Research Article

Formulation and Evaluation of Polyherbal Wound Healing Ointment

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ABSTRACT

Aim: The present investigation was aimed to formulate and evaluate the polyherbal wound healing ointment constituting the methanolic extracts of *Lantana camara* (leaves), *Tamarindus indicus* (leaves), *Psidium guajava* (leaves) and acetone extract of *Curcuma longa* (rhizomes). **Materials and Methods:** Two Formulations (F_1, F_2) were formulated by using fusion method using a mixture of PEG 4000 and PEG 600 as ointment base. The physical evaluation of these polyherbal formulations were made in terms of physical and chemical parameters like pH, physical stability, centrifugation, spreadability, viscosity, extrudability. Assessment of wound healing activity was made by using excision wound model in which the evaluation parameters were wound contraction and epithelialization period in excision wound model by using healthy Albino wister rats. In this model, the animals were divided into 4 groups (n = 6) named as control, standard, formulation1 (F1), formulation2 (F2) receiving vehicle (ointment base), soframycin (2%w/w), F_1, F_2 respectively. The measurement of wound areas were taken on 3rd, 6th, 9th, 12th and 15th day following the initial day of wound. The percentage of reduction in wound area, percentage of protection and period of epithelialization was calculated **Statistical analysis:** Data were analysed by using one way ANOVA followed by Dunnett's comparison test. **Results:** The results were found to be significantly positive ($p<0.05$) with the F_1, F_2. More over F_1 shows more significant positive result than F_2 when compare to standard. **Conclusion:** The results of this study reveals the synergetic effect of the phytoconstituents like tannins, saponins, curcumin, alkaloids present in those plants extracts which help to establish these formulations clinically effective in the wound healing management.

Keywords: Excision wound model, formulations, phytoconstituents, polyherbal, soframycin

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INTRODUCTION

Wounds are the unavoidable events of life which are presented as the loss or breaking of cellular, anatomic, functional integrity of living tissue. These are produced when the integrity of tissue is compromised by physical, chemical, thermal, microbial, immunological agents [1]. These have significant impact on humans life since from the prehistoric times, and the treatment and healing of these wounds is considered as an art as old as humanity [2]. Current reports estimated that about 6 million people suffer from chronic wounds which had immense effect in causing great physiological and mental trauma [3].

Wound healing is a complex process that leads to the contraction and closure of wounds which helps in restoration of functional aspects of damaged tissue [4]. According to the wound healing society it has been defined as a "complex dynamic process that results in restoration of anatomic continuity and function" [5]. It is a physiological process which involves cascade of events like phagocytosis, chemotaxis, mitogenisis, angiogenesis, synthesis of collagen, and other matrix components [6]. Wound repair process is divided into four phases, which are: haemostasis, inflammation, proliferative, angiogenisis and tissue remodeling phases which have specific contributions in the process of wound healing [7].

The immediate phase after injury is the haemostasis in which platelets, other factors like Insulin like and epidermal like growth factors (IGF, EGF), fibronectin, fibrinogen, histamine, platelet-derived

growth factor (PDGF), serotonin, and von Willebrand factor (vWF) starts to migrate at the injury site to aggregate within 24 hours to control bleeding and limit the extent of injury. Clinically inflammation, the second stage of wound healing which is symbolized by the influx of polymorphonuclear leukocytes (neutrophils), macrophages, lymphocytes to the site of wound within 24-36 h of injury by the chemo-attractive agents, to phagocytose the foreign material like bacteria, and also to intiate granuloma tissue formation, and angiogenesis. The proliferation phase is characterized by the formation of granuloma tissue and wound contraction which starts approximately fourth day of wound and usually lasts until 21th day. In the granulation tissue formation, fibroblasts, the predominant cell type, starts to proliferate to produce matrix components and also responsible for wound contraction. Angiogenesis or Neovascularisation intends to promote the nutrient supply to maintain cell metabolism and create intact delivery system. Remodeling, the final stage of wound repair process where granulation tissue is converted into mature connective tissue. During this phase there is decline in the cellularity and vascularity of the wound while the extracellular matrix is remodeled by cross linking of collagen. Finally these phases are eventualised in a fully matured scar with decreased number of cells and blood vessels.

Physiologically wound healing process is a natural process of healing on its own, but there is a need to provide better remedies that can regenerate the damaged tissue. It was estimated that more than 50% of clinically used drugs are of natural origin which shows the importance of phototherapy [8]. It is generally acceptable that herbal products are gaining importance in management of wound healing because of its high acceptability and good toleration [9]. These are not only cheap and affordable but also devoid of hypersensitive reactions which are rarely encountered with these agents.

