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Formulation and evaluation of topical gel using *Eupatorium glandulosum* michx. for wound healing activity

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ABSTRACT

The present work aims to formulate and evaluate the *Eupatorium glandulosum* Michx based topical gel for wound healing activity. The gel was formulated using the methanolic extract of the *Eupatorium glandulosum* michx and carbopol as base. The prepared gel was characterized for their physicochemical constants, preliminary phytochemical analysis, quantitative analysis, spreadability, pH, viscosity and extrudability. Antimicrobial and wound healing studies were carried out for the optimized formulation (F5). The physicochemical constants like total ash, acid insoluble ash, and water-soluble ash are specific identification. The methanolic extract was prepared by maceration method and the extract was formulated into topical gel formulation. The results of preliminary phytochemical investigation showed the presence of carbohydrates, cardiac glycosides, phenols and flavonoids. Quantitative analysis of polyphenolic compounds showed high content of flavonoids. Other parameters such as spreadability, pH, viscosity and extrudability were found to be well within the limits. The antimicrobial activity of optimized formulation was found to be effective in both gram positive and gram-negative organisms. The optimized formulation promotes better wound healing with comparison to that of the marketed product. From the present study, it was concluded that the prepared herbal gel formulation showed significant antimicrobial potential and found to posses better wound healing activity.

Keywords: *Eupatorium glandulosum* michx; carbopol; antimicrobial; wound healing; topical gel

INTRODUCTION

Wound has been defined as the disruption of anatomic or functional continuity of living tissue [1]. In the normal setting wound healing “proceeds through an orderly and timely reparative process that result in sustained restoration of anatomic and functional integrity”. The vast literature on wound healing is focused mainly on skin, which is most susceptible organ in the body that interacts with environment and therefore receives constant insult and damage. Wound healing is a complex phenomenon involving a number of process, including induction of an acute inflammatory process by the wounding, generation of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of extra cellular matrix proteins, remodeling of connective tissue and parenchymal components and collagenization and acquisition of wound strength [2, 3].

All these steps are orchestrated in a controlled manner by a variety of cytokines including growth factors. Some of the growth factors like “Platelet Derived Growth Factor” (PDGF), “Transforming Growth Factor – B” (TGF-B), “Fibroblast Growth Factor” (FGF) and “Epidermal Growth Factor” (EGF), etc., have been identified in self-healing wounds. In chronic wounds, the normal healing process is disrupted due to some unknown reasons and in such cases, exogenous application of some growth promoting agents or some compounds, which can enhance the “*in situ*” generation of these growth factors, is required to augment the healing process [4].

In the present conventional mode of therapy, the pathology of any form of wound healing involves the treatment by application of antibiotics systemically and antiseptics topically. These agents' only help in keeping the wound area sterile, hence preventing any further infection; however they are not involved in the physiological repair process of wound healing.

The 'mother nature' is kind enough to us by creating various herbs in her vast flora, which aid in the secondary intention healing process. The continual usage of certain herbs and chemical agents promotes enhanced "fibrogenetic" and "collagen concentration" synthesizing process, hence resulting in a faster wound healing phenomena. It is therefore highly important to categorize such herbs and shift onto herbal mode of therapy for achieving efficient results.

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration. For the topical treatment of dermatological diseases as well as skin care, a wide variety of vehicle ranging from solids to semisolids and liquid preparation is available to clinician and patients. Within the major group of semisolid preparations, the use of transdermal gels has expanded both in cosmetics and in pharmaceutical preparations. Transdermal application of gels at pathological sites offer great advantage in a faster release of drug directly to the site of action, independent of water solubility of drug as compared to creams and ointments [5, 6].

Eupatorium glandulosum Michx is a profusely branching undershrub upto 90-120 cm in height with a few ascending branches; leaves simple, opposite, subsessile, lanceolate, subentire and glabrous type. The leaves are used as astringent, thermogenic and stimulant in folklore medicine in India [7]. The present study involves the screening of *Eupatorium glandulosum Michx* (Extract of which have been incorporated into a gel base) for their wound healing properties and also for other accessory properties promoting wound healing (wound contraction, period of epithelialization, etc.)

