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Research Article

IN VITRO AND IN VIVO WOUND HEALING ACTIVITY OF *DHATAKYADI YOGAM*

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ABSTRACT

Dhatakyadi Yogam (DK) was formulated as an ointment using hydroalcoholic extracts of *Dhataki* (*Woodfordia fruticosa* (L.) Kurz) flowers and *Lodhra* (*Symplocos racemosa* Roxb.) stem bark with ghee and beeswax. **Materials and Methods:** Hydroalcoholic extracts of *Dhataki* flowers and *Lodhra* stem bark were formulated into ointments at 20% w/w (DKO1) and 10% w/w (DKO2). In-vitro wound-healing activity was evaluated using MTT assay (5–15µg/mL), scratch assay, and chick chorioallantoic membrane assay. In-vivo wound-healing activity was evaluated using an excision wound model in Wistar rats divided into five groups: GI (untreated control), GII (povidone iodine), GIII (DKO1), GIV (DKO2), and GV (ointment base). Wound contraction, epithelialization time, and histopathology were assessed. Data were analyzed using one-way ANOVA, with $p<0.05$ considered statistically significant. **Results:** Pharmacognostical and physicochemical parameters were in accordance with API standards, and phytochemical analysis confirmed the presence of phenols, steroids, flavonoids, and alkaloids. Maximum cell viability was observed at 10µL *(Dhataki),* 5µL *(Lodhra),* and 5µL (DK). DK showed the highest wound closure in the scratch assay at 24 h (98.87±0.85%). Peak angiogenesis was observed in the CAM assay at 5µL *(Dhataki),* 10µL *(Lodhra),* and 5µL (DK). In the excision wound model, DKO2 exhibited maximum wound contraction on day 14 (96.94±0.94%) and the shortest epithelialization period (10.4±0.55 days). **Conclusion:** DKO2 demonstrated significant wound-healing efficacy by enhancing fibroblast activity, angiogenesis, wound contraction, and epithelialization, supporting its potential for further clinical evaluation.

INTRODUCTION

The skin, covering an average surface area of about 1.85 m^2 and accounting for nearly 15% of the total body mass, is the human body's largest organ. Its primary role is to serve as a barrier of defence, protecting the body from microbial invasion, harmful chemicals, ultraviolet radiation, and mechanical damage. Beyond its defensive capacity, the skin also participates in essential physiological functions, including regulation of body temperature, preservation of hydration status, and elimination of certain metabolic wastes.

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Additionally, it acts as an active metabolic organ, contributing to the synthesis of several bioactive molecules, most notably vitamin D.[1,2]

A wound can be described as a disruption in the continuity of the epithelial surface, often extending to involve the underlying tissue architecture and functions.[3] The normal healing response in mammals proceeds through three sequential yet overlapping phases: the inflammatory phase, the proliferative or new tissue formation phase, and the remodelling or maturation phase. In recent years, therapeutic approaches have been investigated for wound management, based on the wound type and its inherent regenerative capacity. While these modalities are well-established and relatively convenient to apply, they are frequently linked with certain drawbacks and limitations. Consequently, the development of cost-effective, non-invasive, and patient-friendly wound care strategies remains a crucial need for achieving optimal healing outcomes.[3]

Medicinal plants are a rich source of diverse bioactive phytochemicals, which can be broadly classified into groups such as alkaloids, carotenoids, phenolic compounds, flavonoids, steroids, saponins, tannins, and terpenoids. These secondary metabolites act through multiple mechanisms at various stages of the wound healing process. They exhibit anti-inflammatory, antimicrobial, and antioxidant properties, while simultaneously enhancing collagen deposition, stimulating cellular proliferation, and promoting angiogenesis.[4]

In Ayurveda, wounds are referred to as *Vrana*. Their definition, classifications, and methods of management are elaborated in classical texts such as *Sushruta Samhita*, *Caraka Samhita*, and *Ashtanga Sangraha*. *Sushruta Samhita* outlines sixty distinct therapeutic approaches (*Upakramas*) for the treatment of *Vrana*. These treatises also describe numerous formulations aimed at promoting *Vrana ropana* (healing of wounds) and *Vrana shodhana* (cleansing of wounds).

The formulation *Dhatakyadi Yogam* is mentioned in *Caraka Samhita*, *Chikitsa Sthana* under "*Dwivraneeya Chikitsa*" and is composed of two main ingredients *Dhataki* and *Lodhra*[5].

Dhataki (*Woodfordia fruticosa* (L.) Kurz.) belonging to the family Lythraceae, is a deciduous shrub abundantly distributed across India and other parts of Asia.

