Formulation and development of polyherbal emulgel containing tridax procumbens l. & vranashodhak oil for wound healing activity.

[1]Karale Ashwini R, [2]Dr. Hingane Lahu D

[2]HOD
Department of Pharmacology
Aditya Pharmacy College, Beed
Maharashtra. 431122.

Abstract*: The goal of this study is to produce a polyherbal wound healing product. Tridax procumbens L. Piperine & Vranashodhak oil were chosen for the current study based on these plants and herbal oil. As a result, these plants and herbal oil were chosen for the research. Piperine was reported as a bioenhancer for the Emulgel formulation, and the leaves of Tridax Procumbens L. and Vranashodhak oil were reported for rapid wound healing activities. The present study was conducted to formulate herbal Emulgel of Tridax procumbens L. Piperine & Vranashodhak oil using gelling agents like Carbopol 934. The prepared Emulgels were evaluated for physicochemical properties and for their pharmacological activity. The formulation has optimum gel consistency, viscosity, Spreadability. Emulgel has also shown reasonable stability in terms of drug content analysis and physicochemical characteristics. The wound healing activity of polyherbal Emulgel formulation was investigated by utilizing excision wound model. Safety evaluations of the Emulgels were proved to be free from skin irritation, the desirability of a topical product. Excision Wound healing studies of the Emulgel formulation revealed that Emulgel of Tridax procumbens L., Piperine, and Vranashodhak oil comparatively grater wound regeneration activity in comparison with Standard (Povidone iodine ointment USP1%), Placebo and untreated control groups. The polyherbal Emulgels, with Tridax procumbens L. leaf extract was successfully prepared and evaluated for assessing their safety, excision wound healing efficacy and pharmaceutical quality.

***Keywords*: Tridax procumbens L., Piperine, Vranashodhak oil, Emulgel, Wound Healing, Excision Wound Model.**

1. INTRODUCTION

In traditional treatments like Ayurveda, Unani, and Siddha, herbal remedies serve as the foundation for the treatment and prevention of a variety of illnesses and disorders. Traditional and Western medicine both heavily rely on plant extracts.(5) Tridax procumbens L. is a member of the Asteraceae family and is known in Marathi as 'Dagdi Pala, kambarmodi' in Ayurvedic as Jayanthi and in English as Coat buttons/Mexican Daisy due to the appearance of its flowers. It is an ethnobotanically important. The plant has been described as a gregarious weed that can be found all across the tropics and subtropics. Tridax procumbens has a long history of use as a medication in Ayurvedic medicine for a number of ailments. Anti-diabetic, anti-inflammatory, anti-cancer, hepatoprotective, blood coagulation, and other pharmacological actions are linked to it. Tridax procumbens leaves have been used as a common cure for cutaneous wounds in India for centuries. Earlier workers have reported that it possesses antidiabetic(6), anti-bacterial, antiplasmodial(7), antihepatotoxic, anti-oxidant(8), antimicrobial(8), immunomodulatory(9), wound healing and anti-cancerous properties (10)

The Vranashodhak oil is folklore ayurvedic formulation that can made from combination of many herbal swaras. This oil is useful in healing the wound quickly. It cleans the wound and prevent infection and pus formation. Each 10 ml of siddha taila is prepared by using aqueous extract of Nirgundi swaras (Vitex negundo)lf., Haritaki kwath (Terminalia chebula) frt. 0.1ml., Kanher swaras (Nerium indicum) lf., Kadulimb pala swasar (Azadirachta indica) lf., Dhotra (Datura metel) lf., 0.1ml each., Base Karanj tail (Pongamia pinnata)ol.10ml ref. Bhavaprakash.

Black pepper contains the presence of an alkaloid known as Piperine. Piperine can be found in the Piperaceae family's black, white, and long peppers. Piperine represents diverse biological activities, such as anti-inflammatory, anticancer, antiviral, anti-larvicidal, pesticide, anti-Alzheimer‘s, antidepressant and most importantly Piperine is known as the bioavailability enhancer.(11)

There are cream, ointment, and gel formulation was present in market for the Tridax procumbens L. but there is no combination of tridax procumbens L. and Vranashodhak Oil is not available in market so need to formulate this combination using Emulgel Formulation. Wound is defined as loss or breaking of cellular and anatomic or functional continuity of living tissue due to physical, chemical, thermal, microbial, or immunological exploitation of the tissues. Wound healing disorders present a serious clinical problem and are likely to increase when they are associated with diseases such as diabetes, hypertension, and obesity, etc.

Emulgel, a biphasic semi-solid formulation comprise of aqueous and non-aqueous phases, delivering both hydrophilic and lipophilic agents(2). Emulgel, are emulsions prepared by mixing with gums.(3) They're also quite good at penetrating the skin, Thixotropic, greaseless, easily spreadable, readily removable, emollient, non-stanning, water-soluble, longer shelf life, bio friendly, translucent & appealing look are only a few of the benefits of Emulgels for dermatological treatment. Topical drug use necessitates an understanding of the factors that influenced percutaneous absorption.(4)

There are basically four stages of wound healing they are Hemostasis, Inflammations, Proliferation and Remouldings.

Plant review:

Table 1.2 Taxonomical Classification of Tridax Procumbens L.

Tridax Procumbens L.	
Kingdom	: Plantae-plants
Sub-kingdom	: Tracheobionta- Vascular plants
Division	: Spermatophyta
Subdivision	: Magnoliophyta- flowering plants
Class	: Magnoliopsida- Dicotyledons
Subclass	: Asteridae
Order	: Asterales
Family	: Asteraceae- Aster Family
Genus	: Tridax L. -tridax
Species	: Tridax procumbens L. -coat buttons

Fig.1.1 Tridax Procumbens L.