MATERIALS AND METHODS

2.1. Collection and Authentication of the plant material

The leaves of *Eupatorium glandulosum Michx* was collected from Nilgiris district, Tamil Nadu in the month of July. The sample was identified and authenticated by Dr. M.N. Naganandini, Assistant Professor, Department of Pharmacognosy, J.S.S. College of Pharmacy, Mysore. After authentication, the leaves were shade dried (25 °C), pulverized separately in a mechanical grinder, passed through a 40-mesh sieve and stored in well closed container till further use.

2.2. Extraction

10 grams of pulverized leaf material were soaked in 100 ml of methanol (LR grade, Merck, India) and kept on a rotary shaker for 24 hours. The extract was filtered through a Whatman No. 1 Filter Paper and the process is repeated until all soluble compounds had been extracted. The collected portion was subjected to screening for further studies [8].

2.2.1. Preliminary phytochemical analysis

Preliminary analysis was carried out to identify the useful constituents like alkaloids, flavonoids, saponins, tannins, phenols and terpenoids using standard methods.

2.3. Materials

Carbopol 934 & propylene glycol was obtained as gift sample from LOBA Chemie Pvt. Ltd, Mumbai, India. Methyl paraben, propyl paraben and triethanolamine were purchased from Merck Specialities Pvt. Ltd, Mumbai, India. All other chemicals used were of analytical grade and obtained commercially.

2.4. Determination of total phenolic content

Total phenols were estimated in the sample *E. glandulosum* by the method proposed by Barreira *et al* [9]. Phenols react with phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium to produce a blue-coloured complex (molybdenum blue) which can be determined spectrophotometrically at 725 nm.

2.5. Determination of total flavonoid method

The flavonoid content was determined by aluminium chloride colorimetric method described by Chang *et al* [10]. Flavonoids react with aluminium chloride and potassium acetate to produce a colored product which can be measured spectrophotometrically at 415nm.

2.6. Preparation of gel containing extract

1 g of Carbopol 934 was dispersed in 50 ml of distilled water. It was kept aside to swell, which was further stirred to form a gel. 5 ml of distilled water and required quantity of methyl paraben and propyl paraben were dissolved with the aid of heat on water bath. Solution was cooled and propylene glycol 400 was added to it. Further, required quantity of methanolic extract of *Eupatorium glandulosum Michx.* at different concentration was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. All the ingredients were mixed properly and with continuous stirring. Triethanolamine was added drop wise to the formulation for the adjustment of skin pH (6.8-7) and also to obtain a gel at required consistency [11]. The same method was followed for the preparation of control sample. Prepared gel was filled in collapsible tubes and stored at a cool and dry place. Physical parameters such as colour, appearance, and feeling on application were recorded. The method describes above and the formulae were tabulated in **Table 1**.

2.7. Evaluation of topical gel formulation

Physical parameters such as color, appearance, homogeneity and grittiness were inspected through visual inspection.

2.7.1. Measurement of pH

pH of the gel was measured by using pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of each formulation was done in triplicate and average values are calculated.

2.7.2. Spreadability

Spreadability was determined by the apparatus consists of a wooden block, which was provided by a pulley at one end. By this method Spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spreadability. Spreadability was calculated using the following formula:

$$S = M \times L / T \quad (1)$$

Where, S = Spreadability, M = weight in the pan (tied to the upper slide), L = length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other.

2.7.3. Viscosity

Viscosity of gel was measured using Brookfield viscometer with spindle.

2.7.4. Extrudability

The gel formulations were filled in standard capped collapsible aluminium tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped [12]. 500 g was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated

>90% extrudability - excellent

>80% extrudability - good

>70% extrudability – fair

2.7.5. Antimicrobial activity for the optimized formulation

The antimicrobial activity of each formulation was carried out [13] using the technique of zone of inhibition, employing *Escherichia coli* and *staphylococcus aureus* as test organisms (**Table 2**).

2.7.6. Preparation of nutrient broth medium

The ingredients such as beef extract, peptone and sodium chloride were dissolved in water with the aid of heat. The pH was adjusted to 7.1 ± 0.1 . Later agar – agar was incorporated into the medium by directly heating at a temperature of 97 °C to 98°C. The prepared media was filtered while hot into an agar tubes to a 20 ml. these tubes were then autoclaved at a temperature of 121°C and 15 lb pressure for 30 minutes. Later, subculture *E.coli* and *S. aureus* were introduced into every agar tubes and were shaken thoroughly at a temperature of 37 °C – 40 °C, employing strict aseptic conditions (under laminar flow bench). The sterilized media were carefully transferred into sterilized petridish and allowed to solidify. Then bore of approximate diameter of 4mm was made in the nutrient culture media. Equal quantity of individual semisolid herbal formulations were added carefully into the bore

made and these petridish were incubated at temperature of 37 °C for 48 hours for the growth of the microorganisms to take place. Later, the zone of inhibition produced by the herbal formulations towards *E.coli* and *S. aureus* organisms were measured individually in all the petridish and photographed.