Dhataki pushpa is described as having *Kashaya rasa*, with *Laghu* and *Ruksha guna, Katu vipaka*, and *Sheeta veerya.* It is considered beneficial for alleviating *kapha* and *Pitta doshas* and is attributed with actions such as *Sandaneeyam* (healing), *Krimighna* (anthelmintic), and *Visarpahara* (useful in skin disorders)[6].

Phytochemical investigations reveal the presence of of bioactive components which include sugars, tannins, β-sitosterol, gallic acid, and ellagic acid. It has been utilized to cure ailments such as *Atisara* (diarrhea), *Vrana* (wounds), and *Arsha* (haemorrhoids). Modern studies further highlight its pharmacological potential, reporting anti-inflammatory, analgesic, antibacterial, and anthelmintic activities[7].

Lodhra (*Symplocos racemosa* Roxb.), a member of the family Symplocaceae, is a medium-sized tree predominantly distributed in the forests of the Western and Eastern Ghats of India.

Lodhra is described as having *Kashaya rasa*, with *Laghu* and *Ruksha guna*, *Sheeta veerya*, and *Katu vipaka*. It is identified to pacify *kapha* and *pitta doshas*, and is attributed with therapeutic properties such as *Chakshushya* (beneficial for the eyes), *Kushtaghna* (useful in skin diseases), and *Vrana ropana* (wound healing)[8].

Phytochemically, the plant has a number of bioactive components including the principal alkaloids loturine, loturidine, and colloturine, along with flavanol and flavonol glycosides, triterpenoids, and glucopyranosides. Pharmacological studies indicate that *Lodhra* possesses significant anti-inflammatory, analgesic, antibacterial, and anthelmintic properties, supporting its traditional applications in wound healing and other therapeutic uses.[9]

MATERIALS AND METHODS

Collection identification, Authentication

The flowers of *Dhataki* (*Woodfordia fruticosa* (L) Kurz) and stem bark of *Lodhra* (*Symplocos racemosa* Roxb) were collected from local herbal market. The plants were authenticated by pharmacognosist, Government Ayurveda College, Poojapura, Thiruvananthapuram. The drugs were washed, shade-dried, ground into a powder, and stored in an air tight container.

Preliminary Physico chemical and Phytochemical analysis

The initial physical and phytochemical evaluations of the drugs in *Dhatakyadi Yogam* were conducted following the standard methodologies outlined in the API;[10,11,12] it includes foreign matter, moisture content, total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and chromatography.

Preparation of extract

Extract was obtained by Soxhlet extraction method. Flowers of *Woodfordia fruticosa* (L) Kurz. and stem bark of *Symplocos racemosa* Roxb. was shade dried and coarsely powdered using a mixer grinder. 200grams of the drug was refluxed in 500ml aqueous alcohol and was heated with a reflux for 3-4 hrs (at 80-85°C). The process was repeated till the chamber becomes colourless. To eliminate the solvents, the extract was filtered out and dried in a hot air oven set at a temperature of not more than 40°C. The concentrated filtrate was subsequently dried using desiccators. The dried extract was then sealed in a container.[13]

Formulation development and evaluation of ointment

Preparation of ointment

For preparation of *Dhatakyadi ointment*, the extracts, plain ghee and bee wax in ratio 2:7.5:1.2. Ointments containing 10% and 20% w/w extract were prepared. Beeswax was melted in a water bath, ghee was added to obtain a uniform molten base, and the extract was incorporated by levigation with a portion of the base. The mixture was then triturated until a smooth and homogeneous ointment was obtained, transferred into sterile containers, and stored under cool and dry conditions until use.

Evaluation parameters for ointment

Preliminary standardization of *Dhatakyadi* ointment was carried out according to CCRAS guidelines which includes pH, acid value, iodine value, saponification value and rancidity.[14]

In vitro study to assess wound healing potential

MTT Assay

L929 fibroblast cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (50 IU/mL and 500mg/L) under standard conditions in 25cm^2 flasks at 37°C with 5% CO_2 in a humidified incubator. The cytotoxicity and proliferative activity of *Dhatakyadi* hydroalcoholic extract (DKE) were assessed using the MTT assay, a colorimetric method that measures cell viability. Briefly, cells were seeded in 96-well plates and allowed to attach for 24 hours, followed by treatment with different concentrations of DKE (5–25µL) in triplicates, while cells treated with 30% ethanol served as a negative control and those maintained in DMEM alone as experimental control. After 24 hours of incubation, the medium was replaced with 20µL of MTT solution (5mg/mL in PBS) and incubated for 4 hours, enabling viable cells to convert MTT into dark blue formazan crystals. Subsequently, 150µL of DMSO was added to dissolve the formazan, and absorbance was measured using an ELISA plate reader (Epoch, BioTek, serial no. 1707274) at 570nm with a reference wavelength of 630nm.(15) The percentage cell survival (CS) was calculated relative to untreated controls, and results were expressed as mean values plotted against drug concentrations.