Phytochemical review:

Flavonoids, alkaloids, carotenoids, hydroxycinnamates, lignans, benzoic acid derivatives, phytosterols, tannins, raw proteins, soluble carbohydrates, and calcium oxide are said to be present in the leaf and other portions of T. procumbens. (12) Croud proteins make up 26% of the leaf of Tridax, along with crude fibre (17%), soluble carbohydrates (39%), calcium oxide (5%), and luteolin, glucoluteolin, quercetin, and isoquercetin, which have all been found in the plant's blossoms. While the plant has also been linked to fumaric acid, fl-sitosterol, and tannin.

2. MATERIAL AND METHODS

2.1. Materials:

Collection of Plant Samples and other excipients

Leaves of Tridax procumbens L. were collected from different localities of Buldana. and its nearby areas and washed thoroughly with distilled water. The cleaned plant parts are then allowed for the complete shade drying and then made to fine powder with a mechanical grinder and stored in an airtight container.(10)

The plant Tridax procumbens L. were identified by D.L. SHIRODKAR BOTANIST Botanical Survey of India Western Regional Centre 7, Koregaon Park, Pune-411001. The Piperine obtained as a gift sample from Amsar pvt. Ltd. Indore., and Vranashodhak oil is a marketed formulation and is collected from Manikarnika Ayurvedalay Pune.

2.2. Preparation of Extract

Add 100 gm of finely divided powdered crude Tridax Procumbens L. in 500 ml of hydroalcoholic mixture (60:40) Keep it in a closed container for 72 hours with stirred time to time and filter it Allow to evaporate filtrate at 60-70^{0}C & Collect the Semisolid extract.(10) The organoleptic/physical properties of Extract and oil was reported.

2.3. Preparation of Tridax procumbens L. & Vranashodhak oil Emulgel :

Gel Phase : Dissolve Carbopol 934 in purified water with Const. stirring at moderate speed using mechanical stirrer and adjust the pH [6-6.5] using triethanol amine.

Oil Phase : Dissolve span 20 (1.5ml) in sufficient quantity of Vranashodhak oil.

Aqueous Phase : a.) Dissolve tween 20 (1ml) in purified water to make Aq. Phase.

b.) Methyl paraben and propyl paraben was dissolved in propylene glycol

c.) Extract (Tridax procumbens L. + Piperine) dissolved in water

Then both b & c were mixed in Aqueous phase (a) Both Aqueous Phase & Oil Phase heated separately up to 70-80^{0}C. Then Oil phase is added in Aqueous Phase with continuous stirring until it get cooled at room temperature Then the obtained Emulsion was mixed with gel in the 1:1 ratio.

2.4. Experimental Design

A central composite design was used to create and optimise the formulations. Nine confirmatory runs were created and their parameters assessed.

The formulation was optimized using a central composite design with two independent variables to estimate various parameters, interaction and also to evaluate the quadratic effects of ingredients on Tridax procumbens L. & Vranashodhak oil emulgel by constructing model using Design Expert (version 8.0.0, Stat-Ease Inc., Minneapolis, Minnesta)
9 runs were generated for the responses alphabetically labelled as F1 to F9 having a concentration of the factors ranging from minimum to maximum as given in Table 1.

Table no. 2.1 Full Factorial Design layout

Factor	Name	Unit	Type	Sub Type	Minimum	Maximum	Coded Low	Coded high	Mean	Std. Dev.
A	Carbopol 934	gm	Numeric	Continuous	0.5858	3.41	-1 ↔ 1.00	+1 ↔ 3.00	2.00	1.0000
B	Vranashodhak oil	mL	Numeric	Continuous	1.48	5.02	-1 ↔ 2.00	+1 ↔ 4.50	3.25	1.25

2.5. Evaluation of Emulgel:

2.5.1. Organoleptic Properties:

The prepared extract and marketed oil and all the formulated Emulgels were visually inspected for colour, homogeneity, and phase separation after they have been settled in the container immediately as well as regularly for an interval of 15 days.(13)

2.5.2. Phytochemical Content of the Leaves Assessment :

Preliminary phytochemical screening of the Hydro-Ethanolic extract of Tridax procumbens L. was carried out by established methods.

2.5.3. HPTLC :

Table 2.2 Optimized HPTLC condition

Stationary Phase	Merck, TLC AI plate silica gel 60 F 254
Plate format	100 x 100 mm
Application type	Band
Application	Position Y: 8.0 mm, length: 8.0 mm, width: 0.0 mm
Track	First position X: 21.5 mm, distance: 11.4 mm
Solvent front position	70 mm
Software	Sever LABSERVER, version 3.1.21109.3
ATS 4	S/N:250243 (NEW ATS4)
Mobile Phase	Toluene : Ethyl acetate (8:2 V/V)
Saturation time	20 min
Mode of Separation	Normal Phase
Sample applicator	Camag Linomat V
Sample Solvent Type	Methanol

2.5.4. Measurement of pH :

The prepared Emulgels were evaluated for pH in triplicate by taking 1 g of each emulgel in 10 mL beaker and measured the digital pH meter at room temperature

2.5.5. Particle size of emulsion and Emulgel:

Characterization of Optimized Formulations: Globule size and its distribution in Emulgels: This study was performed for the optimized batches each from Carbopol 934 based gels. Malvern zetasizer was used to determine globule size and dispersion. A 1.0 gm sample was dissolved in purified water and agitated to get homogeneous dispersion. Sample was injected to photocell of zetasizer. Mean globule diameter and distribution was obtained.

Photomicrography : Morphology of emulsion was studied under light microscope. The form of the Emulgel's optimised batches was examined under a light microscope. The emulgel was appropriately diluted, put on a glass slide, and studied under a 40 X light microscope.