2.8. *In vivo* studies

The study was conducted after obtaining approval from the institutional animal ethical committee of JSS College of Pharmacy, Mysuru. Wister albino rats of 200-250g were used in the study. The surgical interventions are carried out in sterile conditions under general anaesthesia. The predetermined area for the wound infliction at the back of the animal for surgery is prepared by removing the hairs with depilatory cream. The animals were anesthetized using anaesthetic ether by open mask method and placed on the operation table in its natural position.

Skin excision wounds of 1cm x 1cm was created using a punch biopsy needle, and a depth of about 1mm on the dorsal aspect of the thoracolumbar region of the rats, which include a standard group treated with marketed herbal wound healing gel, control group is treated with Carbopol base gel, the treatment group is treated with the optimized formulation. The animals are allowed to recover, housed individually in their cages, and monitored for respiration, colour and temperature. They are maintained under standard husbandry conditions and on a uniform diet and managed throughout the experimental period. Animals are closely observed for any infection; those who show signs of infection are separated and excluded from the study. They are periodically weighed before and after the experiments.

2.9. Excision wound model

Excision wounds are inflicted on the dorsal thoracic region 1–1.5 cm away from the vertebral column on either side and 5 cm away from the ear. An area of about 1 sq. cm was marked with a marker circularly on the shaven back of the animals. The marked area was excised with full thickness with surgical sterile blade and scissors. The respective therapeutic treatment is administered topically to the animals of respective groups until complete epithelialization starting from the day of operation. Collagen estimation, percentage wound contraction, and period of epithelialization parameters are studied [14].

2.10. Percentage wound contraction

The progressive reduction in the wound area is monitored planimetrically by tracing the raw wound boundaries initially on a sterilized transparency paper sheet in mm² without causing any damage to the wound area, and then, the wound area recorded is measured using a graph paper on every 4 days interval. The period of epithelialization is expressed as the number of days required for falling of the eschar (dead-tissue remnants) without any residual raw wound is considered as the end point of complete epithelialization.

$$\text{Percentage closure} = \frac{A-O - A-D}{A-O} \times 100 \quad (2)$$

Where, A – O = wound area on day zero, and

A – D = wound are on corresponding days.

The number of days for complete closure was noted & the scar shape and area were traced and measured on complete closure.

2.11. Measurement of wound area

The dynamic change in the wound area was monitored by camera on foreordained days (4, 8, 12, 16 and 20 days). Later in which, wound area was measured by tracing the wound on a millimetre scale graph paper.

2.12. Period of epithelisation

Falling of scab deserting no raw wound behind was taken as end point of complete epithelisation and the days required for this was taken as period of epithelisation.

2.13. Measurement of wound index

Wound index was measured daily with an arbitrary scoring system i.e., “0” for complete healing, “1” for incomplete but healthy healing, “2” for delayed but healthy but healthy healing, “3” for healing has not yet been started but environment is healthy, “4” for formulation of pus evidence of necrosis.

2.14. Histopathological studies

The regenerated tissue previously collected and preserved in 10% buffered formalin was used for histopathological studies.

2.14.1. Preparation of histological studies

The tissue was removed from buffered formalin after a day or two, dehydrated in ascending grades of alcohol, cleared in chloroform, embedded in paraffin using tissue processor and cut with rotary microton, getting sections of 3 to 5 micrometer in thickness. The section was dewaxed by xylene and the xylene was removed by descending grades of alcohol to facilitate staining procedure.

2.14.2. Staining procedure

Dewaxed section was stained with haematoxylin for 5 to 8 minutes. Washed well in running tap water for 2 to 3 minutes. It was then examined microscopically to confirm a sufficient degree of staining. Remove excess stain by decolourising in 0.5 to 1.0 % by hydrochloric acid in 70% alcohol for a few seconds. The blue stain of haematoxylin was changed to red by the action of the acid. Regained the blue colour and stopped decolourisation by washing in running tap water for at least 5 minutes. Stained in 1% aqueous eosin for 1 to 3 minutes. Dehydrated in alcohol and cleared in xylene for transparency. Mounted in a synthetic resin medium. The Histopathological changes in the regenerated tissue, which took place during the wound contraction or healing phase in both the test, and control, was observed for "epithelisation, fibroblast, collagen, cell infiltration (inflammation) and neovascularization". These parameters were qualitatively assessed and micro photographed under "400X" magnification.

