Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Formulation and evaluation of herbal gel of *Pothos scandens* Linn

Mohammed Haneefa KP[1*], Shahima Hanan K[1], Saraswathi R[1], Guru Prasad Mohanta[2], Chandini Nayar[3]

[1]*Alshifa College of Pharmacy, Poonthavanam Post, Kerala, India*
[2]*Department of Pharmacy, Annamalai University, Tamil Nadu, India*
[3]*School of Chemical & Biotechnology, SASTRA University, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 7 September 2010
Received in revised form 27 September 2010
Accepted 15 November 2010
Available online 20 December 2010

Keywords:
Pothos scandens
Burn wound healing
Herbal gel
Carbopol 934
Carbopol 940

ABSTRACT

Objective: To formulate *Pothos scandens* Linn *(P. scandens)* leaf extract in to a gel and investigate their burn wound healing activity. **Methods:** Ethanolic extract of dried leaves of *P. scandens* were subjected to priliminery phytochemical evaluation and wound healing activities studies. Different gel formulations of ethanolic extract of *P. scandens* (4% w/v) were prepared using polymers carbopol 934 and carbopol 940 by varying their concentration. These formulations were evaluated for physical parameters, drug content, pH, viscosity, extrudability, spreadability, primary skin irritation, pharmacological activity and stability. **Results:** Wound healing studies of ethanolic extract revealed that *P. scandens* treated animals were found to epithelise in 22 days while the solvent control and the untreated rats epithelise within 35 and 40 days respectively. The formulation with 1.5% w/w carbopol 934 was found to be more promising as it shows better physicochemical characteristics, higher pharmacological activity and stability compared to other formulations. **Conclusions:** *P. scandens* alcoholic extract shows significant improvement in burn wound contraction and hence this is a promising candidate in burn wound healing.

1. Introduction

Wound healing is the body's natural process of regenerating dermal and epidermal tissue. Since time immemorial man has used various parts of plants in the treatment and prevention of many ailments[1]. Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc. Today a substantial number of drugs are developed from plants[2] which are active against a number of diseases.

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions[3]. Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin[4]

Wound healing process holds several steps which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and acquisition of wound strength[5]. Research on wound healing agents is one of the developing areas in modern biomedical sciences and many traditional practitioners across the world particularly in countries like India and China which have valuable information of many lesser-known hitherto unknown wild plants for treating wounds and burns[6]. Traditional forms of medicine practiced for centuries in Africa and Asia are being scientifically investigated for their potential in the treatment of wounds related disorders[7].

Our particular interest was *P. scandens* (Family-Araceae), traditionally used to treat skin disorders. An ethnobotanical survey was carried out among the ethnic groups (Kani/Kanikaran) in Southern Western Ghats of India described that leaf of this plant mixed with the fruits of *Capsicum annum* and rhizome of *Allium sativum* is applied topically on affected places to heal wounds created during delivery[8]. Srilankan tribal people use leaves of *P. scandens* to reduce swelling speedily in trauma area[9]. GC-MS analysis of ethanolic extract of *P. scandens* revealed the presence of compounds like 2-Hexadecanol, 9,12-Octadecadienoic acid, 9,12,15-Octadecatrienoic acid etc which has antiseptic and anti-inflammatory activity respectively[10]. Although *P. scandens* is known to possess wound healing properties,

*Corresponding author: Mohammed Haneefa KP, K P S Manzil, Near Nila Hospital, Pattambi (PO) Palakkad (Dt) Kerala, India Pin 679303.
Tel:9447350096, 04662212247
E-mail: haneefa001@gmail.com

no systematic studies have been carried up to now on the clinical evaluation of the burn wound healing potential of the *P. scandens* leaf extract. Thereof, its effect was investigated using thermal wound models in rat.

In the present study alcoholic extract of *P. scandens* is incorporated in to carbopol gel base and investigated for burn wound healing activity. The gels are becoming more popular due to ease of application and better percutaneous absorption, than other semisolid preparations. Gels can resist the physiological stress caused by skin flexion, blinking and mucociliary movement, adopting the shape of applied area.

2. Materials and methods

2.1. Materials

Carbopol 934 and carbopol 940 were obtained from Loba Chem. Pvt Ltd, Mumbai. Methyl paraben sodium and propyl paraben sodium were obtained from Hi Media laboratories. Glycerol and triethanolamine were obtained from Nice chemicals Pvt. Ltd, Mumbai.