2.5.6. Determination of Spreadability:

On the wooden block covered with an excess of the emulgel being studied, a ground glass slide was fixed. This slide is sandwiched with another second slide. The second slide is attached to a hook of the balance. In order to expel the air, a weight of 250 - 500 mg was placed on the top of the two slides for 5 min. The required quantity of weight was placed in the other pan which is attached to the pulley through a hook. It was noted how long it took the top slide to travel 5 cm. A shorter interval indicates a better spreading coefficient.(14)

2.5.7. Viscosity :

The viscosity of the formulated preparations was determined using brook field viscometer with spindle no. 64 (Brookfield Engineering Laboratories). The formulation whose viscosity was to be determined was added to a beaker covered with a thermostatic jacket. Spindle was allowed to pass into the emulgel, and the reading was noted at 30 rpm, the apparatus being maintained at 25 °C.(15)

2.5.8. Drug Content:

1g of prepared emulgel was mixed with methanol and was kept for shaking for 30 min. The solution was filtered and was diluted suitably using a phosphate buffer of 6.8 pH, and the absorbance was measured at 267 nm. From the absorbance reading percentage, drug content was calculated

2.5.9. **In-vitro Release Studies :**

The in vitro drug release from emulgel was carried out using Franz diffusion cell at 50 RPM and $37^0C \pm 0.5^0C$. Phosphate buffer was used as the dissolution medium. 1 ml of dissolution medium was withdrawn at predetermined time intervals and fresh dissolution medium was replaced. The sample were withdrawn at regular interval and analyzed by UV spectrophotometer at 267 nm for the presence of the drug.

2.5.10. **Stability studies :**

The prepared Emulgels were packed in aluminium collapsible tubes (5 g) and subjected to stability studies at 5^OC, 25 ^OC/ 60% RH, 30 ^OC /65% RH, and 40 ^OC /75% RH for a period of 3 months. Samples were withdrawn at 15-day time intervals and evaluated for physical appearance, pH, rheological properties and drug content (16)

2.5.11. **Antimicrobial study**:

We undertake a batch optimization observational study. First, the extract's MIC (minimum inhibitory concentration) was determined using the serial dilution method against Escherichia coli and Candida albicans. Using an agar disc- diffusion experiment, the Polyherbal Emulgel was tested against an E. Coli bacterial strain and Candida Albicans. The inhibitory zone was measured. The antibacterial activity of the polyherbal Emulgel was compared to a standard. A nutritional agar medium solution of 20 mL was made and placed in Petri plates. Allow some time for the media to be established. After that, we drilled 8 mm holes in Petri plates for our formulation, extract, and standard medicine to be poured into. All of the items that we will be using should be sterilized. After that, we performed bacterial inoculation. For bacteria and fungus, we need to incubate for 24 hours, and for their growth in Petri plates, we need to incubate for 48 hours. After that, a set incubation time zone was calculated

2.6. In vivo study for wound healing :

2.6.1. **Experimental Design :**

The rat were divided into four Group each consist of 6 rats

I. Group I (Normal control) : No topical treatment was given and its % Wound Contraction was calculated.
II. Group II (Placebo control): The rat was treated with Placebo Emulgel and its % wound contraction was calculated.
III. Group III (Standard control): The rat was treated with Povidone iodine Ointment 5% and its % wound contraction was calculated.
IV. Group IV (Optimized Formulation): The rat was treated with Tridax Emulgel Formulation and its % wound contraction was calculated.

2.6.2. **Wound Healing model in rats:**

Rats: Wister albino rats of either sex (body weight between 150-250 g) were used for wound healing activity. They were housed in standard environmental conditions, fed with standard pallet diet and water ad libitum.

Excision Wound Model :

In this model, a typical wound is created by cutting a circle skin in the dorsal region of the experimental animals under light ether anesthesia. A wound of 500 mm2 is usually formed by marking a margin on a pre-shaved region using indelible ink and sealing it with a rubber closure. Morton and Malone used planimetric measures to assess the vulnerary activity of an open wound in rats. This model can also be used to investigate the wound healing phase. This is accomplished by photographing the wound at various time intervals during the healing process.(17)The percentage of the original wound that closes after a predetermined lap of time (in days) is used as a criterion. Separate cages are used to keep the animals. The time has come. The period of epithelization, that is, day of fall of eschar and the scar area, was also noted down.(18) (19)

$$\% \text{ Wound Contraction} = \left[\frac{\text{Initial Day Wound Size} - \text{Specific Day Wound size}}{\text{Initial Day Wound Size}}\right] \times 100$$

2.6.3. **Determination of period of epithelization:**

The dropping of the scab from the wound is considered as the wound healed, or as the end point of complete epithelization. The number of days required for these, are known as epithelization period.(20)

2.6.4. **Histopathological study** :

The healing tissues obtained on the 15th day from all four groups of animals of the incision wound model were processed for histopathological study (**PRADO, Preclinical Research and Development Organization, Pvt. Ltd. Pune**). The amount of collagen was quantified using Vangeison stain

3. RESULT AND DISCUSSION

3.1. Evaluation Parameters:

3.1.1 Organoleptic Properties:

the prepared extract and marketed oil were checked visually for physical appearance, texture, odor, nature are as given in following table no.

Table 3.1 Organoleptic Properties of Drugs

Physical properties	Tridax procumbens L.		Vranashodhak oil	
	Observed	Reported	Observed	Reported
Appearance	Dark greenish	Green	Yellow	Yellow
Texture	smooth	Smooth	Less viscous	Less viscous

Odor	Characteristics	Characteristics	Characteristics	Characteristics
Nature	Semi-solid	Powder	Liquid	Liquid

Various formulations of the emulgel were checked visually for their homogeneity, color, phase separation and consistency, and it was observed that all the formulation F1 to F9 appeared homogenous, pale yellow having a smooth consistency without having any phase separation.

Table 3.2 Organoleptic properties of Emulgel Formulation.

Formulation	Color	Homogeneity	Consistency
F1	Greenish yellow	+	Uniform
F2	Greenish yellow	+	Uniform
F3	Light yellow	+	Uniform
F4	Yellow	+	Uniform
F5	Light yellow	+	Uniform
F6	Light yellow	+	Uniform
F7	Light yellow	+	Uniform
F8	Yellow	+	Uniform
F9	Yellow	+	Uniform

1.1. Phytochemical Study of Tridax procumbeans L. Hydro-Ethanolic Leaf Extract

The phytochemical screening of Tridax procumbens Hydro-Ethanolic extract showed the presence of Alkaloids, Carotenoids, Flavonoids, Saponins, Tannins, were presented (Table). The phytochemical constituents indicate.