2.15. Stability study

The stability study was performed as per ICH guidelines. The optimized gel formulation was filled in the collapsible tubes and stored at different temperature and humidity conditions, viz. $25^{\circ}\text{C} \pm 2^{\circ}\text{C}/ 60\% \pm 5\% \text{ RH}$, $30^{\circ}\text{C} \pm 2^{\circ}\text{C}/ 65\% \pm 5\% \text{ RH}$, for a period of three months and studied for various parameters.

RESULTS AND DISCUSSION

3.1. Extraction of plant material

The simple maceration method was used for the preparation of the extract. The percentage yield of methanolic extract of *Eupatorium glandulosum Michx* was found to be 27.32%.

3.2. Organoleptic properties

The organoleptic properties of the plant extracts are mentioned in **Table 3**.

3.3. Determination of physicochemical constants

The physicochemical constants like ash value, extractive value, moisture content and foreign matter was determined. The dried aerial parts of *Eupatorium glandulosum Michx* showed higher percentage of total ash (13.71% w/w) and water-soluble ash (6.24% w/w). Higher yields of alcohol soluble extractive (18.59% w/w) and water-soluble extractive (28.56% w/w) values were observed. Moisture content and foreign matter was found to be 7.49% w/w and 0.92% w/w respectively. The results are shown in the **Table 4**.

3.4. Preliminary phytochemical analysis

Preliminary phytochemical studies of *Eupatorium glandulosum Michx* reveal the presence of alkaloids, carbohydrates, cardiac glycosides, tannins, saponins, phenols and flavonoids. The results obtained were summarised in the **Table 5**.

3.5. Estimation of total phenol and flavonoid content

The concentration-absorbance calibration curve for 5 sequentially and separately prepared standards stock of gallic acid solution measured absorbance values at 765 nm. Total phenol content in the aqueous extract of *Eupatorium glandulosum Michx*, using the calibration curve, was found to be 34.0 mg gallic acid equivalents/g dry weight of extract. Total flavonoid content in the methanolic extract of *Eupatorium glandulosum Michx* was found to be 476.0 mg/g equivalent to quercetin (QE), i.e., 1 g of the extract contains 476 mg of quercetin equivalent.

3.6. Formulation aspects

Topical gels were prepared with different concentration of the aqueous extract of *Eupatorium glandulosum Michx*. Different combinations were tried to formulate a best suitable base and the base gel with carbapol 934 was found to be more stable and smooth. Hence further evaluation studies are carried out for the same.

3.7. Evaluation of topical gel

3.7.1. Physical evaluation

The physical parameters such as appearance, homogeneity and grittiness were checked by visual examination and the results are given in the **Table 6**.

3.7.2. Measurement of pH

The pH of various gel formulations were determined by using digital pH meter. 2.5gm of gel was accurately weighed and dispersed in 25ml of distilled water and stored for two hours. The measurement of pH of each formulation was done and the results were shown on the **Table 7**.

3.7.3 Determination of spreadability of the formulations

The spreadability of formulations was evaluated at ambient conditions. The spreading diameter of 2 ± 0.01 g of formulation was placed between two horizontal glass plates and was measured after 1 minute. The data was reported in the following **Table 8**. It was observed that the gel belongs to the semi fluid category and observed that increase in concentration of plant extract decreases the spreadability of the formulation.

3.7.4 Determination of the viscosity of the formulation

The viscosities of gel based formulations were determined using Brookfield viscometer. Using the spindle number S95 at 100 rpm and temperature was maintained at $30 \pm 1^\circ\text{C}$. It was observed that with increase in the concentration of the plant extract also increases the viscosity. The viscosities of the formulations were reported in the **Table 9**.

3.7.5 Determination of extrudability

The amount of the extruded gel was collected and weighed. The percentage extrudability results are shown in the **Table 10**.

3.8 Antimicrobial Activity

Antibacterial activity of all the prepared formulation was carried out using *E.coli* (**Table 11**) and *S.aureus* (**Table 12**). The results were found to be encouraging. The control samples did not show any zone of inhibition (**Figure 1**).