$$CS = \frac{\text{Mean absorbance of drug exposed well at specific concentration}}{\text{Mean absorbance of the control well}} \times 100$$

Scratch Assay

In vitro wound healing of DKE was evaluated by scratch assay in L929 fibroblast cells using a 1:1 dimethyl formamide solution. Cells were seeded in 6-well plates, grown to 90% confluence at 37°C with 5% CO_2, and a linear scratch was made with a 200µL pipette tip. After washing with PBS to remove debris, cells were treated with DMEM containing 10% FBS and DKE at concentrations derived from the MTT assay, while controls received serum-free medium. Wound closure was observed at 0, 6, and 24 h under an inverted microscope, and the wound area was quantified using ImageJ software, with the baseline area at 0 h considered 100% for calculating percentage reduction.[16]

Percentage of wound closure was calculated as follows:

$$\frac{\text{Area of initial wound at time t0} - \text{Area of wound at time t1}}{\text{Area of initial wound at time t0}} \times 100$$

CAM Assay

Grouping of eggs

Fertilized chicken eggs were grouped into 7 groups as described in table 1

Table 1: Grouping of Eggs

Group	Treatment	No. of Eggs
Group 1	Negative control (200µl)	2
Group 2	*Dhataki* Extract 5µl	2
Group 3	*Dhataki* Extract 10µl	2
Group 4	*Lodhra* Extract 5µl	2
Group 5	*Lodhra* Extract 10µl	2
Group 6	DK extract 5µl[i]	2
Group 7	DK extract 10µl	2

i- Dhatakyadi extract

Fertilized eggs were disinfected with 70% ethanol and incubated at 37°C with constant humidity. On day 2, 2.5–3mL of albumin was withdrawn through a pinhole at the sharp end to improve CAM visibility, and the hole was sealed with parafilm. A 1×1cm window was then cut in the shell, and sterile filter paper discs were placed over the CAM before resealing. On day 3, test drug concentrations were applied onto the discs using a micropipette, and eggs were re-incubated for 72 h. After reopening, the CAM was photographed, and vessel density and length density were quantified using ImageJ software.[17]

Acute Dermal toxicity

The OECD guideline No. 402 defines acute dermal toxicity as adverse effects observed within a short period after dermal application of a single-dose of a test substance.(18)

Animals

Five female albino rats (Weight:200-300g; Age: 8-12 weeks) were purchased from the Animal House Faculty, Department of Biochemistry, University Campus, Karyavattom, Thiruvananthapuram.

Dose of test drug

An ointment containing 2,000mg/kg of the 20% w/w crude extract of *Dhatakyadi Yogam* was applied uniformly, covering at least 10% of the body surface area.

Body surface area (BSA) is determined using Meeh's formula. [19]

Total BSA (cm^2) = K·w^2/3, where K = 9.83 and *w* = body weight (g).

For a 200 g rat → Total BSA = 342.08 cm^2.

Shaved area (1/10th of BSA) = 34.21 cm^2.

Procedure

Under general anaesthesia induced by intraperitoneal ketamine (10%), fur covering ~10% of the dorsal/flank region was clipped 24 h before

application. The test compound was applied evenly over the exposed skin, covered with sterile gauze and non-irritant adhesive tape for 24 h, with animals housed individually to prevent ingestion. Residual material was removed after exposure, and animals were monitored frequently during the first 24 h (especially 2–6 h) and daily for 14 days for skin, mucous membranes, behaviour, and systemic signs. Body weights were recorded prior to dosing, weekly thereafter, and at study termination. Gross necropsy was performed on all animals, and microscopic examination of affected organs or application sites was undertaken when needed. Animals showing severe distress were humanely euthanized, and all observations were systematically documented.

In vivo wound healing potential in albino rats

The experiment protocols were reviewed by the Institutional Animal Ethics Committee (IAEC/C/03/23) conducted on 08/06/2023, Govt. Ayurveda College, Thiruvananthapuram, and were in accordance with the guidelines of CPCSEA.

Animals

Wistar albino rats of both sexes weighing 150-200 grams were purchased from the Animal House Faculty, Department of Biochemistry, University Campus, Karyavattom, Thiruvananthapuram. All animals were kept under ideal conditions and provided with standard diet and water. The animals were acclimatized 14 days prior to the experiment.

Excision wound model

25 healthy Wistar albino rats of both sexes weighing 150-200 grams were kept in different cages and randomly selected into each group.

