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RESEARCH ARTICLE

Development and Evaluation of Polyherbal Gel Formulation for Wound Healing Activity

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ABSTRACT

The plant *Azadirachta indica* belonging to family Meliaceae traditionally used in Ayurvedic system as insect repellant, antibacterial, anti-microbial effect, anti-fungal, anti-viral, anti-inflammatory and also strengthens the body's overall immune responses Symplocos racemosa Roxb belonging to family Styraceae posses Antioxidant activity. Vitex Negundo Linn posses' Antimicrobial activity, Antibacterial activity, Tinospora Cordifolia belonging to family Menispermaceae curative of dermatosis, Curcuma longa belonging to family posses wound healing activity. The aim of present study was to evaluate physical parameter with the wound healing activity of Polyherbal gel; the different concentration of gel base Aqupec 505 HV HC with *Azadirachta indica* extract, Symplocos racemosa Roxb extract Vitex Negundo extract, Tinospora Cordifolia and Curcuma longa extract on the basis of physical parameter and *in vitro* drug release optimized batch was selected for wound healing activity by using a Partial thickness burn wound model in wistar males rats. All animals were divided into three groups. Group I was control. Group II was Test gel. Group III were treated by (Reference Standard) for 21 days all gel was applied topically. The wound healing activity was evaluated by physical parameters namely wound contraction and epithelialization. The polyherbal gel showed significant wound healing activity.

KEYWORDS

Azadirachta Indica Extract, Symplocos Racemosa Roxb Extract, Vitex Negundo extract, Tinospora Cordifolia and Curcuma Longa, Aqupec 505, Mechanical Stirrer

INTRODUCTION

Natural source is one of the most important sources for the herbal drug. Because of its non-toxicity, easily availability and biocompatible nature, the herbal drugs have their own choice in preparation of various dosage forms.

Topical application of drugs offers potential advantages of delivering the drug directly to the site of action and acting for an extended period of time.

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Gels have better potential as a vehicle to administered drug topically in comparison to other dosage form, because they are non-sticky requires low energy during the formulation. Natural source is one of the most important sources for the herbal drug. Because of its non-toxicity, easily availability and biocompatible nature, the herbal drugs have their own choice in preparation of various dosage forms.

Gel: Gels are semisolid systems in which a liquid phase is trapped within an interlocking, tree dimension polymer matrix of a natural or synthetic gum. A high degree of physical or chemical cross linking of the polymer is involved

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.where polymers or long-chain molecules in the internal phase are capable of cross-linking and interacting with themselves to entrap the external phase within a web like structure.

The classic example of a gel is dissolved gelatin in water forming a highly viscous gel structure. Reversible gels are reproducible, semisolid, highly viscous structures at low temperatures, and colloidal solutions and/or liquids at higher temperatures. Most gels, whether aqueous or nonaqueous are prepared with the aid of heat and high shear agitation. Suspensions, milks, and magmas of clays and inorganic hydroxides are sometimes referred to as gels because of their high viscosity. [Alexander T. *et al*]

A gel is an intermediate state of matter possessing property of a solid and a liquid, termed as viscoelasticity.

1. The structural materials that form the gel network can be composed of inorganic particles or organic macromolecules, primarily polymers
2. Cross links can be formed via chemical or physical interactions. This leads to gel classification into chemical and physical gel systems, respectively.

Chemical gels are associated with permanent covalent bonding while physical gels result from relatively weaker and reversible secondary intermolecular forces such as hydrogen bonding, electrostatic interactions, dipoledipole interactions, Vander Waals forces.

The **U.S.P. defines** gels as a semisolid system consisting of dispersion made up of either small inorganic particle or large organic molecule enclosing and interpenetrated by liquid. The inorganic particles form a three-dimensional "house of cards" structure. Gels consist of two phase system in which inorganic particles are not dissolved but merely dispersed throughout the continuous phase and large .organic particles are dissolved in the continuous phase, randomly coiled in the flexible chains. [Roychowdhury S. *et al* 2012]

Wound: Wounds may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissue. It may be produced by physical, chemical, thermal, microbial, or immunological damage to the tissue. When skin is torn, cut, or punctured it is termed as an open wound and when force trauma causes a contusion, it is called closed wound, whereas the burn wounds are caused by fire, heat, radiation, chemicals, electricity, or sunlight.

