



Research Article

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FORMULATION AND EVALUATION OF CREAM PREPARED FROM *CROTON SPARSIFLORUS* MORONG AND THEIR WOUND HEALING ACTIVITY

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ABSTRACT

Topical application of cream prepared from the chloroform extract of *Croton sparsiflorus* Morong were formulated and evaluated for its efficacy and safety. Creams were prepared at different concentrations i.e. 5%, 7.5% and 10% (w/w) by fusion method using different excipients. These topical formulations were tested for pH, viscosity, spreadability and drug contents uniformity. The stability study was carried out at 4, 25 and 37 °C. The drug content uniformity of creams was found within the range of 98.09 to 98.21%. All the formulations were evaluated for its acute skin irritancy, wound healing activity in Swiss Albino rats. These formulations did not produce any skin irritation for about a week when applied over the skin. Comparative studies showed that the viscosity of the formulations increases, spreadability decreases and vice versa. From the stability studies, creams showed no changes in properties after keeping at different temperatures for 90 days. All the formulations and the normal chloroform extracts of *C. sparsiflorus* Morong showed significant ($P < 0.001$) wound healing activity by excision wound model and comparable with that of the reference standards and control bases. The measurement of the wound areas were taken on 3rd, 6th, 9th, 12th, 15th and 18th days and the percentages of wound closures were calculated.

Keywords: Chloroform extract, *C Sparsiflorus* Morong leaves, Cream, Wound healing

INTRODUCTION

Croton sparsiflorus Morong belongs to the plant family Euphorbiaceae is a small annual herb, growing up to 1-2 ft tall. Alternately arranged leaves, 3-5 cm long, are lance-shaped, with a toothed margin. Small white flowers are borne in 3-8 cm long racemes at the end of branches. Flowers have 5 sepals and 5 petals and numerous long stamens protruding out. Fruit is a 5 mm oblong capsule, with a warty surface. It is grown abundantly in the rural areas of Malda, West Bengal. It is sufficiently found in the Coastal east Orissa of India. The plant is well known under vernaculars as 'BanTulasi' in Hindi, 'Banamaricho' in Oriya¹.

The powdered leaves are useful in controlling high blood pressure and used for the treatment of skin diseases, cuts and wounds²⁻⁴. This plant contains main chemical constituents like glycosides, saponins, tannins, flavanoids, terpenoids and alkaloids^{5,6}. The antimicrobial activities against bacteria and fungi confirm the presence of broad spectrum antibiotic compounds in the leaves of this species⁷. The literature quercitol reveals that the *C. Sparsiflorus* Morong leaves are used as antiseptic and antidote also²⁻⁴ and most of the phytoconstitutes were isolated from leaves of *C. Sparsiflorus* Morong. Hence, the leaves of this plant have been used for all pharmacological activities. We found limitations of opioid and NSAID therapy and hence it has become necessary to continuing search for new analgesics and there is no scientific proof in traditional treatment of this plant in some painful and inflammatory conditions. Hence, an endeavor has been made to establish the scientific validity to explore the possible antinociceptive, anti-inflammatory and also behavioral study of the crude

chloroform extract of *C. Sparsiflorus* Morong leaves in animal models.

MATERIALS AND METHODS

Plant Material and Extraction

Croton Sparsiflorus Morong leaves were collected during the month of September from the rural belt of Chapada village, Jagatsinghpur Dist, Orissa India, identified and authenticated by Prof. S. K. Dash, HOD, PG Department of Bioscience, College of Pharmaceutical Sciences, Mohuda; comparing with the voucher specimen (CSML-I) present in the herbarium, has been kept in the laboratory for future references. The collected plants were washed and air-dried under the shade, cut into tiny pieces, powdered by a automatic grinder and passed through 40-mesh sieve and stored in a closed vessel for future use. The dried, powdered leaves of *Croton sparsiflorus* Morong (1 kg) were extracted successively with 1,200 ml of petroleum ether (60 - 80°C) and 1200ml of chloroform in soxhlet apparatus by following standard TAPPI test Method. A dark greenish black colored petroleum ether extract was obtained. The same powdered leaves (marc), after proper drying, were extracted with chloroform (5 hr) to produce a greenish brown semisolid mass. The extractions were carried out until the solvents became colourless. These extracts were again dried and concentrated by evaporating the solvent completely under vacuum at the range of boiling points of solvent (Chloroform at 62°C) using rotatory evaporator (Jain Scientific glass works, DTC 201, Ambala cant, India). The chloroform extract (yield 5.60 % w/w with respected to dry powdered plant material) was selected for all experimental procedure. The chemical constituents of the

extract was identified by qualitative analysis and established by the thin layer chromatography (i.e. hRf values). CSCE (*Croton Sparsiflorus* Chloroform Extract) was prepared an emulsion by triturating the accurately weighed quantity of the extract with 0.025% w/v of carboxyl methyl cellulose (CMC) used for the study. All extractive solvents are of analytical grade reagents (AR).