Procedure

Rats were anaesthetized with intraperitoneal ketamine (10%), the dorsal fur was shaved, and the site sterilized with ethanol. A full-thickness excision wound (≈450 mm^2) was created on the marked area, and bleeding was controlled with saline-soaked cotton. Animals were randomly divided into five groups (Table 2), and the respective test or control formulations were applied topically once daily for 14 days.[20] In this model, % wound cutting, epithelialization time, and Hydroxyproline content were tested, performed for histopathological examination.

Table 2: Grouping of Animals

Group No.	Intervention
GI-C	Disease control- no topical application
GII-PI	Treatment with 10% Povidone iodine ointment (Standard care)
GIII-DKO1	Treatment with DKO1
GIV-DKO2	Treatment with DKO2
GV-Oint Base	Treatment with Ointment base

Rate of wound contraction

Wound margins were traced using transparent paper and area (in millimeter square) was calculate using a graph paper. Measurements and photographs of each were taken on day 0 and on 2nd, 4th, 6th, 8th, 10th, 12th and 14th day. Rate of wound contraction (percentage) was calculated using the formula.[20]

$$\text{Rate of wound contraction} = \frac{\text{(initial wound area − specific day wound area)}}{\text{initial wound area}} \times 100$$

Period of epithelization

The endpoint of complete reepithelization is considered when eschar falls of and leaving no exposed wound area and the days required for this was considered as period of epithelization.[20]

Tissue Collection

On 15th day after taking the wound measurements the animals were exposed with high dose of diethyl ether. The granulation tissue/healing tissue were excised and fixed in 10% buffered formalin solution.[20]

Histopathology

Paraffin-embedded wound tissue sections (5–6 µm thick) were prepared, stained with hematoxylin and eosin, and examined microscopically to assess histopathological changes, including fibroblast proliferation, collagen deposition, epithelial regeneration, and neovascularization.[20]

Statistical Analysis

Using One way analysis of variance (ANOVA) the data was analysed and presented as mean±SD followed by Tukey post-test. Value of $p<0.05$ were considered significant.

Results

Plant Material Collection

The crude drugs, *Dhataki* (Woodfordia fruticosa (L)Kurz flowers and *Lodhra* (Symplocos racemosa Roxb) bark, were brought from local herbal market. Following cleaning and drying, the net weight obtained for *Dhataki* was 200g, and for *Lodhra* was 200g.

Physico chemical analysis

The physicochemical evaluation of flowers of *Woodfordia fruticosa* (L.) Kurz and stem bark of *Symplocos racemosa* Roxb were done as per the standard procedures. The results obtained are given in the table 3.

Table 3: Preliminary Physicochemical Analysis of Flowers of *Woodfordia Fruticosa* (L.) Kurz and Stem Bark of *Symplocos Racemosa* Roxb

S.No	Physiochemical Parameters	Observation (%)			
		Dhataki	**API**	***Lodhra***	**API**
1.	Foreign matter	Nil	≤ 2	Nil	Nil
2.	Loss on drying	13.07	-	11.4	-
3.	Water soluble extractive	37.3	≥ 28	15.48	≥ 15
4.	Alcohol soluble extractive	21	≥ 7	10.757	≥ 9
5.	Total ash	5.07	≤10	7.6	≤ 12
6.	Acid insoluble ash	0.12	≤ 1	0.07	≤ 1
7.	Water insoluble ash	3.16	-	0.33	-

Preparation of extracts

Both *Dhataki* (*Woodfordia fruticosa* (L). Kurz) and *Lodhra* (*Symplocos racemosa* Roxb) were extracted using 200 g of dried drug material. The extracts obtained, their respective extractive values, and colour observations are presented in table no. 4

Table 4: Characteristic Features of Extracts

Plant Material	Weight of Dried Drug (g)	Extract Obtained (g)	Extractive Value (% w/w)	Colour of Extract
Dhataki (*Woodfordia fruticosa* (L) Kurz)	200	26	13	Dark brown
Lodhra (*Symplocos racemosa* Roxb)	200	18	9	Dark brown

Preliminary phytochemical analysis

Preliminary phytochemical analysis was done on the flowers of *Woodfordia fruticosa* (L.) Kurz and stembark of Symplocos racemosa Roxb as per standard methods which showed the presence of phenols, alkaloids, steroids and flavonoids.

Preparation of ointment

Two ointments were prepared for the purpose of the study. For the 10% formulation (DKO2), a total of 4 g extract was incorporated into a base containing 31.05g ghee and 4.97g beeswax, yielding a smooth, homogeneous ointment of 40g. For the 20% formulation (DKO1), 8g extract was incorporated into 27.61g ghee and 4.42g beeswax, producing 40g of finished ointment.

Preliminary standardisation was carried out on the ointments to evaluate their physical and physicochemical characteristics and the results are enlisted in table no. 5 and 6.