Wound Healing

Wound healing, or wound repair, is the critical physiological process by which the body repairs skin or organ tissue after injury. Wound healing is a complex process that results in the contraction and closure of the wound and restoration of functional barrier:

1. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue.
2. Repair of injured tissues includes inflammation, proliferation, and migration of different cell types.
3. Inflammation, which constitutes a part of the acute response, results in a coordinated influx of neutrophils at the wound site.

Process of Wound Healing

The healing process can be categorized into primary and secondary healing. Primary healing, or first intention, is the least complex as it refers to the healing together of the edges of clean, closely opposed wound edges. Secondary healing or second intention involves not only apposition of edges, but also the filling of a soft tissue defect as seen in traumatic, infection or disease induced wounds. Delayed primary closure or third intention is a combination of the first two.

Phases of Wound Healing

There are mainly 3 phases of wound healing there response to injury, either surgically or traumatically induced, is immediate and the damaged tissue or wound then passes through three phases in order to affect a final repair.

1. The inflammatory phase

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2. The fibroplastic phase

3. The remodelling phase

Inflammatory Phase

The inflammatory phase starts immediately after the injury that usually last between 24 and 48 hrs and may persist for up to 2 weeks in some cases the inflammatory phase launches the haemostatic mechanisms to immediately stop blood loss from the wound site. This phase is characterized by vasoconstriction and platelet aggregation to induce blood clotting and subsequently vasodilatation and phagocytosis to produce inflammation at the wound.

Fibroplastic Phase

The second phase of wound healing is the fibroplastic phase that lasts upto 2 days to 3 weeks after the inflammatory phase. This phase comprises of three steps viz., granulation, contraction and epithelialisation. In the granulation step fibroblasts form a bed of collagen and new capillaries are produced. Fibroblast produces a variety of substances essential for wound repair including glycosaminoglycans and collagen.

Remodeling Phase

This phase last for 3 weeks to 2 years. New collagen is formed in this phase. Tissue tensile strength is increased due to intermolecular cross-linking of collagen via vitamin-C dependent hydroxylation. The scar flattens and scar tissues become 80% as strong as the original.

MATERIAL AND METHODS

Table 1: Materials used

Sr. No.	Name of material
1	Aquapac 505 HV HC
2	Triethanolamine
3	Methyl paraben
4	Glycerin
5	Ethanol
6	Diethyl ether
7	Molten wax
8	Distilled water

Animals

Wister Males rats (150-200gm) were procured from Animal House of Anuradha College of Pharmacy Chikhli, (Dist-Buldhana) & were fed a standard diet, water was provided *ad libitum* & they were acclimated 7 days before entry into subsequent study. The protocol was approved by Institutional Animal Ethics Committee (IAEC).

Procurement of Herbal Extracts

Herbal Extracts of *Vitex Nigundo, Curcuma longa, Tinospora Cardifolia, Symplocus Racemosa, Azadirachta indica* Collected as gift sample from **Kisalaya Herbals LTD Indore** three different formulations were prepared. The formulae of prepared herbal gel formulations incorporating the herbal drugs extract is given below.

Table 2: Different herbal gel formulations

Ingredients	F1	F2	F3
Vitex Negundo (0.200mg), Tinospora cordifolia (0.200 mg), Symlocus Racemosa (0.200mg), Curcuma Longa (0.200mg), Azadirachta Indica (0.200mg),	1%	1%	1%
Aquapac HC 505 HV	2%	2.5%	3%
Propylene Glycol	10%	10%	5%
Glycerine	5 %	5%	10%
Methyl Paraben	0.2	0.2	0.2
Triethanolamine	q.s	q.s	q.s
Water	q.s	q.S	q.s

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Procedure

Phase A- Take Required quantity of Water & add glycerine on continued Stirring was done then add Propylene Glycol on continued Stirring then Add weighed quantity of Aqupec 505 slowly & periodically in purified water until the uniform dispersions was produced by continuously stirred by mechanical stirrer.