Preliminary Phytochemical Analysis

The chloroform extract of *C. sparsiflorus* Morong was subjected to preliminary phytochemical screening for detection of major chemical groups. In each case test 10 %w/v solution of the extract in chloroform was used and unless otherwise mentioned in individual test⁸. Results of different chemical tests on the chloroform extract of *C. sparsiflorus* Morong showed the presence of phytoconstituents viz., steroids, terpenoids, flavonoids, saponins and tannins.

Determination of Maximum Absorption of *C. sparsiflorus* Morong L. leaves Extract

To determine the maximum absorption, 100mg of extract was placed in 1000ml flask and 1L of chloroform was added. The flask was agitated to get a concentration of 100µg/ml. The absorption spectrum of the solution of the extract was recorded using a UV spectrophotometer (Model: SL-159-Shimadzu-1700, SI-164 Double Beam) and the wavelength for maximum absorption was determined. Various concentrations of the chloroform extract of *C. sparsiflorus* Morong L. leaves were prepared to contain between 0.01 to 0.1mg/ml of extract in chloroform. The absorbance of each concentration was taken at 271nm and plotted against the various concentrations to obtain the calibration curve for the extract.

Chemicals Used

Chlorocresol, Stearic acid, Sorbitan monostearate (span-60), EDTA (Loba Chemie Pvt. Ltd, Mumbai), Mineral oil, Sodium metabisulfite (Merck specialities Pvt. Ltd, Mumbai), Twin 80(Polysorbates 80) (Acros organics, Belgium). All the chemicals were purchased through the local suppliers.

Animals

Swiss Albino rats of either sex weighing 150-200g obtained from M/s Ghosh & Ghosh Enterprises., Kolkata, India, were housed in standard polypropylene cages at room temperature of 30 ± 2 °C and 60-65% relative humidity and had free access to food and water ad libitum. The animals were fed with a commercial diet (Hindustan Lever Ltd., Bangalore).

Formulations

The dried chloroform extracts of *C. sparsiflorus* Morong was taken for the preparation of creams. Three different formulations were prepared using a cream base according to the formula given in the Table 1^{9,10}. Appropriate standard methods of fusion were adopted, where the solid fats were melted and mixed, and trituration was followed for preparation of the creams^{11,12}. The chloroform extract of *C. sparsiflorus* Morong was incorporated in the bases to get three different concentrations (5%, 7.5% and 10%). All preparations were packed in wide-mouthed plastic jars with screw-capped lid. The following tests were carried out on all the preparations.

Physical Evaluations

Preliminary evaluation of formulations at different concentrations was carried out as follows:

pH

The pH of various formulations was determined by using Digital pH meter (Digital pH meter 335, Systronics, Noroda, Ahmedabad). One gram of cream was dissolved in 100ml of distilled water and stored for two hours^{13,14}. The measurement of pH of each formulation was done in triplicate and average values were depicted in Table 1.

Viscosity

The measurement of viscosity of prepared creams was carried out with Brookfield Viscometer (model LV-DV-II, Helipath-spindle type S-96). The values of each formulation were done in triplicate and average values were depicted in Table 1. The viscosity values are expressed as Mean \pm Standard deviation¹⁵.

Spreadability

Spreadability is a term expressed to denote the extent of area to which the cream readily spreads on application to skin or affected part. A special apparatus has been designed by Multimer¹⁶ to study the spreadability of formulations. The spreadability is expressed in terms of times in seconds taken by two slides to slip off from cream and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, resultant the better spreadability¹⁷. Spreadability was calculated by using the formula. ($S = M.L/T$). Where S= spreadability, M= Weight tied to upper slide, L= Length of glass slides and T= Time taken to separate the slides completely from each other. In this present experiment M= 250gm, L= 3.8 cm and T was recorded in the Table 1.