Table 5: Characteristic Features of *Dhatakyadi* Ointments and Ointment Base

S.No	Characters	Observation		
		DKO1	**DKO2**	**Ointment base**
1.	Texture	Smooth	Smooth	Smooth
2.	Colour	Dark Brown	Dark Brown	Yellowish white
3.	Odour	Characteristic	Characteristic	Odour of ghee
4.	Form	Semi solid	Semi solid	Semi solid
5.	Touch	Unctuous	Unctuous	Unctuous

Table 6: Results of Physico Chemical Analysis of *Dhatakyadi* Ointments

S.No	Characters	Observations	
		DKO1	**DKO2**
1.	Ph	5.02	5.17
2.	Refractive Index	1.467	1.396
3.	Rancidity	Not rancid	Not rancid
4.	Acid Value	6.2	5.8
5.	Saponification value	23.4	23.2
6.	Iodine value	32.05	33.12

In vitro study to assess wound healing potential

MTT Assay

Mean absorbance and percentage viability of different concentrations is listed in table no. 7

Table 7: Results of MTT Assay

Concentration in µl	Mean absorbance			% viability		
	Dhataki	***Lodhra***	**DKE**	***Dhataki***	***Lodhra***	**DKE**
Control	0.6385	0.6385	0.6385	100	100	100
5	0.5525	0.63225	0.63225	86.53	99.02	93.87
10	0.78525	0.62885	0.593	90.74	98.4	88.04
25	0.51775	0.532	0.545	81.08	83.32	80.92

DKE- *Dhatakyadi* extract

On the basis of MTT Assay, the L929 fibroblast cells showed maximum cell survival at 10µl concentration in *Dhathaki* extract and 5µl concentration in *Lodhra* extract and in DKE.

Scratch Assay

Mean percentage of wound contraction of the test group and control group are explained in table no. 8 and fig.1. Shapiro-Wilk test shows the data in all groups not significantly deviated from normality (p-value > 0.05).

Table 8: Mean Percentage of Wound Contraction of Test Group and Control Groups at Various Time Interval

Hours	Mean absorbance				F value (p- value)
	Control	***Dhataki***	***Lodhra***	**DKE**	
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
6	4.76^{a}±.45	18.79^{b}±6.62	$14.74^{a,b}$±3.91	59.02^{c}±5.34	77.85 (<.01)
24	37.98^{a}±2.19	97.82^{b}±1.28	97.50^{b}±.78	98.87^{b}±0.85	1397.5 (<.01)

a, b, c are homogeneous subgroups by the post hoc multiple comparison test Tukey HSD at 5% level of significance.

DKE- *Dhatakyadi* extract

ANOVA again confirmed significant differences among groups at the 1% level ($p<0.01$). Tukey's test showed that the control group was significantly different from all other treatment groups. The DKE demonstrated superior wound closure at both 6th hr (mean 59.02 & SD 5.34) and 24th hr (mean 98.87 & SD 0.85), showing statistically significant improvement over individual treatments and control.

Results of CAM Assay

Vessel density and vessel length density of the CAM were analysed using Image J software and the results are given in table 9 and fig.2.

Table 9: Vessel Density and Vessel Length Density of the Eggs

Group	Vessel density	Vessel length density
Normal	10.912	1.052
Dhataki (5 µl)	14.867	1.758
Dhataki(10µl)	10.435	1.112
Lodhra (5µl)	12.427	1.111
Lodhra (10µl)	16.253	2.201
DKE (5µl)	20.457	3.693
DKE (10µl)	20.282	3.446

The maximum value of vessel length density was obtained at 5 µl *Dhataki*,10 µl *Lodhra* and 5µl of DKE.

Acute Dermal Toxicity

The acute dermal toxicity study was initiated with a single rat at a dose of 2000mg/kg body weight and monitored for 24 hours. As no toxic signs or behavioural changes were observed, the same dose was administered to four additional rats. All animals were observed intensively during the first 30 minutes and 2–6 hours post-administration, followed by daily monitoring for 14 days. No toxicity, behavioural abnormalities, or mortality were recorded.

The mean body weight of all rats in the groups are presented in table 10. All animals exhibited a normal and consistent increase in body weight, indicating stable health status.

Table 10: Mean Body Weight of Rats

Group	Mean body weight in g	
	Day 0	Day 15
Acute Dermal toxicity	201.2	212

The skin from treated animals revealed normal histoarchitecture without any pathological alterations. No signs of inflammation, necrosis, or structural damage were observed.

Wound Contraction

Normality assessment using the Kolmogorov–Smirnov and Shapiro–Wilk tests showed that most time points had p-values above 0.05 in at least one of the tests, indicating no significant deviation from normality. Thus, the parametric tests One way ANOVA and repeated one way ANOVA was done to test the hypothesis. The percentage of wound contraction on day 4, day 10, and day 14 is given in table no. 11 and fig.3.