Table. 3.3 Qualitative profile of phytochemicals founds in Tridax Procumbens Leaves.

Sr. no.	Phytochemicals	Status
1.	Alkaloid	+
2.	Carotenoids	++
3.	Flavonoids	+
4.	Saponins	+
5.	Tannins	++

1.2. HPTLC :

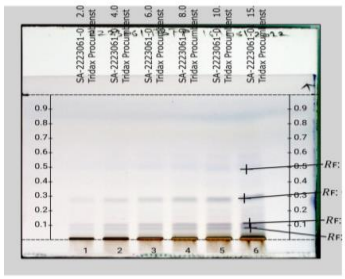


Image at R white after derivatisation

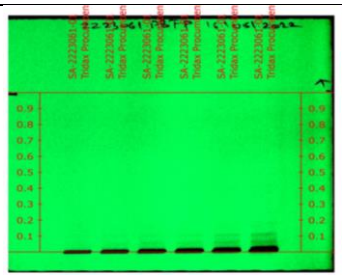


Image at R 254 nm after derivatisation

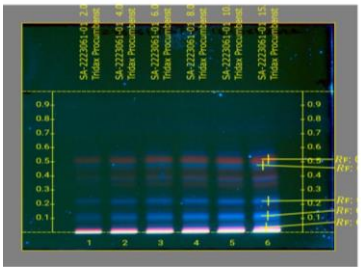


Image at R 366 nm after derivatisation

Fig. 3.1 Photo-documentation of Tridax procumbens L.

Description: Tridax procumbens L. 200mg/10ml in water : Ethanol 60:40 v/v
Volume: 15.0 µl

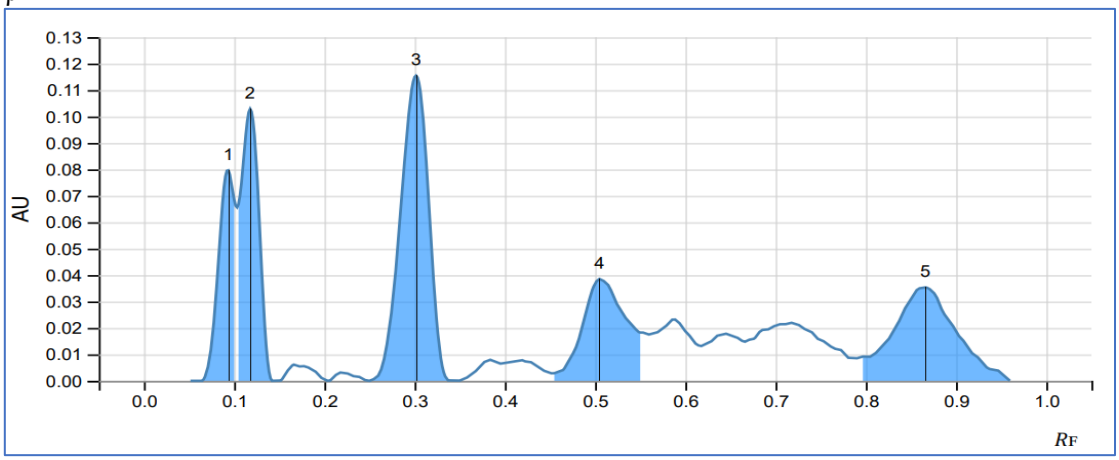


Fig.3.2 HPTLC Profile of Hydro-Ethanolic Extract of Whole Plant of Tridax procumbens Linn

Table. 3.4 Track 6. Data of Tridax procumbens L.

Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.065	0.0000	0.094	0.0797	21.42	0.102	0.0663	0.00160	12.16	No	
2	0.103	0.0657	0.118	0.1029	27.66	0.142	0.0000	0.00242	18.35	No	
3	0.250	0.0000	0.302	0.1155	31.05	0.340	0.0000	0.00412	31.33	No	
4	0.453	0.0028	0.505	0.0385	10.36	0.550	0.0183	0.00214	16.25	No	
5	0.795	0.0090	0.866	0.0353	9.50	0.960	0.0000	0.00288	21.90	No	

1.3. Measurement of pH: The pH readings were taken as an average of three sample readings. The values exhibited by the Emulgels are found to be in the range of 5.85 to 6.87. Hence all the formulations were in the normal pH range of the skin

1.4. Particle size of Emulsion and Emulgel:

Globule size and its distribution in emulgel Mean globule size in formulation was found to be 386.6 nm and zeta potential was -19.6 mv.

Photomicrography The suitably diluted emulsions of optimized batches were observed under light microscope at 40X. From the photomicrograph, nearly spherical globules of emulsion were observed. Though this study does not give any exact estimate of size however it gives a general idea about formation of emulsion and success of the method used.

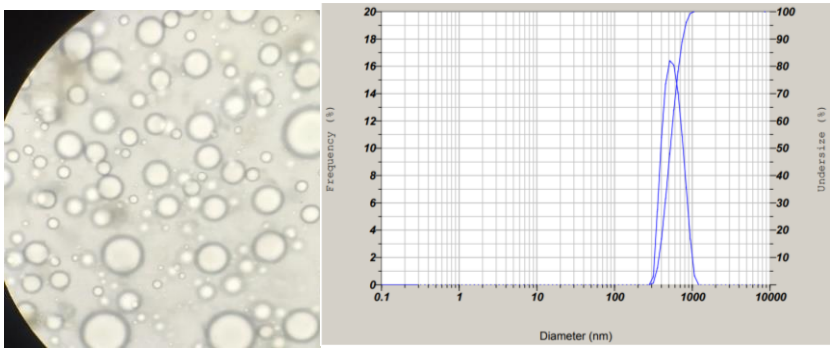


Fig.3.3 Photomicrograph of Formulation

1.5. Spreadability Studies:

Spreadability is one of the essential criteria for an emulgel. Spreadability is influenced by the formulation's viscosity. The **Model F-value** of 13.99 implies the model is significant. Only 2.73 percent of the time is there a probability that noise will cause an F-value this large. Model terms are considered significant when the P-value is less than 0.0500. In this instance, key model terms were A, A2, and B2. Model terms are not significant if the value is higher than 0.1000. Model reduction may enhance your model if it has a lot of unnecessary terms (except those needed to maintain hierarchy). Experimentally Spreadability estimation of all the runs was done was found to be in the range of 12.17 to 20.97 Gm.cm/Sec . fig 2.