3.9. Percentage Wound Contraction for the optimized formulation

The reports of the wound contraction studies in rats are reported in **Table 13** and their profiles are given in the **Figure 2**. The results indicate that the rate of wound contraction was maximum on the 12th day in the treated group. Wound contraction studies depicted that Formulation F5 showed the highest wound contraction when compared with that of standard. Since the wound contraction ceases after 16 days, the treatment was undertaken only for 16 days. The scars left behind F5 and standard were very light, but in control the scars left behind were very thick **Figure 3**.

3.10. Period of Epithelialization

The values of period of epithelialisation for the control and the treated groups are depicted in **Table 14**.

3.11 Wound Index

Wound index was measured daily with an arbitrary scoring system and reported in **Table 15**.

3.12 Histopathological Studies

Microscopical examination of the sections prepared from the wounds of normal, standard, control and treated are depicted as follows,

Normal – The tissue is composed of dense collagen fibres, fibroblasts with round to oval nuclei and blood vessels. Epithelialization is not seen in the given section.

Control – The tissue shows densely inflamed connective tissue with chronic inflammatory cells interrupted between the collagen fibres, this shows the incomplete healing of wound. Many thin walled blood vessels are seen.

Standard – The tissue shows dense fibrous connective tissue with fibroblasts and collagen fibres.

Treated – The tissue shows dense fibrous tissue with fibroblasts and collagen fibres which is more than that of the standard. The characters are almost to that of the normal (**Figure 4**).

3.13 Stability Studies

There was no change in the physical appearance of the optimized formulation as mentioned in **Table no. 16A and 16B**.

Table 1: Formulation chart for preparation of gel

INGREDIENTS	F1	F2	F3	F4	F5	C
Carbopol 934 (1%) (gm)	1	1	1	1	1	1
Plant extract (%)	1%	2%	3%	4%	5%	-
Propylene glycol (ml)	5	5	5	5	5	5
Methyl Paraben (0.2%) (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (0.15%) (ml)	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine (q.s)	0.5	0.5	0.5	0.5	0.5	0.5
Distilled water	Up to 100ml					

*C - Control***Table 2: Ingredients used to prepare nutrient broth**

Sl.No	Ingredients	Quantity / 250ml
1.	Beef extract	5 g
2.	Peptone	5 g
3.	Sodium chloride	1.25 g
4.	Agar - agar	1.5 %
5.	Water	q.s to 250 ml

Table 3: Organoleptic properties of the plant extracts

Sl.No	Description	Crude drug	Methanolic extract
1.	Colour	Light green	Brown
2.	Odour	Characteristic	Characteristic
3.	Taste	Bitter	Bitter

Table 4: Physicochemical constants of the aerial parts of EG

Name of the plant	<i>Eupatorium glandulosum</i> Michx
Total Ash (%w/w)	13.71
Acid Insoluble Ash (%w/w)	5.39
Water Soluble ash (%w/w)	6.24
Alcohol soluble extractive values (%w/w)	18.59
Water soluble extractive values (%w/w)	28.56
Moisture content (%w/w)	7.49
Foreign matter (%)	0.92

Table 5: Preliminary phytochemical analysis

Sl.No	Plant Constituents	Test / Reagent	Methanolic extract
1.	carbohydrates	Molish's	+
		Fehling's	+
		Benedict's	+
2.	proteins	Biuret	-
		Millon's	-
3.	Amino acids	Ninhydrin	-
4.	Sterols	Salkowski	-
5.	Saponins	Foam	+
		Haemolysis	+
		Wagner's	+
6.	Alkaloids	Mayer's	-
		Dragendroff's	+
		Hager's	+
		Ferric chloride	+
7.	Tannins	Gelatin	-
		Vanillin	+
		Shinoda	+
8.	Flavonoids	Ferric chloride	+
		Lead acetate	+
		Zinc-HCl	+
		NaOH	+
9.	Glycosides	Antraquinone	+
		Cardiac	+

+ = Present, - = Absent

Table 6: Physical evaluation of the Prepared Gel formulation

Sl.No.	Appearance	Homogeneity	Grittiness
F1	Light brown	Good	Nil
F2	Light brown	Good	Nil
F3	brown	Good	Nil
F4	brown	Good	Nil
F5	brown	Good	Nil
C	White	Good	Nil