Table 11: Percentage of Wound Contraction

Days	Mean ±SD					F-value (p-value)
	GI-C	GII-PI	GIII-DKO1	GIV-DKO2	GV–Oint.base	
4	46.07^{a}± 3.39	44.87^{b}±6.41	44.20^{a}±6.44	$56.09^{a,b}$±4.86	44.87^{a}±6.41	6.07(<.01)
10	79.15^{e}± 2.07	80.39^{e}±5.57	83.15^{e}±4.43	87.46^{e}±.70	85.37^{e}±3.88	4.18 (<.05)
14	91.20^{a} ±2.00	95.31^{b} ±2.74	96.17^{b}±1.00	97.94^{b}±0.94	$94.07^{a,b}$±0.75	9.04(<.01)

a ,b, c are homogeneous subgroups by the post hoc multiple comparison test Tukey HSD.

Overall, the results indicate that both Betadine and the plant extract treatments, particularly at 10% concentration (DKO2), demonstrated superior wound healing compared to the control and ointment base groups, with having variations that are statistically significant throughout most of the observation period.

Period of epithelialization

The distribution of the data was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Both tests indicated a significant deviation from normality ($p < 0.05$) for all groups, except group "N". Therefore, the non-parametric Kruskal–Wallis test, followed by post hoc pairwise comparisons, was applied, and the outcomes are presented in table no.12.

Table 12: Period of Epithelialisation

Group	Mean	Min	Max	SD	Kruskal – Wallis test
					Chi square value (p-value)
GI – C	12.2	11	13	0.8	11.67* (0.020)
GII – PI	11.4	11	12	0.5	
GIII – DKO1	11.6	11	12	0.5	
GIV-DKO2	10.4	10	11	0.5	
GV-Oint base	11.6	11	12	0.5	

*Significant at 5% level of significance

a, b are the homogeneous groups of post hoc pairwise comparison of Kruskal-Wallis test at 5% level of significance.

The mean days of epithelialization is minimum in DKO2(10.4) and maximum in N (12.2). The Kruskal-Wallis test shows there is significant difference in mean days among groups at 5% level of significance (p-value<.05).

Histopathological Studies

In histopathological study, all treatment group demonstrated improved outcomes in comparison to the control, as illustrated in figure. Histopathological results are given in table no. 13. And fig.4.

Table 13: Interpretation of Histopathological Findings

Group	Pathology
Normal Skin	Epidermis showed stratified squamous epithelium. A well-defined border was demarcating between the epidermis and dermis was seen. Dermis consists of compactly packed and well positioned thick bundles of collagen. Hair follicles and sebaceous glands were present. Hypodermis appears normal.

GI – C	Epithelium becomes thick and hyperplasic (EH). Dermis (D) and deeper layers thick and inflammatory (I), some area shows mild collagen proliferation.
GII – PI	Epithelium shows mild hyperplasia; deeper layers show mild inflammation and collagen proliferation (CP).
GIII – DKO1	Dermis and deeper layers become thick and infiltrated with inflammatory cells (I). Mild vascular proliferation (VP) also noted.
GIV – DKO2	Mild epithelial hyperplasia of epidermis with loose and inflammatory deeper layer.
GV – Oint. base	Mild epithelial hyperplasia of epidermis with loose and inflammatory deeper layer. Mild CP noted.