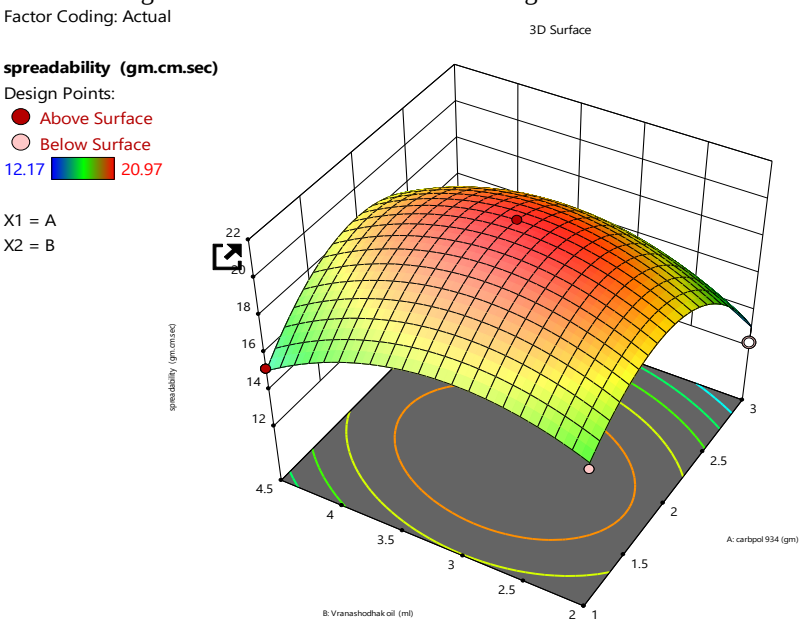


Fig 3.4. Plot for spredability coefficient

1.6. Determination of Viscosity of the Formulation:

Formulated Emulgels were evaluated for viscosity using Brooke field viscometer using spindle no 4 at 30 rpm**.** The **Model F-value** of 10.26 implies the model is significant. There is only a 1.16% chance that an F-value this large could occur due to noise. **P-values** less than 0.0500 indicate model terms are significant. In this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. Experimentally viscosity estimation of all the runs was done was found to be in the range of 10348 to 23507 Cps. Fig 3.

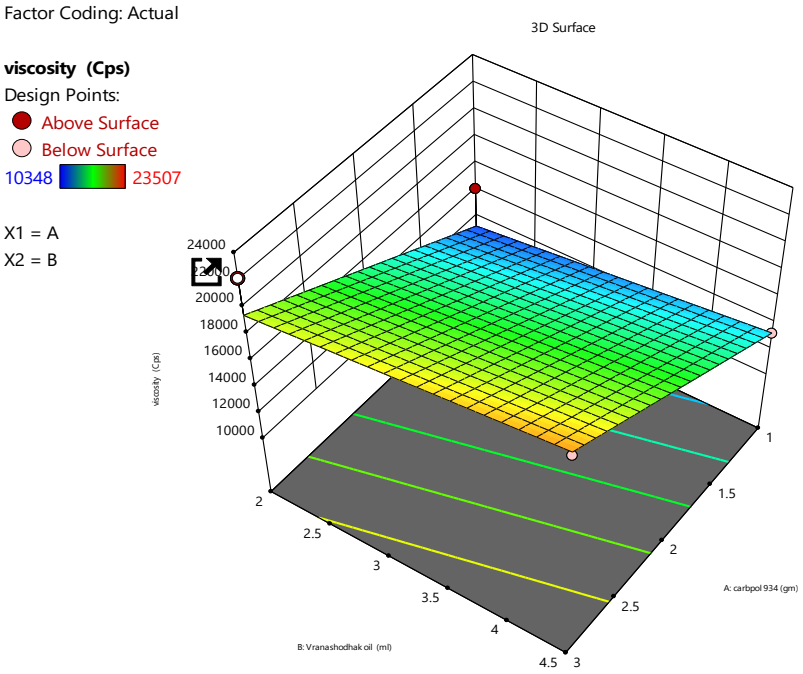


Fig. 3.5 Plot for Viscosity

3.2 % drug release of the formulation:

The software created a quadratic model, and the model's Model F-value of 23.67 indicates that it is significant. The likelihood of noise producing an F-value this large is merely 1.29 percent. Model terms are considered significant when the P-value is less than 0.0500. B and B2 are important model terms in this instance. Model terms are not significant if the value is higher than 0.1000. Model reduction may enhance your model if it has a lot of unnecessary terms (except those needed to maintain hierarchy). Fig. 1. Experimental estimates of the drug content in all the runs were found to range from 49.53 to 83.9 percent.