(C) – Control

Table 7: pH Measurement

Formulation	Trial I	Trial II	Trial III	MEAN \pm SD
F1	6.5	6.7	6.5	6.5 \pm 0.11
F2	6.5	6.8	6.5	6.6 \pm 0.17
F3	6.7	6.7	6.6	6.6 \pm 0.05
F4	6.8	6.5	6.8	6.8 \pm 0.15
F5	6.7	6.7	6.8	6.8 \pm 0.05
C	6.8	6.7	6.6	6.7 \pm 0.10

S.D* = Standard Deviation, n=3

Table 8: Measurement of spreadability

Formulation	Spreadability (g.cm/Sec)			
	Trial I	Trial II	Trial III	Mean \pm SD
F1	31.59	31.43	31.67	31.56 \pm 0.12
F2	31.02	31.34	31.52	31.29 \pm 0.25
F3	30.22	30.31	30.66	30.39 \pm 0.23
F4	30.43	30.33	30.21	30.32 \pm 0.11
F5	29.32	29.15	29.42	29.29 \pm 0.13
C	25.20	24.09	24.57	24.62 \pm 0.55

S.D* = Standard Deviation, n=3

Table 9: Measurement of viscosity

Formulation	Viscosity (CPS)			
	Trial I	Trial II	Trial III	Mean \pm SD
F1	15441	15430	15446	15439 \pm 08.18
F2	15900	15923	15947	15923 \pm 23.50
F3	16434	16457	16425	16438 \pm 16.50
F4	16876	16825	16853	16851 \pm 25.54
F5	17205	17247	17214	17222 \pm 22.11
C	13302	13315	13312	13309 \pm 06.80

Table 10. Measurement of extrudability

Extrudability	Mean \pm SD
Net formulation in the tube (g)	25.34 \pm 1.32
Weight of gel extruded (g)	23.91 \pm 0.43
Extrudability Amount percentage	92.07 %

S.D* = Standard Deviation, n=5

Table 11. Zone of inhibition shown by the formulation for *E. coli*

Sl.No.	Formulation	Zone of Inhibition for <i>E.coli</i> (cm)
1.	F1	0.4
2.	F2	0.7
3.	F3	0.7
4.	F4	0.9
5.	F5	1.2
6.	Standard	1.3

Table 12. Zone of inhibition shown by the formulation for *S. aureus*

Sl.No.	Formulation	Zone Of Inhibition for <i>S. aureus</i> (cm)
1.	F1	0.2
2.	F2	0.6
3.	F3	0.8
4.	F4	1.3
5.	F5	1.6
6.	Standard	1.5

Table 13. Excision wound studies showing percentage reduction in wound size, when treated with F5, control and standard

Days	% Contraction		(Mean \pm S.E.M)
	Control	Standard	Treated
4	15.60	20.58	19.21
8	39.42	52.41	51.94
12	56.31	76.34	83.12
16	72.14	93.57	94.36

S.E.M* = Standard Error mean, n=6

Table 14: Period of epithelisation

Sl.No.	Groups	Epithelialization Period (Days)
1	Control	22.03 \pm 0.92
2	F5	13.45 \pm 1.23
3	Standard	15.23 \pm 0.72

S.D* = Standard Deviation, n=6

Table 15: Measurement of Wound index

Sl.No.	Experimental Groups	Wound Index
1	Control	2.92 \pm 0.22
2	F5	1.55 \pm 0.77
3	Standard	1.75 \pm 0.55