2.2. Plant materials and extract preparation

P. scandens Linn. (Family: Araceae) were collected from local areas of Perinthalmanna, kerala and was authenticated by Dr. A. K. Pradeep, curator, Department of Botany, University of Calicut and a voucher specimen was deposited at the University of Calicut herbarium(No.113056). The foreign, earthy matter and residual materials were removed carefully from the leaves and then cleaned and dried in the shade. It was then powdered and used for extraction. Powdered herb was placed inside a thimble made from thick filter paper, which was loaded into the main chamber of the soxhlet extractor. The soxhlet extractor was placed onto a flask containing the extraction solvent until it get exhausted. The extract is filtered and concentrated under reduced pressure. It was stored at 4- 8 ℃ until use.

2.3. Animals

A total of 78 12-week old healthy wistar strain rats weighing 150-200 g of either sex, bred locally in the animal house of Alshifa College of Pharmacy, Perinthalmanna, were selected for the wound healing studies and 9 albino rabbits (average wt 2.2 kg) were used for primary skin irritation test. They were housed under controlled conditions of temperature (23±2) ℃, humidity (50±5) ℃ and 10-14 hours of light and dark cycles. The animals were housed individually in polypropylene cages containing sterile paddy husk bedding and free access to food and water ad libitum.

The study was conducted after obtaining the approval from Institutional Animal Ethical Committee.(Reg No: 1195/ac/08/CPCSEA).

2.4. Study design

The animals were randomly allocated in to 3 groups of six animals each for the wound healing studies of the extract and 10 groups of six animals each for the wound healing studies of the formulation as follows:

(1)For wound healing studies of extract: Group I: Solvent control - treated with the solvent alcohol; Group II : Test - treated with 4% alcoholic extract of *P. scandens*; Group III: Untreated control - assigned as negative control.

(2)For wound healing studies of the formulation: Group I: A1 treated with gel formulation of 4% alcoholic extract with 1% carbopol 934; Group II : A2 treated with gel formulation of 4% alcoholic extract with 1.5 % carbopol 934; Group III : A3 treated with gel formulation of 4% alcoholic extract with 2% carbopol 934; Group IV: A4 treated with gel formulation of 4% alcoholic extract with 2.5% carbopol 934; Group V: B1 treated with gel formulation of 4% alcoholic extract with 1% carbopol 940; Group VI: B2 treated with gel formulation of 4% alcoholic extract with 1.5% carbopol 940; Group VII: B3 treated with gel formulation of 4% alcoholic extract with 2% carbopol 940; Group VIII: B4 treated with gel formulation of 4% alcoholic extract with 2.5% carbopol 940; Group IX: Control of Carbopol 934 treated with dummy gel of carbopol 934; Group X : Control of Carbopol 940 treated with dummy gel of carbopol 940.

2.5. Wound healing studies of extract[11]

Rats were anaesthetized with ketamine+xylazine (50 mg/kg+5mg/kg) and the hair on the back was clipped with electric clippers. Burn wounds were created by using a device with an iron piece and a wooden handle placed on the back of the rat. It was heated to red hot over flame and was placed in contact with the back of anaesthetized rat up to 10 seconds without any pressure. After this, each animal was placed in a separate cage for full recovery from anesthesia before being returned to holding rooms. The wound of the test animal was applied with extract in glycerol and the control group I was applied with glycerol as the solvent control and control group 2 were left untreated. The application was repeated daily for the next 20 post operative (1)Epithelization period: It was monitored by noting the number of days required for scar to fall away, leaving no raw wound behind; (2)Wound contraction: To monitor this, progressive changes in wound area were followed planimetrically. Leaving the wounding day, wounds were traced on a transparent paper on an alternate day. The animal was restrained in proper position during tracing. The tracings were then transferred to 1 mm^2 graph sheet. From this, wound areas were read and the percentage of wound contraction was calculated taking the initial size of wound (250 mm^2) as 100%.

$$\%\ \text{Wound contraction} = \frac{\text{Initial wound size} - \text{Final wound size}}{\text{Initial wound size}} \times 100$$

2.6. Formulation of gel

Eight different formulations were prepared using different

concentration of carbopol 934 and carbopol 940. Accurately weighed carbopol was taken in a beaker and dispersed in distilled water with constant stirring using a mechanical stirrer for 30 min at 1 200 rpm. After all the carbopol was dispersed, the extract dissolved in ethanol and the preservatives were added and mixed well. The pH was adjusted to neutral using triethanolamine until a clear consistent gel was obtained.