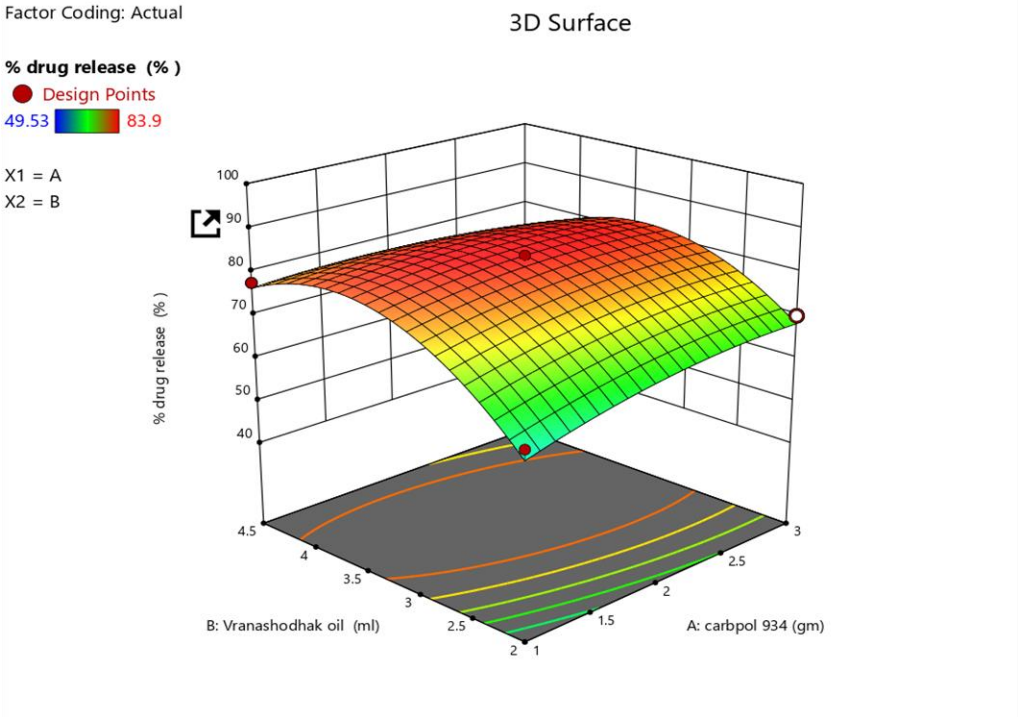
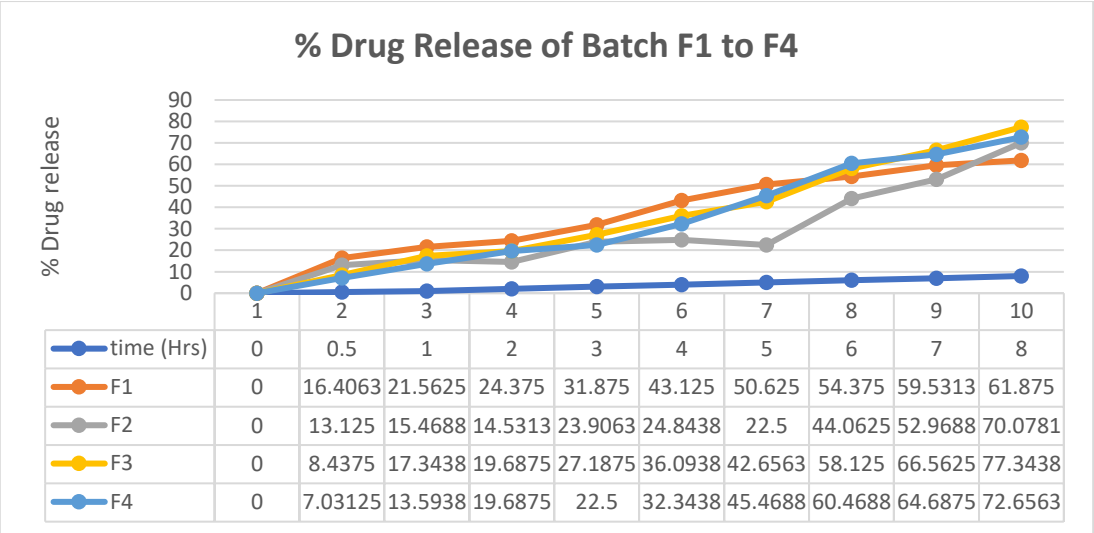
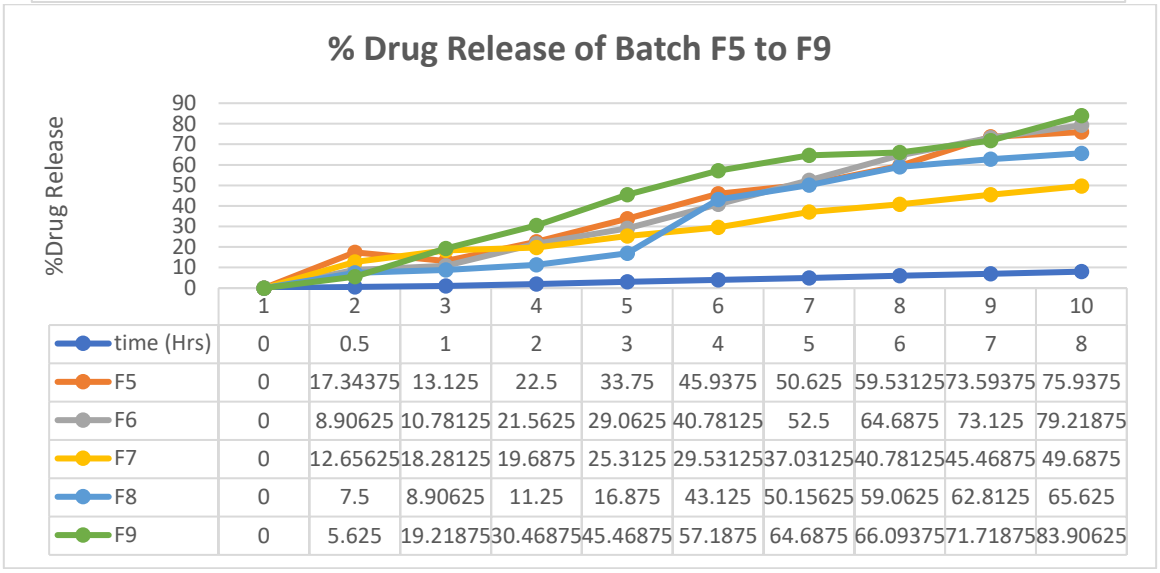