Phase B - Heat and mixed the other ingredients of Phase B together and finally add all the uniform dispersion of phase B into Phase A on continued stirring. The mixture was neutralized by drop-wise addition of 50% triethanolamine (w/w). Mixing was continued until a transparent gel was formed. [Misal G.*et al* 2012]

% Drug Content

The drug content of the gel formulations was determined by dissolving an accurately weighed quantity 1 gm of gel was taken from each batch according to dose of extract dissolved in 10ml of solvent phosphate buffer pH 6.8. The solutions were kept for shaking for 4hrs and then kept for 6hrs for complete dissolution of the formulations. Then the solutions were filtered through 0.45mm membrane filters and proper dilutions were made and solutions were subjected to the Spectrophotometric analysis. [Jaiswal N.R. *et al* 2014]

% Drug Release= test abs./std. abs×1/dilution factor×100

Evaluation of the Formulation (*In-vitro*)

The prepared herbal gel formulations incorporating herbal extracts were subjected for the in vitro evaluation and stability studies by using the various parameters.

Physical Evaluation

The colour, appearance and the feel on application of the prepared herbal gel formulations were noted and the results are shown in Table 1 (Aly Usama Farghaly *et al* 2012).

Subjective Properties

Subjective properties such as consistency, texture and irritation are observed and shown in Table 1

pH Measurement

The pH of the gel was determined by using a digital pH meter (Systronics pH meter type) instrument reading in terms of pH are tabulated in the Table 1. The pH was studied for 30 days. (Karade Preeti G. *et al* 2012)

Grittiness

All the formulations were evaluated for the particulate matters under light microscope.

Determination of Spreadability

Spreadability is expressed in terms of time in seconds taken by two slides for detachment by placing gel in between the slides [Syed U. F. *et al* 2015]

$$S = M. L / T$$

Where,

M = Wt. tied to upper slide

L = Length of glass slides

T = Time taken to separate the slides

Viscosity Study

Viscosity of gel was determined by Brookfield viscometer (Dial type). [Himaja N. 2015]

***In- Vitro* Diffusion Drug Release Study**

The in vitro diffusion studies of the gel formulations were performed using a vertical Franz diffusion cell whose diffusion area was 1.59 cm^2, and by using dialysis membrane dry, unwashed, pre-cut and open ended; flat width: 35 mm; inflated diameter, 21mm; Length: 30mm). The membrane soaked in phosphate buffer pH 6.8 for 3 hour was clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter, 4.16 cm^2 area). 100 ml of phosphate buffer was taken in a beaker, which was used as receptor compartment for the study. 1gm of each formulation was spreaded uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.5^{0}C. The solutions on the receptor side were stirred by externally driven Teflon-coated magnetic bars. At pre-determined time intervals, 1 ml of solution from the receptor compartment was

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pipetted out and immediately replaced with 1 ml fresh phosphate buffer solution. The drug concentration of the receptor fluid was determined spectrophotometrically at 250.2nm, 253.8nm, 344.4nm, 425.3nm and 533.11nm against appropriate blank. The amounts of drug permeation of all the formulations were calculated.

Antimicrobial Study

Antimicrobial activity was checked by disc gel diffusion method. The cultures were grown in nutrient broth and incubated at 37°C for 24 h. After incubation period is over, the O.D. of the culture was adjusted to 0.1 with sterile nutrient broth. 20 ml molten Mueller-Hinton agar medium was poured into sterile petri plates and allowed to solidify. The discs (6mm diameter) impregnated with 6 batches of gel formulation were placed on the surface of the petri plates seeded with 0.1 ml of microbial suspension (5 x 105 CFU/ml). Soon afterwards the plates were kept at 100C for 30 min. After it normalized to room temperature the plates were incubated at 37 0C for 24 h. After incubation period the zone of inhibition was measured. [Valgas C. *et al* 2007]

Burn Wound Model

A cylindrical metal rod (10mm diameter) is heated over the open flame for 30 secs and pressed to the shaved and disinfected surface for 20 secs on selected dorsal area of animal under light anesthesia whereas a partial thickness burns wounds can also be inflicted upon animals starved overnight and under mild anaesthesia, [Subalakshmi M. *et al* 2014] Then burn wound was poured by pouring hot molten wax (2gm) at (80^0C). The wax was poured on the shaven back of the animal through a metal cylinder of 3^2 circular opening. The wax was allowed to remain on the skin till it gets solidified. Immediately after the injury and on subsequent days, all the gel was daily applied topically for 21 days or till complete epithelization. (Kotade Kiran *et al* 2008). After animal recovered completely from anesthesia, they were kept in individual cages and followed all norms of good laboratory practice in carrying the animals. (Meena K. *et al* 2011).