2.7. Evaluation of the gel

2.7.1. Estimation of drug content[12]

Each formulation (1 g) containing approximately 40 mg of drug was taken in a 50 mL volumetric flask and diluted with ethanol and shaken to dissolve the drug in ethanol. The solution was filtered through whatmann filter paper; 0.1 mL of the filtrate was pipette out and diluted to 10 mL with ethanol. The content of the drug was estimated spectrophotometrically by using standard curve plotted at 270 nm (λ max of extract). The gel formulations were observed for their visual appearance, transparency and homogeneity.

2.7.2. Extrudability[13]

A closed collapsible tube containing about 20 g of gel was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and the gel was extruded until the pressure was dissipated.

2.7.3. pH measurements[14]

pH measurements of the gel were carried out using a digital pH meter by dipping the glass electrode completely in to the gel system to cover the electrode.

2.7.4. Viscosity[15]

Viscosity of the gels was determined using Brookfield viscometer (Spindle type,S-24; model LVDV-E) at 10 rpm. 200 g of the gel was taken in a beaker and the spindle was dipped in it for about 5 minutes and then the reading was taken.

2.7.5. Spreadability[16]

Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slide. 100 g weight was placed upon the upper slides so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 20 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated by three times and the mean time taken for calculation.

Spreadability was calculated by using the following formula:

$$S = m \times l/t$$

Where, S - Spreadability, m - Weight tied to the upper slide (20 g), l - Length of the glass (7.5 cm), t - Time taken in seconds.

2.8. Pharmacological studies of formulation

2.8.1. Primary skin irritation test[16,17]

The study employed nine rabbits (white, average weight of 2.2 kg) to test for the skin irritation. They were kept carefully following an acclimation period of 7 days to ensure their suitability for the study. Test animals were kept within a limited-access rodent facility with environmental conditions set to a temperature of (25 ±2) ℃, a humidity of 60%-90% RH and a 12-hours light / dark cycle. Animals were provided *ad labium* access to a commercial rabbit-diet and drinking water was supplieds to each cage. The area on the back of each rabbits was shaved prior to the experiment. The back was divided into five marked areas for the topical application of the gel containing various concentrations of extract. Test product was placed on each area for 24 hours using adhesive tape. Scoring of the erythema and edema was preformed at 24 and 72 hours with Draize technique[16,17]. The positive control of this experiment was 98% lactic acid.

2.8.2. Wound healing studies of the formulation[18]

Wound healing studies of formulation were prepared similar to wound healing studies of the extract in which the control groups of rats were applied with dummy gel. The application was repeated daily for the next 20 post operative days and the following parameters were studied, epithelization period and wound contraction.

2.9. Stability studies of formulation[13]

Formulated gel preparations were kept at different temperature condition like ambient temperature (R.T), (8± 1) ℃ (refrigerator temperature), (45±2) ℃ at 75%±5% R.H. (condition of accelerated stability testing) for span of three months. The following parameters of the gel such as color, pH, viscosity, spreadability, extrudability and drug content were studied.

2.10. Statistical analysis

Differences between the control and the treatments in these experiments were tested for significance using student *t* test. Data were considered significant if $P < 0.05$.

3. Results

3.1. Wound healing studies of extract

Table 1 shows the effect of *P. scandens* leaf extract

administered topically on wound healing in rats with burn wound. In this model, *P. scandens* treated animals were found to epithelise in 22 days while the solvent control and the untreated rat's epithelise with 35 and 40 days respectively. On the 20th day, the percentage wound area reduction in solvent controls, Untreated rats and the extract treated rats were 64 %, 54% and 95%, respectively and the difference were statistically significant ($P<0.05$).

3.2. Formulation and evaluation of gel

Herbal gels of *P. scandens* Linn were prepared successfully by using different concentration of carbopol 934 and carbopol 940. It was found that all the formulations were green in color and the physical evaluations were given in Table 2. The *P. scandens* alcoholic extract shows λ_{max} at 270 nm. All the formulations were found to be neutral (pH 6.2 to 7.2) and drug content was found to be in the range of 90%-102 % w/w (Table 2). Viscosities of all the formulations are given in Table 3. Spreadability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. The bioavailability efficiency of a gel formulation also depends on its spreading value. Maximum spreading value was found for formulation with 1% carbopol 934. The extrudability reflects the capacity of the gel, to get ejected in uniform and desired quantity when the tube is squeezed. The results of extrudability are shown in Table 2.

3.3. Stability studies

The formulated gels were subjected for stability studies. No color fading was observed for all prepared gels. The pH of all formulations were not affected and found to be within the range of 6.2-7.2. The viscosity and spreadability of all gels was found to be same especially at ambient and 8 ℃ temperature, but at 45 ℃ slight decrease in viscosity was found. The drug content was found to be in the limit 90%-103% for all gel formulation at all temperature conditions.