S.D* = Standard Deviation, n=6

Table no 16 A: Stability data of Gel formulation (F5) at 25 °C and 40 °C

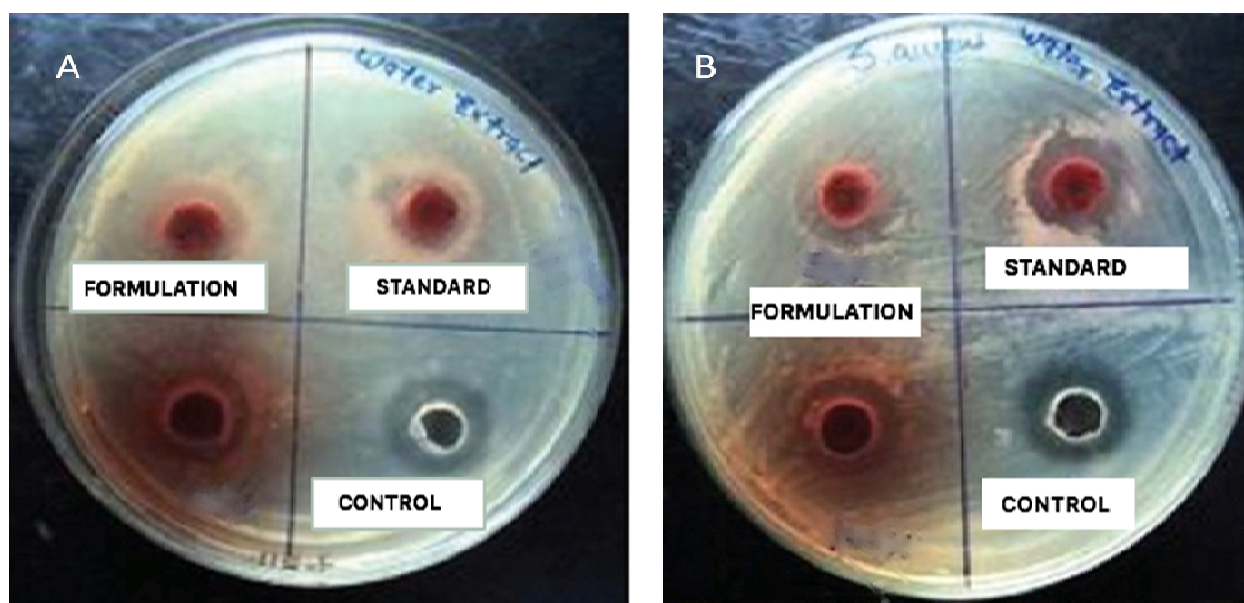
Days	Formulation	pH \pm S.D	Homogeneity	Grittiness	Report
0	F5	6.7 \pm 0.10	***	Nil	Complies
15		6.7 \pm 0.02	***	Nil	Complies
30		6.6 \pm 0.17	***	Nil	Complies
60		6.6 \pm 0.11	**	Nil	Complies
90		6.6 \pm 0.15	**	Nil	Complies

S.D* = Standard Deviation, n=6

Table no 16 B: Stability data of gel formulation (F5) at 40 °C

Days	Formulation	pH	Homogeneity	Grittiness	Report
0	F5	6.7 \pm 0.10	***	Nil	Complies
15		6.6 \pm 0.08	***	Nil	Complies
30		6.6 \pm 0.01	**	Nil	Complies
60		6.5 \pm 0.03	**	Nil	Complies
90		6.5 \pm 0.01	**	Nil	Complies

S.D* = Standard Deviation, n=6

Figure No 1: Zone of inhibition shown by the formulations for *E.coli* (A) and *S.aureus* (B)

0TH DAY

CONTROL	STANDARD	TREATED
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8TH DAY

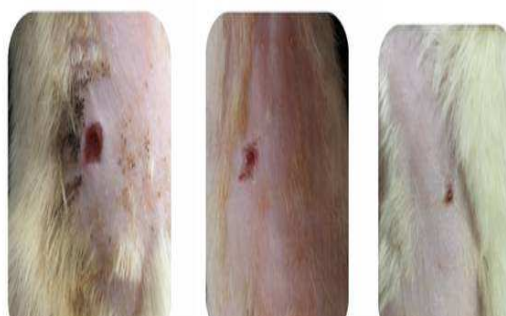
CONTROL	STANDARD	TREATED
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4TH DAY

CONTROL	STANDARD	TREATED
---------	----------	---------

12TH DAY

CONTROL	STANDARD	TREATED
---------	----------	---------

16TH DAY

CONTROL	STANDARD	TREATED
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Figure No 2: Percentage Wound contraction treated with formulation F5, control and standard