Fig 3.6 plot for drug content



	1	2	3	4	5	6	7	8	9	10
time (Hrs)	0	0.5	1	2	3	4	5	6	7	8
F1	0	16.4063	21.5625	24.375	31.875	43.125	50.625	54.375	59.5313	61.875
F2	0	13.125	15.4688	14.5313	23.9063	24.8438	22.5	44.0625	52.9688	70.0781
F3	0	8.4375	17.3438	19.6875	27.1875	36.0938	42.6563	58.125	66.5625	77.3438
F4	0	7.03125	13.5938	19.6875	22.5	32.3438	45.4688	60.4688	64.6875	72.6563



	1	2	3	4	5	6	7	8	9	10
time (Hrs)	0	0.5	1	2	3	4	5	6	7	8
F5	0	17.34375	13.125	22.5	33.75	45.9375	50.625	59.5312	73.59375	75.9375
F6	0	8.90625	10.78125	21.5625	29.0625	40.78125	52.5	64.6875	73.125	79.21875
F7	0	12.65625	18.28125	19.6875	25.3125	29.5312	37.0312	40.7812	45.46875	49.6875
F8	0	7.5	8.90625	11.25	16.875	43.125	50.15625	59.0625	62.8125	65.625
F9	0	5.625	19.2187	30.4687	45.46875	57.1875	64.6875	66.0937	71.7187	83.90625

TABLE 3.4 POINT PREDICTION :

Analysis	Predicted mean	Predicted median	Std. Dev.	SE mean	95% CI low for mean	95% CI high for mean	95% TI low for 99% Pop	95% TI high for 99% Pop
% drug release	83.9	83.9	2.68676	2.68676	75.3495	92.4505	57.3688	110.431
Viscosity	16447.7	16447.7	2692.85	897.618	14251.3	18644.1	1417.38	31478
spredability	20.97	20.97	0.914101	0.914101	18.0609	23.8791	11.9434	29.9966
pH	6.18333	6.18333	0.405401	0.135134	5.87171	6.49495	4.14533	8.22134

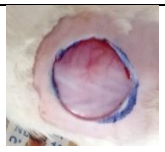
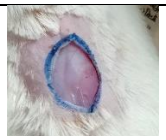
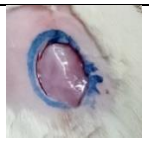
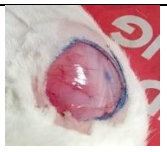
3.2 Stability study:

After being stored for three months, it was discovered that all of the created emulgel formulations were stable; there had been no change in their pharmacological composition, pH, rheological qualities, or outward appearance.

3.3 Skin irritation test (Patch Test) :

No allergic symptoms like inflammation, redness, irritation appeared on rats up to 24 hr.

% wound contraction :

	Control	Placebo Control	Standard Control	Formulation
Day 0				

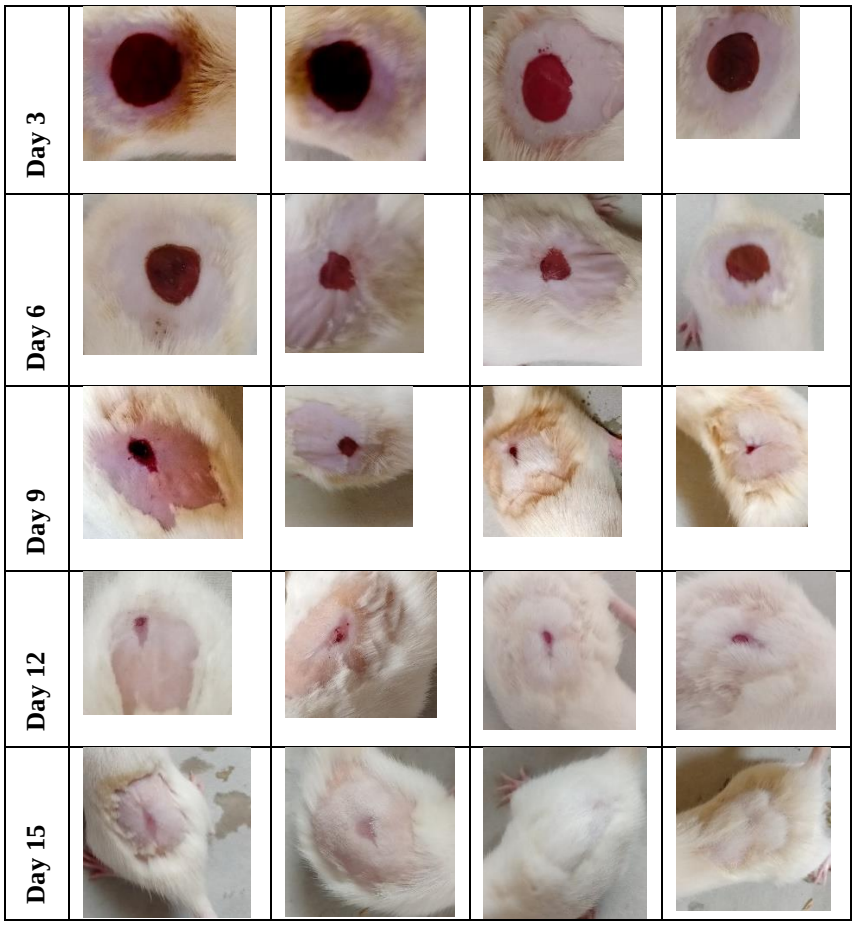


Fig. Photographic representation of contraction rate showing percent wound contraction area on different post-excision days of control, Placebo control, Standard and Emulgel Formulation treated rats.

