significant differences between the groups at any of the time points (p>0.10) were observed.

After 12 days, the wound area was significantly smaller in the 5th treated groups than in the control group, although the difference was not statistically significant. Furthermore, 50% of animals with excision wound in G2 and G5 had reached 100% recovery by day 20, whereas only 10.66% of rats with excision wound in G1 had reached the same status. Further, the injured areas were smaller in the treated groups at the conclusion of the evaluation period, and the G5 produced a smaller injury area than those in the other groups. Wound healing graph shows the rate of wound healing of each group up to 20 days (Figure 1).

Hydroxyproline

During the healing process, collagen is synthesized in the tissue. To confirm the synthesized collagen the hydroxyproline content of the wound areas was measured. Hydroxyproline content shown in Table 5.

Table 6: Table showing Injure area and their Standard Error.

Day	Injury area (cm^2)							Standard Error	*p* Value
	G1	G2	G3	G4	G5	G6	G7		
Day 0	49.01	46.5	51.34	53.06	53.5	48.93	65.91	2.4069	>0.10
Day 4	38.18	33.87	36.62	35.47	32.69	35.04	37.19	0.7239	>0.10
Day 8	30.68	25.17	27.78	24.68	24.41	26.92	27.53	0.8366	>0.10
Day 12	22.02	14.17	16.57	15.29	12.02	15.89	16.16	1.1577	>0.10
Day 16	14.68	5.7	8.85	7.02	4.85	7.1	8.23	1.2184	>0.10
Day 20	6.33	1.17	3.64	2.66	0..83	2.49	4.822	0.7416	>0.10

Multiple comparisons were performed using the Tukey-Kramer test. The analysis of the data was carried out using ANOVA. $p<0.05$ and $p<0.01$ were considered significant.

Histopathological studies

On the 21st day of wound healing, samples were collected and stained with haematoxylin and histopath was taken from these samples. In Figure 2A, that neovascularisation was slow in the skin, and the number of blood vessels were also low. Figure 2B shows the photomicrograph of the animal's skin after treatment with the standard gel, an epidermis with a prominent epithelium and a faster rate of epithelisation. The number of neutrophils and fibroblasts were greater than the number of hair follicles. In Figure 2C, a great deal of neutrophils and connective tissues were present on the skin after treatment with Formulation-I. In Figure 2D, the photomicrograph shows that the number of macrophages and fibroblasts were smaller in the skin treated with Gel-II. Figure 2E of the treated skin revealed a well-organised and abundant macrophage population and fibroblast proliferation. Excision of tissue and the resulting collagen formation are seen as the development of new blood vessels, the movement of extracellular matrix, keratin interlinks, and cell proliferation. Figure 2F, the skin treated with Gel-IV had fewer macrophages and fibroblasts than untreated skin. Figure 2G, the skin that had been treated with Formulation-V had a greater number of neutrophils and Fibroblast. These all are shown in Figure 2.

DISCUSSION

Wound healing process includes ensemble of numerous biochemical and cellular activities only wound healing is not the ultimate aim of researchers now a day. More affective, rapid wound healing with hydration, antimicrobial and without scar is the goal of every scientist in wound healing therapy. Wound healing agent derived from natural source are chosen to over orthodox allopathic wound healing agent due to lower risks of side effect. Gels are used as topical formulations because they are more stable and easier to apply and wash in the wound area.