As the rhizomes of *Curcuma longa* (family: Zingiberaceae) possess anti-inflammatory [10] and anti-microbial properties [11], leaves of *Tamarindus indica* (family: Fabaceae- Caesalpinioideae) possess anti-fungal, anti-inflammatory [12], leaves of *Lantana camara* (family: Verbenaceae) leaves possess wound healing activity [13], leaves of *Psidium guajava* (family: Myrtaceae) possess wound healing activity [14], the present investigation was aimed to formulate and evaluate the synergetic potential of polyherbal wound healing ointment constituting the methanolic extracts of *Lantana camara* (leaves), *Tamarindus indicus* (leaves), *Psidium guajava* (leaves) and acetone extract of *Curcuma longa* (rhizomes) as they possess the wound healing as well as antibacterial and anti-inflammatory properties individually.

MATERIALS AND METHODS

1. PLANT MATERIAL COLLECTION AND IDENTIFICATION:

The rhizomes of *Curcuma longa,* leaves of *Tamarindus indica*, *Lantana camara, Psidium guajava* were collected from surroundings of S.V. University, Tirupati, situated in Chittoor district of Andhra Pradesh in the month of December, 2011 and they were identified and authentified by the taxonomist, Dr. P. Jayaraman, Director of Plant Anatomy Research Centre (PARC), Chennai with Rg. no of authentification certificates: PARC/2012/1130, 1124, 1127, 1120 respectively. The authentified plant portions were cut and deposited in a polythene bag. Formalin (2%) was poured into this bag to keep the specimen in fresh condition. These specimens were deposited at the herbarium of the Department of Pharmacognosy, Sree Vidyanikethan College of Pharmacy, Tirupathi.

2. EXTRACTION:

These plant materials were shade dried, powdered and made to pass through sieve no.40. Then the powdered rhizomes of *Curcuma longa* with the solvent acetone, leaves of *Tamarindus indica*, *Lantana camara, Psidium guajava* made to undergo soxhelation with the methanol for 72 hours individually. These extracts were filtered and concentrated to dryness under reduced pressure by using rotary evaporator at a temperature not exceeding 40^0c.

3. PRELIMINARY PHYTOCHEMICAL ANALYSIS:

The crude extracts were screened for the presence of phytochemicals like alkaloids, glycosides, carbohydrates, sterols, phenolic compounds and tannins, flavonoids, saponins, proteins, and amino acids by using the standard procedures [15].

4. FORMULATION:

Formulations (F1, F2) of these ointments were prepared by fusion method by using a mixture of PEG 4000 and PEG 600 in the ratio of 3:7 as ointment base [16]. The prepared formulations F1, F2 were composed of extracts in the following manner as specified in (**Table 1, 2**).

Table 1: Formulation 1 (F1)

S. No	Ingredients	Quantity (100g)
1.	*Lantana camara* (extract)	2 % w/w
2.	*Curcuma longa* (extract)	2 % w/w
3.	*Tamarindus indica* (extract)	1 % w/w
4.	*Psidium guajava* (extract)	1 % w/w
5.	Methyl paraben	0.01% w/w
6.	Ointment base	95% w/w

Table 2: Formulation 2 (F2)

S. No	Ingredients	Quantity (100g)
1.	*Lantana camara* (extract)	2 % w/w
2.	*Curcuma longa* (extract)	1 % w/w
3.	*Tamarindus indica* (extract)	1 % w/w
4.	*Psidium guajava* (extract)	1 % w/w
5.	Methyl paraben	0.01% w/w
6.	Ointment base	95% w/w

4. EVALUATION OF FORMULATIONS (F1, F2)

4.1 Physical evalution

The physical evaluation of these polyherbal formulations were made in terms of physical and chemical parameters like pH, physical stability, centrifugation, spreadability, viscosity, extrudability which are likely to affect the stability and acceptability of the formulations.

4.1.1 pH: The pH of the prepared polyherbal formulations (F1,F2) were determined by using digital pH meter.

4.1.2 Physical stability: Ointment formulations were evaluated in terms of physical changes like phase separation and changes in color, odour, consistency etc. of the formulations which effects their stability and other desired formulation properties. Samples of the oformulations were kept at different temperature conditions like 50^0c, 40^0c, 37^0c temperature for a period of 45 days. They were periodically observed for physical changes like phase separation and development of objectionable color and odour [17].

4.1.3 Centrifugation: It is believed to be an unique tool for the evaluation of accelerated deterioration of ointments. It was determined by using Remi centrifuge in 10 ml-graduated cylinder at 10,000 rpm for 10 min [17].

4.1.4 Viscosity: By using Brookfield viscometer (Model RVTDV II) at 100 rpm using spindle no. 6, the viscosity of the prepared formulations (F1, F2) was assessed.

4.1.5 Spreadability: Assessment of the spreadability of the prepared formulations were determined individually by measuring the spreading diameter of 1gm of ointment between two glass plates (20cm × 20cm) by having a standard weight of 125gm on the upper plate.

4.1.6 Extrudability: The formulations were filled in the standard collapsible alluminium tubes which were sealed at the end. The weight of each tube was determined and recorded. Then the tubes were placed in between two glass slides which were clamped by having standard weight of 0.5 kg over the glass plates. Then the cap made to remove and weigh the extruded ointment

from the tube. The percentage of extruded ointment was calculated.