Table 1
Wound healing effect of *P. scandens* extract in burn wound model.

Treatment (n=6)	% of wound contraction (mean±SD)					Epithelization time (days)
	Day 4	Day 8	Day 12	Day 16	Day 20	
Solvent control	3.10±0.72	24.25±1.24	32.56±2.01	48.10±1.59	64.43±2.70	35.00±1.69
Alcoholic extract	5.25±1.36	28.60±2.13	58.10±3.69[a,b]	87.10±1.88[a,b]	95.50±2.37[a,b]	22.00±2.43[a,b]
Untreated control	2.90±1.21	12.40±0.92	24.20±0.81	34.25±1.29	54.50±2.02	40.00±1.06

[a] $P< 0.05$ *vs*. solvent control, [b] $P <0.05$ *vs* untreated control.

Table 2
Characteristics of *P. scandens* gel formulations.

Sl No.	Formulation	pH	Viscosity(cps)	Spreadability gm cm/sec	Drug content %w/w	Extrudability	Nature of gel
1	A1	6.8	32170	37.50	92.26	+++	Green transparent, homogenous
2	A2	6.9	48240	25.46	101.97	+++	Green transparent, homogenous
3	A3	6.4	53180	22.12	89.47	+++	Green slightly translucent gel, homogenous
4	A4	6.5	64250	18.47	97.86	++	Green translucent gel, homogenous
5	B1	7.2	55380	30.00	102.50	+++	Green transparent , homogenous
6	B2	7.0	65640	24.07	91.61	+++	Green transparent, homogenous
7	B3	6.4	78720	17.79	103.61	++	Green slightly translucent gel, homogenous
8	B4	6.2	81548	16.44	95.55	++	Green translucent gel, homogenous

+++ Excellent, ++ Good.

Table 3
Wound healing effect of formulations in burn wound model.

Formulation	% of wound contraction (mean±SD)					Epithelization time
	Day 4	Day 8	Day 12	Day 16	Day 20	
Control of Carbopol 934	4.95±1.05	11.96±1.68	22.11±2.08	52.20±2.60	65.80±1.28	35.00±1.65
A1	7.81±1.02	22.50±1.80	47.70±1.61	86.10±2.49	92.26±1.30[a]	24.00±1.80[a]
A2	5.88±1.06	30.13±1.56	59.96±1.72	88.03±0.77	96.66±1.51[a]	22.00±1.02[a]
A3	6.91±1.04	24.01±1.36	51.96±1.69	75.93±2.66	90.23±1.43[a]	25.00±1.54[a]
A4	6.00±1.44	26.00±2.17	52.00±3.16	73.00±1.35	88.00±1.54[a]	27.00±1.45[a]
Control of carbopol 940	2.70±1.03	9.05±2.38	23.56±1.65	57.00±2.34	71.83±1.50	33.00±1.98
B1	5.03±0.62	24.10±2.53	52.55±2.45	74.30±2.67	93.53±2.81[b]	24.00±2.13[b]
B2	5.50±1.73	27.16±1.68	45.88±2.02	73.10±1.50	90.41±2.45[b]	25.00±2.06[b]
B3	6.16±1.33	26.26±2.47	45.88±2.02	74.06±1.87	88.63±1.92[b]	28.00±1.54[b]
B4	5.11±0.52	25.03±2.68	42.20±2.27	71.81±1.48	87.95±2.34[b]	27.00±1.80[b]

[a] $P< 0.05$ *vs* control of carbopol 934, [b] $P<0.05$ *vs* control of carbopol 940.

3.4. Pharmacological studies of formulation

3.4.1. Primary skin irritation test

The skin irritation studies revealed that all formulations were non sensitizing and safe for use.

3.4.2. Wound healing activity studies of the formulation

In this model, formulation with carbopol 934 treated animals shows better healing compared to carbopol 940 treated rats. Formulations with 1.5% carbopol 934 treated rats were found to epithelise in 22 days while the control group epithelise with 35 days. On the 20th day, the percentage wound area reduction in control, and the formulation with 1.5% carbopol 934 treated rats were 65.8 and 96.6% respectively and the difference were statistically significant. Results are shown in Table 3.

4. Discussion

Ethanolic extract of *P. scandens* is known to posses antiseptic and anti-inflammatory activity[10] but its wound healing activity were not studied extensively. The major finding of the study is to demonstrate the significance of *P. scandens* alcoholic extract in burn wound healing. The preliminary pharmacological study showed that ethanolic extract of *P. scandens* Linn leaves possess wound healing effect in rats and the effects produced were maximum with 4% alcoholic extract. Eight different gel formulations were prepared using 4% alcoholic extract by varying the concentration of C934 and C940. All the formulations were evaluated for their physicochemical characteristics and pharmacological activity. Formulation A2 (1.5% C934) was found to be optimum in terms of gel consistency, spreadability and extrudability. Pharmacological studies of the formulation revealed that formulation A2 has maximum burn wound healing activity.