Days	Wound Area in mm^2 (% contraction)						Epithelization period	Scar area (mm^2)
	Day 0	Day 3	Day 6	Day 9	Day 12	Day15		
Control(induction)	400±4.472	379±5.785	329.3333±3.450	244.333333±6.552	117.333333±12.060	38±3.335	12.7±0.67	98.5±4.92
Placebo	403.333333±3.33	376.16667±5.750 ns	304.1667±25.773 ns	190.6667±7.001 ***	92.833333±8.382 ns	17.166667±1.276 ***	11.46±0.21	91.8±7.34
Standard	400±7.303	349.3333333±4.708 **	286.83333±5.322 ns	176±6.203 ***	28.33333±3.827 ***	8.16667±0.7032 ***	10.21±0.75	78.8±3.65
Formulation	386.66667±6.657	348.8333±5.603 **	282.5±4.008 ns	140±3.603 ***	23.166667±3.038 ***	7.66667±0.8819 ***	10.0±0.58	75.4.04

Values are the mean ± S.E.M. of six rat /treatment. Significance $^{ns}P>0.05$, *P<0.05, **P<0.01, ***P<0.001, compared vs placebo, standard and formulation.

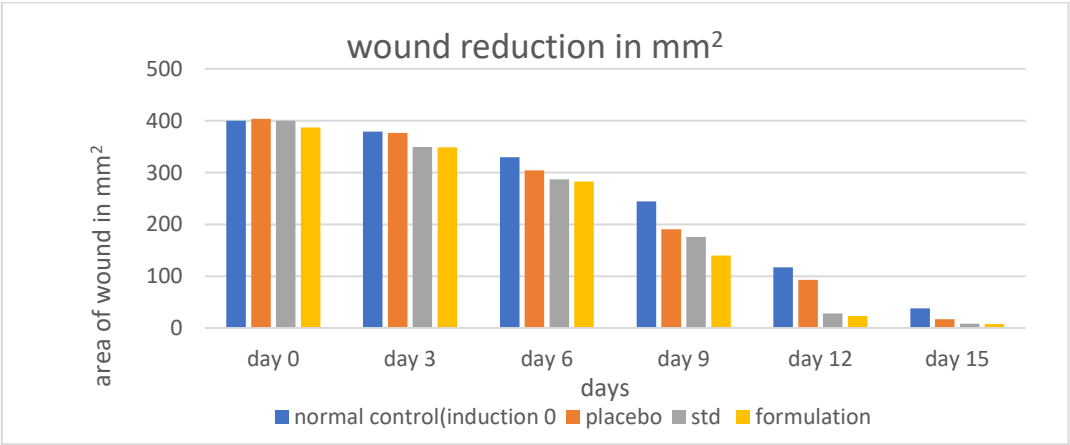
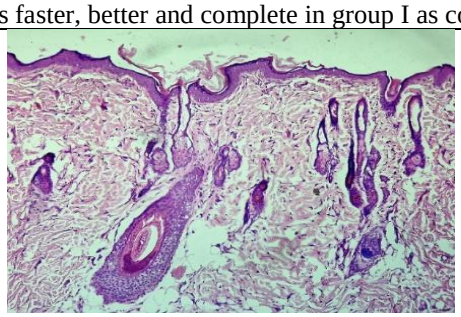
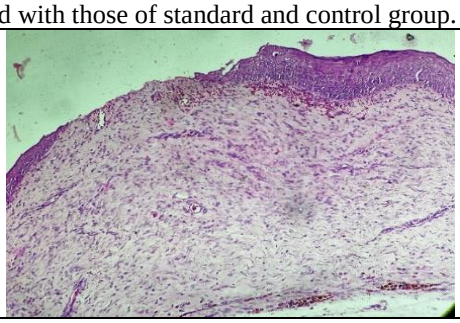
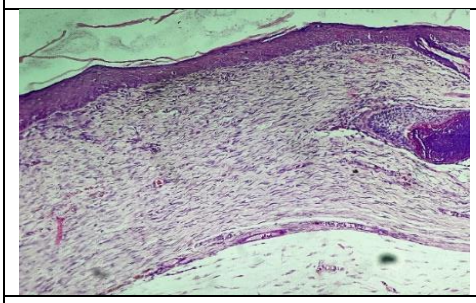
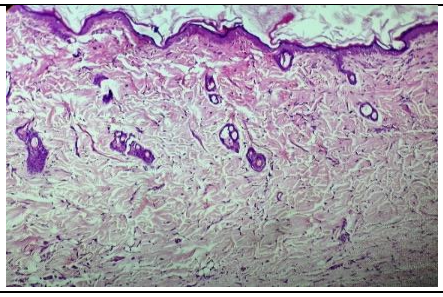


Fig. Wound Reduction in mm^2

Epithelization period : The epithelization period for the Emulgel Formulation were better than the Normal control, placebo control, Standard control.

Histopathology : Based on the above observations of histopathology it can be concluded that wound healing potential of herbal formulation was faster, better and complete in group I as compared with those of standard and control group.

	
Control: Note complete epidermis with secondary structures and hair follicles. H & E, 10X	**Placebo**: Note Incomplete epidermal closure (black arrow). Minimal angiogenesis (Yellow arrow), Minimal Horizontal collagen orientation(star), H & E, 10X
	
Standard: Note complete epidermis, Minimal infiltration of inflammatory cells (star). H & E, 10X	**Emulgel Formulation** : Note Moderate mixed collagen orientation (star), H & E, 10X

Based on the above observations of histopathology it can be concluded that wound healing potential of herbal formulation was faster, better and complete in Emulgel formulation as compared with those of standard and control group.

CONCLUSION :

The Emulgel Formulation were developed and evaluated successfully with conclusion that Carbopol-934 with Aqueous extract of Tridax procumbens L., and Piperine shows excellent gelling property. Vranashodhak oil in emulsion shows good dispersion property in gel formulation. For assessment of wound healing Excision wound model were used and conclude that Optimized formulation shows better wound healing property than the control group, standard group, and placebo control group. Hence, at last it was concluded that emulgel formulation is the one of the better choices for combine use of oil and drug with promising effect in drug release as transdermal drug delivery system.

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