The animals were randomly divided into 3 groups and each group containing 2 animals. The treatments of each gel (500mg/rats) were applied topically once a day. (Ahmad N. S. *et al* 2005)

1. Group I :- Control group
2. Group II:- Test group Treated with Formulation II
3. Group III:- (Reference Standard) Heal-O-Kind Gel (Nano crystalline silver)

Assessment of Burn Healing

Animal will be inspected every alternate day and the healing will be assessed based on physical parameters namely.

1. Wound contraction and
2. Epithelialization

Wound Contraction

Wound contraction was monitored by measuring the progressive changes in row wound area, plan metrically on a transparent paper, from which the wound surface area was evaluated. The tracing was then transferred to 1 mm^2 graph sheet, from which the wound surface area was evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound, 2cm, as 100% by using the following equation.

$$\text{Wound contraction (\%)} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Epithelialization

Time taken for full epithelialization was measured by recording the days. (Meena K. *et al* 2011)

Statistical Analysis

Experimental data are expressed as Mean ± (SEM) standard error of mean. Statistical analysis was performed by using one way ANOVA followed by Dunnet's test.

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RESULTS AND DISCUSSION

All results of different parameters of evaluation are recorded. The physical parameter such as color, appearance, feel on application are observed and shown in Table 3 All gel Formulations F1 to F3 were prepared as per the procedure and from the physical evaluation mention in Table 3 the color of prepared herbal gels was dark Brownish Yellow, Light Brownish Yellow and Brownish yellow as the color of extracts was brown and yellow. Appearance of gel was translucent and it was smooth on application. So it shows significant physical evaluation parameters. The subjective properties mention in Table 3 such as consistency was good and texture of prepared herbal gel was found to be smooth. No skin irritation was there on application of gel to the skin surface. The pH was studied for 30 days at room temperature.

All six formulations were in range of 4.92 – 5.85. All the prepared herbal gel formulations show desirable spreadability values. The percentage drug released by formulation I and II have more ability to drug released compare to other.

Formulation III was low as compared to I and II formulation, it might be due to gelling agent it have drug holding capacity, Percentage Drug released of F1, F2, F3 mention in Table 4, 5, 6 respectively. Antimicrobial Study carried out by disc diffusion method with *Staphylococcus aureus and Escherichia coli culture* was used in that it shows average same zone of inhibition on both culture of micro-organization. It had good antimicrobial activity on both culture showed in Table 7 In pharmacological evaluation the wound healing activity in this animals were divided into 3 groups. The observations were made on 4 day, 8 day, 12 day, 16 day and 21 day respectively and the observations were mentioned in Table 8.

Table 3: Evaluation of Herbal Gel

Physical Parameter	**F1**	**F2**	**F3**
Color	Dark Brownish Yellow	light Brownish Yellow	Dark Brownish Yellow
Appearance	Translucent	Translucent	Translucent
Feel on application	Smooth	Smooth	Smooth
Consistency	Good	Good	Good
Texture	Smooth	Smooth	Smooth
Skin Irritation	--	--	--
pH	5.78	5.85	5.17
Spredibility	26.66	29.23	30.46
Viscosity	84730	128,000	330,000

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Table 4: % Drug release at different time interval of F2

Time	F1-V	F1-S	F1-T	F1-C	F1-A
0	0	0	0	0	0
15	19.68±0.61	17.10±0.97	18.97±0.73	16.28±0.96	11.89±0.91
30	22.93±0.34	33.56±0.49	29.13±0.51	18.83±0.49	17.15±0.60
60	26.56±0.47	37.52±0.99	31.60±0.48	26.86±0.87	23.22±0.56
90	32.68±0.62	44.72±0.62	37.86±0.84	32.35±0.60	34.19±0.58
120	37.006±0.87	51.26±0.55	43.78±0.54	38.75±0.62	41.02±0.77
150	43.02±0.79	57.41±0.50	49.16±0.76	45.85±0.60	46.80±0.58
180	52.88±0.93	70.10±0.97	67.85±0.40	55.39±0.94	63.76±0.31

Table 5: % Drug release at different time interval of F2

Time	F2-V	F2-S	F2-T	F2-C	F2-A
0	0	0	0	0	0
15	13.09±0.33	9.93±0.91	16.87±0.37	12.59±0.36	14.76±0.14
30	19.97±0.46	15.02±0.67	22.18±0.85	16.69±0.43	19.20±0.76
60	26.83±0.33	20.18±0.82	30.32±0.56	20.83±0.97	21.13±0.71
90	32.87±0.56	29.02±0.57	34.94±0.78	26.85±0.85	29.97±0.57
120	36.79±0.98	36.31±0.79	41.26±0.70	32.42±0.52	41.33±0.47
150	41.70±0.67	43.93±0.30	48.91±0.95	37.86±0.48	46.82±0.39
180	54.85±0.48	53.33±0.67	51.22±0.66	49.04±0.97	52.17±0.60