Acute Skin Irritation Study

The primary skin irritation test was performed on albino rats weighing about 150-200gm. The animals were maintained on standard animal feed and had free access to water *ad libitum*. The animals were kept under standard laboratory condition. The total mass was divided into four batches, each batch containing seven animals. Two batches of each were used for control and test. Dorsal hairs at the back of the rats were clipped off one day prior to the commencement of the study. Animals showing normal skin texture were housed individually in cages with copography meshes to avoid contact with the bedding. 50mg of the each formulation of different concentrations were applied over one square centimeter area of intact and abraded skin to different animals^{18,19}. Aqueous solution of 0.8% formalin was applied as standard irritant. The animals were observed for seven days for any signs of oedema and erythema.

Extrudability

A simple method was adopted for this study. The formulations were filled in the collapsible tubes after the creams were set in the container. The extrudability of the different cream formulations was determined in terms of weight in grams required to extrude a 0.5cm ribbon of cream in 10 second^{15,20}.

Drug Content

Each formulation (1gm) was accurately weighed and transferred to 100ml volumetric flask to which about 70ml of chloroform was added. After shaking, the volume

was made up to 100ml with chloroform. The content was filtered through a suitable filter paper. 1ml filtrate was taken, then diluted and the drug content (extract) was estimated by using UV/Visible spectrophotometer, (SL-159-Shimadzu-1700, SI-164 Double Beam) at 271nm. Results given in Table 1 are the average of triplicate values. Drug contents values are expressed as Mean ± Standard deviation.

Stability Studies

The stability studies were carried out in all formulations at different temperature conditions (4, 25 and 37 °C) for 3 months²¹⁻²³. All the evaluation parameters i.e. pH, viscosity, spreadability, extrudability, drug contents, consistency and phase separation studied at different time intervals i.e. 15th, 30th, 60th and 90th days.

Evaluation of Wound Healing Activity (Excision Method)

The rats were inflicted with excision wounds as described by Morton and Malone²⁴. The rats were anaesthetized with ether solution prior to creation of the wounds. The dorsal thoracic region of the animal was shaved with electric clipper and the area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 500mm² was created

along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open to the atmosphere^{25,26}. The animals were divided into seven groups of six each. The animals of Group I were applied with cream base and considered as the control I, Group II served as reference standard I (Neosporin; Neomycin and Polymyxin B Sulfates and Bacitracin Zinc M/S Glaxo Smith Kline Pharmaceuticals Limited, Mumbai), Group III served as reference standard II (Betadine; Povidone-Iodine IP 5% w/w, M/S Win-Medicare Pvt. Ltd, New Delhi). The animals of Group IV, V and VI were treated with cream 5% (C-I), 7.5% (C-II) and 10% (C-III) respectively. The animals of Group VII were treated with pure extract with 1% caboxy methyl cellulose base (E-I). The cream was topically applied once in a day, starting from the day of the operation, till completion of epithelialisation. The measurement of the wound areas of the excision wound model were taken on 3rd, 6th, 9th, 12th, 15th and 18th days. Thereafter on alternate days until healing were complete; the percentage of wound closure was calculated. All the protocols were reviewed and permitted by the Institutional Animal Ethical committee (CPCSEA approval no. 1018/c/06/CPCSEA) of Royal College of pharmacy and Health Sciences, Berhampur , Orissa .

Table 1 : Physicochemical Evaluations Of Different Formulation Of Creams

Formulations	Ingredients	Concentration (% ,m/m)	Drug content % mean±SD	pH	Viscosity(cps) Mean±SD	Spreadability (sec)
C -I	Extract	5	98.09±0.46	6.60	12.100±0.50	14
	Liquid Paraffin	50				
	White bees wax	15				
	Span 60	5				
	Purified water	30				
C -II	Extract	7.5	98.12±0.30	6.55	12.200±0.100	15
	Liquid Paraffin	50				
	White bees wax	15				
	Span 60	5				
	Purified water	30				
C-III	Extract	10	98.21±0.24	6.41	12.250±0.50	16
	Liquid Paraffin	50				
	White bees wax	15				
	Span 60	5				
	Purified water	30				

Table 2 : Topical Application Of Creams in Rats Prepared From Extracts Of *Croton sparsiflorus* For Wound Healing Activity [% Of Wound Healing = (1-T/C)X100]