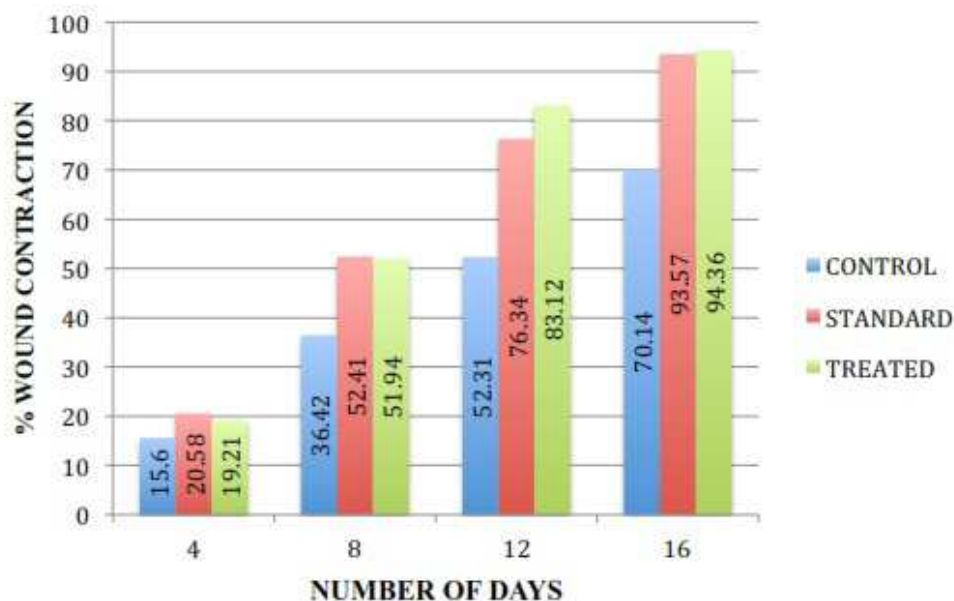


Figure No 3: Comparison of Percentage Wound contraction treated with formulation F5, control and standard

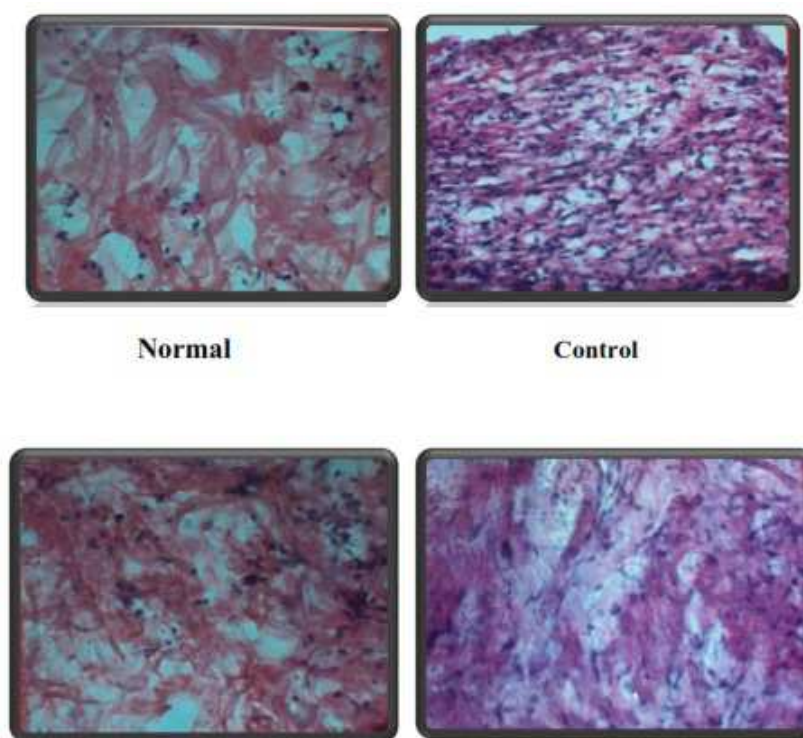


Figure 4: Histology of regenerated tissue of open wounds as on the 16th groups day at 400x of normal, control, standard and treated

CONCLUSION

Eupatorium glandulosum extracts contains several active constituents. Wound healing activity of *Eupatorium glandulosum* was investigated by formulating it into gel formulations. Initial investigation studies showed that formulation F5 exhibited promising results for pH, viscosity, spreadability and anti-microbial studies. *In vivo* studies of the prepared gel formulation was investigated by applying the gels formulations in albino wistar rats. It was observed that formulation F5 showed a shorter period of epithelization and greater rate of wound contraction. The optimized formulation was compared with the (5 % w/w aqueous leaf extract gel) marketed product. It was found that wound contraction, i.e. the process of wound area shrinkage was significantly greater for optimized formulation (F5) than that of marketed product. The wound closure time was lesser, as well as the percentage of wound

contraction was much more with the marketed product. It was concluded that the *Eupatorium glandulosum Michx* gel using carbopol base has higher wound healing properties than that of marketed product.

Declaration of Interest

The authors report no conflicts of interest.

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