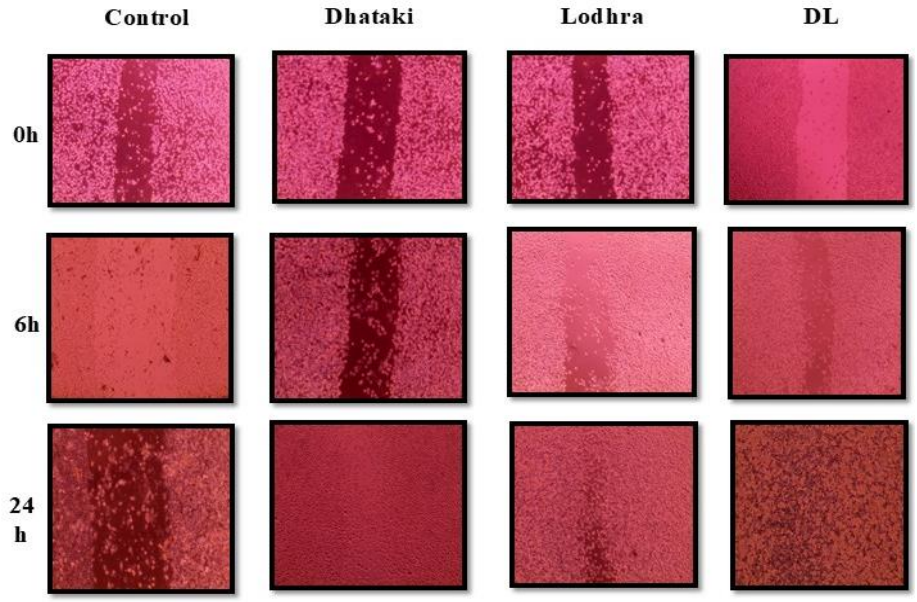

Fig No:1 Results of Scratch Assay at different hours

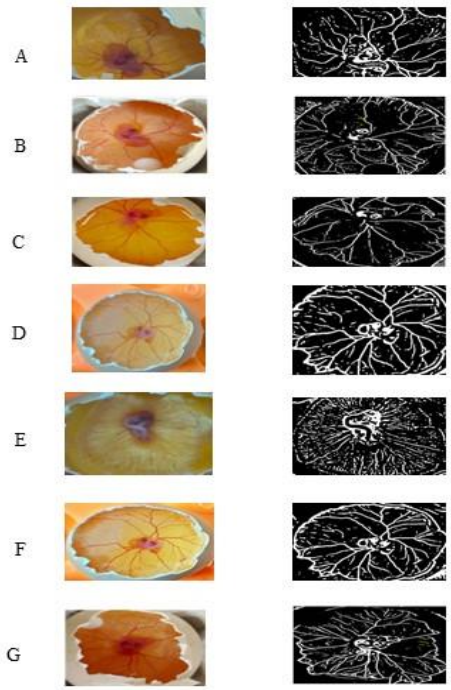

Fig No:2 Results of CAM Assay; A) Untreated group; B) *Dhataki* - 5µl; C) *Dhataki* - 10µl; D) *Lodhra* – 5µl; E) *Lodhra* - 10µl; F) DKE - 5µl; G) DKE – 10µl