Table 6: % Drug release at different time interval of F3

Time	F3-V	F3-S	F3-T	F3-C	F3-A
0	0	0	0	0	0
15	13.97±0.67	17.75±0.89	20.48±0.66	12.89±0.52	14.92±0.65
30	16.88±0.53	21.27±0.72	25.65±0.18	17.65±0.20	18.75±0.39
60	20.08±0.29	27.58±0.50	30.99±0.69	21.12±0.91	22.29±0.61
90	25.03±0.39	32.81±0.92	36.43±0.47	26.07±0.67	29.04±0.34
120	32.16±0.89	38.77±0.90	41.22±0.81	30.13±0.81	36.37±0.67
150	36.66±0.79	45.06±0.65	44.27±0.98	34.95±0.79	45.31±0.43
180	44.18±0.43	49.03±0.67	52.88±0.56	40.18±0.77	56.78±0.60

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Antimicrobial Study

Table 7: Zone of inhibition (mm)

Formulation	*Staphylococcus aureus* Zone Of inhibition (mm)	*Escherichia coli* Zone Of inhibition (mm)
F1	10	11
F2	9	9
F3	8	9

Table 8: Evaluation of wound healing activity

Gr. No.	Treatment	Area of wound during different days of observation (%)				
		4	8	12	16	21
1	Control	7.50±0.70	24.50±2.82	27.50±0.70	42.50±0.70	64.50±1.41
2	Standard	22.50±0.70	32.5±0.70	42.5±0.70	68.50±1.42	90.50±2.82
3	Test	18.5±1.41	27.50±0.70	37.50±0.70	62.50±0.70	88.50±1.41

Epithelialization Period (days)

Table 9: Epithelialization period

Group No.	Groups	Epithelialization period (days)
1	Control	32.50±0.70
2	Test	26±1.41
3	Standard	22.5±0.70

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Stability Testing

Result of stability study shows a good stability even at different temperature

Table 10: Stability testing

Formulation	Initial color	At a room temperature			
		Ist week	IInd week	IIIrd week	IVth week
F1	Light Yellow	+	+	+	+
F2	Light Yellow	+	+	+	+
F3	Light Yellow	+	+	+	+
F4	Light Yellow	+	+	+	+
F5	Light Yellow	+	+	+	+
F6	Light Yellow	+	+	+	+

Heal O Kind Gel (Nano Crystalline silver 1% w/w) manufactured by Mankind Pharma (P) Ltd., Mumbai, (MH) was used as a standard for comparisation of positive control with prepared formulations. Marketed Heal O Kind Gel showed 90.50% wound contraction after 21 days with respect to initial wound size 20mm as 100%, whereas optimized formulation showed 88.50% wound contraction and control group showed 64.50% wound contraction as showed in Table 8. Thus due to increase in concentration of extracts activity increased.

The mean epithelialization period of marketed heal o kind gel was 22 days where as the mean epithelialization period of optimized formulation (F1) was 25 days, days as showed in Table 9. Wound healing potential of polyherbal gel is nearly equivalent with compared to standard. The prepared herbal gel formulations were subjected to stability testing Table 10.

The prepared herbal gel were store at room temperature for a period of 30 days to study effect of temperature. The physical parameter were evaluated during study period the result of study indicates that preparation are physically stable at room temperature.

CONCLUSION

Natural remedies are more acceptable in the belief that they are safer with no or fewer side effects than the synthetic ones. Herbal gel formulations were prepared successfully**.** Herbal extract of Nirgundi (*Vitex Negundo*), Lodhra (*Symlocus Racemosa*), Gulvel (*Tinospora cardifolia*), Haldi (*Curcuma Longa*) and Neem (*Azadirachta Indica*) shown excellent wound healing activity. Also said herbal extracts showed antimicrobial activity. Existing antimicrobial and wound healing activity of various herbal extracts in combination in gel preparation may show synergistic action. Stability studies over a period of four weeks proved that, the all preparations were stable and effective.

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