Group	Post wounding days						
	0 day	3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day
Control	510.84±0.97* (0.00)	480.44±1.05* (5.95)	397.58±1.36* (22.17)	352.72±0.97* (30.95)	269.22±0.87* (47.30)	187.84±0.65* (63.23)	85.22±0.59* (83.32)
Standard -I	508.78±1.53* (0.00)	406.25±1.19* (20.15)	323.56±1.64* (36.40)	252.13±0.77* (50.44)	135.69±0.54* (73.33)	9.25±0.26* (98.18)	0 (100)
Standard -II	510.56±1.64* (0.00)	415.75±1.41* (18.57)	333.34±1.25* (34.71)	259.83±0.62* (49.11)	144.56±0.58* (71.69)	19.52±0.47* (96.18)	0 (100)
C-I	504.41±1.08* (0.00)	457.69±0.99* (9.26)	369.99±1.08* (26.65)	302.24±0.59* (40.08)	183.48±0.59* (63.62)	81.530.56* (83.84)	18.10±0.42* (96.41)
C-II	506.39±1.08* (0.00)	454.34±0.95* (10.28)	350.22±1.36* (30.84)	290.11±0.62* (42.71)	166.87±0.58* (67.05)	64.67±0.51* (87.23)	7.67±0.30* (98.49)
C-III	508.24±1.65* (0.00)	442.96±1.35* (12.84)	331.79±1.20* (34.72)	269.19±0.80* (47.03)	142.96±0.51* (71.87)	19.93±0.45* (96.08)	0 (100)
E-I	506.27±1.07* (0.00)	454.98±0.87* (10.13)	361.22±0.73* (28.65)	297.67±0.56* (41.20)	180.42±0.43* (64.36)	77.98±0.37* (84.60)	18.06±0.41* (96.43)

Results are expressed mean ± SEM of six readings; Significance evaluated by One-way analysis of variance (ANOVA) followed by Dennett's t-test versus control group, *P<0.001, (n=6). Figures in parentheses indicate the percentage of wound contraction. The cream formulation of different concentrations comparable with the control, standard -I and standard-II.

RESULTS AND DISCUSSION

The measurement formulations showed good homogeneity and extrudability. The pH of all the formulations was in between 6.4 to 6.7, which lie in the normal pH range of the skin. The drug content was in the range of 98.09 - 98.21%. The formulations did not produce any skin irritation, i.e., erythema and edema for about a week when applied over the skin. The rheological behaviors of the cream formulation were studied with Rotational Brookfield Viscometer. The result indicated that as torque increases, shear stress increases and viscosity decreases. A comparative study of viscosity and spreadability showed that as viscosity of the formulation increases, spreadability decreases and vice versa. All the creams showed only slight difference in release profile at the particular time period. From the stability studies cream-5%, 7.5% and 10%w/w showed no changes in pH, viscosity, spreadability, extrudability, drug content, consistency, and phase separation after keeping at different temperatures for 90days. All the formulations and the normal chloroform extracts of *C. sparsiflorus* Morong L. showed significant promotion of wound healing activity with statistically significant (*P<0.001) in all the seven groups of animal which were depicted in the Table 2. The mean percentage closure of wound area was calculated on the 3rd, 6th, 9th, 12th, 15th and finally 18th days. Post wounding days also shown in Table 2. The wound-healing activity was found to be comparable with that of the reference standards and control base. The percentage closure of excision wound area in animals treated with C-I, C-II and C-III were found to be 96.41%, 98.49% and 100% respectively. The animals treated with normal chloroform extract with 1% caboxy methyl cellulose base were found to be 96.43%. The cream formulation containing 10% chloroform extract of *C. sparsiflorus* Morong L. showed significant wound healing activity and comparable with that of the commercial products of Neosporin and Betadine. The mechanical properties of pharmaceutical creams are important tests that often form part of a manufacturer's own specification which are quantifiable by the viscosity, spreadability, extrudability, consistency of the creams. The viscosity provides a measure of cream strength, spreadability expressed to denote the extent of area to which the cream readily spreads on application to skin or affected part, extrudability is a measure of removal of cream from the orifice of the container, consistency is a measure of flow when stress is applied. Creams prepared by using all the excipient on the other hand, showed acceptable viscosity, spreadability and extrudability, values at the concentrations employed, indicating the suitability of method for the production of the creams with the chloroform extract of *Croton sparsiflorus* Morong leaves. Thus, the mechanical properties of the creams were affected by the type and concentration of excipient employed. The results indicate that the creams were generally stable under tropical storage conditions. Thus, the methods of preparation of the creams with the chloroform extract of *Croton sparsiflorus* Morong leaves need to be carefully selected to ensure the production of creams with adequate viscosity, spreadability, extrudability and at the same time release the active

compound(s) for biological action. Furthermore, the type and concentration of excipient employed in the formulations the chloroform extract of *Croton Sparsiflorus* Morong leaves need to be carefully chosen to enable the production of suitable creams.

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