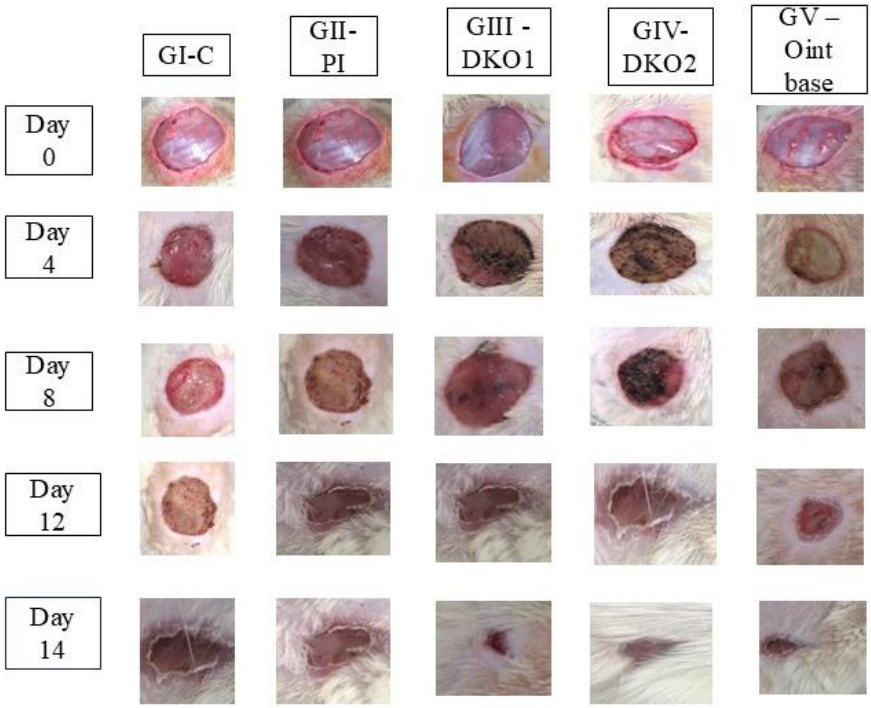

Fig No:3 Wound image of rats on different days in the in vivo study

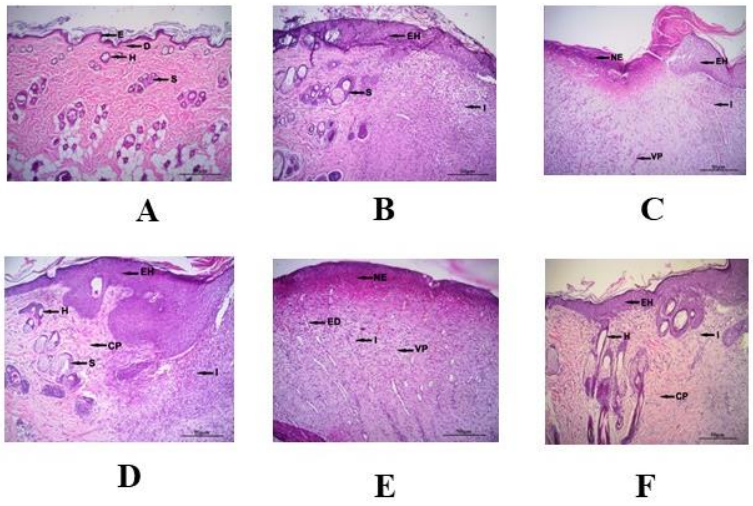

Fig No:4 Histopathology of skin A)Normal Skin B) GI-C C) GII-P D) GIII-DKO1 E) GIV-DKO2 F) GV- Oint base

E – epidermis; **D** – Dermis; **H** – Hypodermis; **S** – Squamous epithelium; **I** – Cell infiltration **EH** – Epithelial hyperplasia; **CP** – Collagen proliferation

DISCUSSION

Wound healing remains a significant global challenge, particularly in chronic cases such as diabetic ulcers and pressure sores. It is a multistage process involving hemostasis, inflammation, proliferation, and remodeling; disruption of these stages can result in delayed or non-healing wounds. Plant-based compounds, known for their antioxidant, anti-inflammatory, and antimicrobial activities, are increasingly explored as safer alternatives in wound management.

In Ayurveda, several formulations are described for *Vrana ropana* (wound healing). *Dhatakyadi Yogam*, comprising *Woodfordia fruticosa (Dhataki)* flowers and *Symplocos racemosa (Lodhra)* bark, is one such classical preparation. Both plants contain flavonoids, alkaloids, tannins, and phenols, which collectively contribute antimicrobial, antioxidant, and collagen-promoting effects. These constituents accelerate wound contraction,

epithelialization, and granulation tissue formation, supporting their traditional use.

The hydroalcoholic extracts of both drugs were formulated into ointments (10% and 20% w/w) using ghee and beeswax as base materials, chosen for their antioxidant, antimicrobial, and emollient properties. The formulations were stable, homogenous, and free from rancidity, with physicochemical parameters within acceptable limits. HPTLC profiling confirmed the retention of phytoconstituents in the ointment.

In vitro assays supported the wound-healing activity of the extracts. MTT assay showed >90% fibroblast viability, indicating safety and proliferative potential. Scratch assay demonstrated significantly enhanced cell migration in treated groups, while CAM assay revealed notable angiogenic activity, particularly at lower concentrations.

In vivo excision wound model further confirmed these results. Both ointments accelerated wound contraction and epithelialization compared to controls, with the 10% formulation (DKO2) showing contraction of 96.17±1.00% by day 14 and epithelialization completed in 10.6 days, comparable to povidone-iodine. Histopathological analysis revealed collagen deposition, fibroblast proliferation, and neovascularization, especially in DKO2, aligning with the positive control. Acute dermal toxicity tests confirmed safety, with no signs of irritation or systemic toxicity.

From an Ayurvedic perspective, both Dhataki and *Lodhra* possess *Kashaya rasa*, *Sheeta virya*, and *Laghu–ruksha guna*, attributes linked to hemostatic, anti-inflammatory, and wound-cleansing actions. These qualities promote *Sandhana karma* (edge contraction), *Rakta sthambhana* (arrest of bleeding), and *Mamsa utpatti* (tissue regeneration). The observed reduction in healing time and improved tissue repair in the experimental groups are consistent with these classical properties, thereby validating the traditional claim of *Dhatakyadi Yogam* as a potent *Vrana ropaka* formulation.

CONCLUSION

The collected drugs were confirmed genuine, and their physicochemical parameters complied with API standards. The prepared ointment had a smooth, non-irritant, and easily spreadable texture, with no signs of rancidity throughout the study, confirming its suitability for topical use. Standardization revealed the presence of flavonoids, tannins, and phenolic compounds, all recognized for their wound-healing potential. In vitro assays substantiated this activity: the MTT assay demonstrated enhanced cell viability, the scratch assay promoted fibroblast migration, and the CAM assay facilitated angiogenesis, collectively supporting tissue regeneration. In vivo studies further highlighted the efficacy of the formulation, where the 10% ointment showed faster wound closure and superior histopathological recovery compared to the standard treatment, while the 20% ointment exhibited similar though slightly less pronounced effects. Overall, *Dhatakyadi Yogam* significantly accelerated wound contraction, reduced epithelialization time, and improved restoration of skin architecture in treated groups, thereby validating its wound-healing potential.

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