In this study, three polyherbal formulations of *Azadirachta indica A. Juss.* and *Tridax procumbens* L. with varied proportions of both plants (Table 2) constructed and appearance, nature, grittiness, phase separation, pH, viscosity and extrudability of all prepared formulations were compared with control, standard (Betadine 5%) and individual formulations of these plants (Table 3). The lowest viscosity of the polyherbal gel formulation F3 with equal proportion of both plants is 11372 cps, whereas the viscosity of F1 with *Azadirachta indica* to *Tridax procumbence* 3:1 proportion and F2 with *Azadirachta indica* to *Tridax procumbence* 1:3 proportion is 11438 and 11460 cps, respectively. Also, spreadability of Formulation F3 (29.56) is higher than F1 (28.33) and F5 (27.33) formulations (Table 3). The appearance, nature, grittiness, phase separation, pH, viscosity, extrudability, and spreadability of F3 formulation were checked at 40°C and 75±1% relative humidity in three-month stability studies and the results were within acceptable limits (Table 4). After evaluation of all gel formulations, excision wound healing studies were carried out. After 16 days therapy, wound healing studies demonstrated that for F3 gel formulation, healing was 98%, compared to 88% and 76% in the betadine and control groups, respectively (Figure 1). The average injured area of F3 group on 20th day is significantly less than control group and other groups which are treated with formulated drugs, and its results near to standard betadine formulation (Table 5). Based on results, F3 formulation showed much shorter epithelialization times and higher wound contraction rates. Hydroxyproline is essential part of protein collagen which is required for collagen stability. The plant boosted the hydroxyproline content of the excision wound's granulation tissue, indicating rapid collagen formation. Both extracts had a rise in hydroxyproline content, (Table 6) which contributes to faster wound healing.[17] Histopath of the F3 formulation demonstrated a well-organized and plentiful macrophage population as well as fibroblast growth (Figure 2). The growth of new blood vessels, the movement of extracellular matrix, keratin interlinks, and cell proliferation are all visible as a result of tissue excision and the ensuing collagen production. It was a better result compared to other formulations Figure 2.

CONCLUSION

Tridax procumbence leaf chemical constituents like kaempferol, apigenin, catechin, quercetin, naringenin, hydroxybenzoic acid, and caffeic acid have antimicrobial, antioxidant, and anti-inflammatory activities which help in the wound

healing process. *Azadirachta indica* leaves have azadirachtin, nimbolide, ascorbate, quercetin, nimbosterol, and liminoid as chemical constituents with antimicrobial, antioxidant, and anti-inflammatory properties. Most of the chemical components in both extracts have antioxidant properties which preserve nontoxic ROS levels in the wound tissues and accelerate the wound healing. Antimicrobial and anti-inflammatory properties of chemical compounds in both extracts give an additional advantage in wound healing treatment.

After formulation, evaluation and comparison of polyherbal formulations of *Azadirachta indica* and *Tridax procumbence* with varied proportions. Appearance, nature, grittiness, phase separation, pH, viscosity and extrudability of polyherbal gel formulation of *Azadirachta indica A. Juss* and *Tridax procumbens* L. in equal proportion was found to be better than other formulations. After excision model animal studies, wound healing activity of hydroalcoholic extracts of *Azadirachta indica A. Juss.* and *Tridax procumbens* L. in equal proportion has demonstrated the best efficacy. Different physical, histological, and biochemical characteristics support this action. Hence the above results suggest that this polyherbal formulation could potentially be included in fast, effective and better treatment of wounds.

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AUTHOR CONTRIBUTIONS

Dr Aishwarya Balap have contributed in suggesting design of the work, preparation and analysis of the results, interpretation of data and discussion. Mr. Suraj Satpute has performed the practical part. All authors are in agreement with the contents of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPLC: High Performance Liquid Chromatography; **°C:** Degree Celsius; **hr:** Hours, **%:** Percentage; **mL:** Milliliter; **rpm:** Revolutions per minute, **g/gm:** Gram; **pH:** Potential of Hydrogen; **cm:** Centimeter; **S:** Spreadability; **L:** Length; **M:** Mass; **T:** Time; **SQ:** Square; **ANOVA:** Analysis of Variance; **N:** Normality; **NM:** Nanometer; **PPM:** Parts per million; **RH:** Relative Humidity; **G:** Groups; **CPS:** Centipoise; **w:** Weight; **Hm:** Humidity.

SUMMARY

Azadirachta indica A. Juss. and *Tridax procumbens* L. have been known for its wound management potential since ancient times. This study includes formulation and evaluation of polyherbal formulations of *Azadirachta indica A. Juss.* and *Tridax procumbens* L. with varied proportions of both plants and its comparison with control and standard (Betadine 5%). Excision wound model was used to study wound healing action of prepared formulation.

Formulation with equal proportions of *Azadirachta indica A. Juss.* and *Tridax procumbence* L. extracts showed the best results in formulation parameters. As per wound healing results, there was an increase in wound contraction as well as an increase in wound strength for this formulation which was confirmed with histopathology study. It was observed that the wounds treated with an equal proportion of plant extract minimized scar formation and improved fibroblast proliferation, angiogenesis, keratinization, and epithelialization. Hence the above results suggest that this polyherbal formulation could potentially be included in fast, effective and better treatment of wounds.

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