From the results it can be concluded that Pothos scandens alcoholic extract when formulated as gel shows significant improvement in burn wound contraction and hence this is a promising candidate in burn wound healing.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Chah KF, Eze CA, Emuelosi CE, Esimone CO. Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. *J Ethnopharmacol* 2006; **104**: 164 –7.

[2] Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environ Health Pers* 2001; **109** (Suppl 1): 69–75.

[3] Senthil Kumar M, Sripriya R, Vijaya Raghavan H, Sehgal P. Wound healing potential of *Cassia fistula* on infected *albino* rat model. *J Surg Res* 2006; **131**: 283–9.

[4] Meenakshi S, Raghavan G, Nath V, Ajay Kumar SR, Shanta M. Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm. et Lind. *J Ethnopharmacol* 2006; **107**: 67–72.

[5] Suresh Reddy J, Rao PR, Reddy MS. Wound healing effects of *Heliotropium indicum, Plumbago zeylanicum* and *Acalypha indica* in rats. *J Ethnopharmacol* 2002;**79**: 249–51.

[6] Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. Ethnopharmacological approaches to wound healing – exploring medicinal plants of India. *J Ethnopharmacol* 2007; **114**: 103–13.

[7] Krishnan P. The scientific study of herbal wound healing therapies: current state of play. *Curr Anaes Crit Care* 2006;**17**: 21–7.

[8] Ayyanar M, Ignacimuthu S. Traditional knowledge of Kani tribals in Kouthalai of Tirunelveli hills, Tamil Nadu, India. *J Ethnopharmacol* 2005;**102**: 246–55.

[9] Ediriweeraa ERHSS, Grerub DD. Traditional medical practices of srilanka in orthopaedic treatment. *AYU* 2009; **30**: 147–52.

[10]Lalitharani S, Mohan VR, Regini GS, Kalidass C. GC-MS analysis of ethanolic extract of Pothos scandens leaf. *J Herbal Med & Toxicol* 2009; **3**(2):159–60.

[11]Shila Gurunga, Natasa Skalko-Basnet. Wound healing properties of *Carica papaya* latex: *in vivo* evaluation in mice burn model. *J Ethnopharmacol* 2009; **121**(2): 338–41.

[12]Tanaji Nandgude, Rahul Thube, Nitin jaiswal, Pradip deshmukh, Vivek chatap, Nitin hire. Formulation and evaluation of pH induced insitu nasal gel of salbutamol sulphate. *Int J Pharma Sci & Nanotechnol* 2008 ; **1** (2): 177–83.

[13]Benoy Brata Bhowmik, Bhabani Shankar Nayak, Arkendu Chatterjee. Formulation development and characterization of metronidazole microencapsulated bioadhesive vaginal gel. *Int J Pharma and Pharma Pract* 2009; **1**(1): 240.

[14]Maria BR Queiroz, Natália B Marcelino, Marcos V Ribeiro, Laila S Espindola, Franciscor Cunha, Monica V da Silva. Development of gel with *Matricaria recutita* L. extract for topic application and evaluation of physical-chemical stability and toxicity. *Lat Am J Pharma* 2009; **28** (4): 574–9.

[15]Panigrahi L, Ghosal SK, Pattnaik S, Maharana L, Barik BB. Effect of permeation enhancers on the release and permeation kinetics of lincomycin hydrochloride gel formulations through mouse skin. *Indian J Pharm Sci* (2006); **68**: 205–11.

[16]Srisombat Nawanopparatsakul, Jeeratikorn Euasathien, Chuwit Eamtawecharum, Porntip Benjasirimingokol, Sakdanai Soiputtan, Photchanart Toprasri, et al. Skin irritation test of curcuminoids facial mask containing chitosan as a binder. *Silpakorn Uni versity J* 2005;**5**(1–2): 140–7.

[17]Kirwin CJ. *Eye and skin local toxicity testing in toxicology: principles and practice*. Sperling F. (ed.) New York: Wiley-Interscience Publication;1984, p. 169–75.

[18]Farnood Shokuhi Sabet Jalali, Hossein Tajik, Shahram Javadi. The efficacy of alcoholic extract of garlic on the healing process of experimental burn wound in the rabbit. *J Anim & Vet Adv* 2009; **8** (4): 655–9.