4.2 Pharmacological evaluation

4.2.1 Animals: Healthy wister rats of either sex weighing between 150-220gm were procured from Sri venkateswara enterprises, Banglore. The rats were housed as per CPCSEA guidelines in the animal house of Sree Vidyanikethan College of pharmacy; Tirupati and they were acclimatized to the laboratory conditions seven days before the study. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) with the approval no SVCP/IAEC/I-001/2011-12.

4.2.2 Skin irritation studies: By using healthy wister rats of either sex, skin irritation studies of the prepared formulations were made to evaluate. The animals were treated daily individually with the formulations upto seven days and finally the treated skin was evaluated visually for erythema and edema.

4.2.3 Effect on excision wound model: The animals were anaesthetized prior to the creation of the wounds with anaesthetic ether. By using electric clipper, the dorsal fur of the animals was shaved at the wound creating area. An outline was created on the shaved area prior to the wound creation with marker. A full thickness of the excision wound of 2.5 cm in width (circular area = 5.0 cm^2) and 0.2 cm depth was created along the markings. Then the animals were divided into four groups of 6 animals in each group. In the following way the experimental protocol was executed [18].

Group 1: Control group receiving PEG ointment base for 15 days

Group 2: Standard group receiving marketed Soframycin ointment (2% w/w) for 15 days

Group 3: Test group receiving F1 for 15 days

Group 4: Test group receiving F2 for 15 days.

The parameters studied were reduction in wound area and epithelialization time. From the initial day of wound, the measurement of wound area was made on 3rd, 6th, 9th, 12th and 15thday using transparent paper, permanent marker and graph paper. The epithelialization time was calculated as the number of days required for falling off the dead tissue remnants without any residual raw wound. The percentage of protection was calculated by using the following formula [19].

$$\text{Percentage of protection} = \frac{Initial - Final}{Initial} \times 100$$

The results were then analyzed by using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test with equal sample size. The difference was considered as significant when *P*-values < 0.05. All the values were expressed as mean±SEM.

RESULTS

The preliminary phytochemical analysis of the crude extracts reveals the presence of carbohydrates, triterpenoids, volatile oils in the acetone extract of *C. longa* rhizomes; alkaloids, glycosides, carbohydrates, saponins, tannins, flavonoids in the methanolic extract of *L. camara* leaves; alkaloids, saponins, tannins, flavonoids in the methanolic extract of *T. indica* leaves; glycosides, carbohydrates, triterpenoids, tannins, flavonoids in the methanolic extract of *P. guajava* leaves.

Formulations complied with the physical evaluation parameters like pH, physical stability, centrifugation, viscosity, spreadability, extrudability were found to be acceptable which were notified in (**Table 3**).

Table 3: Results of physical evaluation parameters of the formulations (F1, F2)

Formulations	pH	Physical stability	Centrifugation	Viscosity	Spreadability	Extrudability
F1	6.9	Acceptable	No phase separation	4900 cps	65 mm	68 % (fair)
F2	7.0	Acceptable	No phase separation	4800 cps	60 mm	65 % (fair)

As the skin irritation studies on the animals didn't show any significant effects like erythema, edema, itching, etc., it was stated to be safer in clinical practice. By using parameters like reduction in wound area, percentage of protection, period of epithelialization, assessment of wound healing activity was made to exploit in the present study. The results were found to be positive in the present study with the formulations (F1, F2) which was shown in table no.4. It was found that all the four groups showed decreasing of wound area from day to day. However on 15th day, the Group – I showed 64.84% (Table 4) protection which may be due to self-immunity of animals whereas the Group – II (i.e., standard) showed 91.13% (Table 4) protection. On the other hand Group – III showed 93.03% (Table 4) protection, which is significant to that of standard (Soframycin 2% w/w) indicating significant wound healing activity ($P<0.05$), whereas Group – IV (i.e., formulation 2) exhibited appreciable wound healing activity ($P<0.05$) of 87% (Table 4) protection as compared to control and the results were even comparable with that of standard. In the other way the period of epithelialization was found to be 23.33, 17.67, 17.17, 19.00 days ($P < 0.05$) for control, standard, F1, F2 respectively which was shown in (**Table 4**).

Table 4: Effect of polyherbal formulation on wound area and percentage of protection in excision wound model

	Area of wound closure (sq mm ± S.E.M)					
Groups	**3rd day**	**6th day**	**9th day**	**12th day**	**15th day**	**Epithelization period (Days)**
I Control	445.2±1.447 (10.96%)	389.2±2.386 (22.16%)	355.2±1.447 (32.96%)	294.7±1.667 (41.06%)	175.8±2.386 (64.84%)	23.33±0.2108
II Standard	345.2±1.833[a] (30.96%)	253.8±1.537[a] (49.24%)	197.3±0.955[a] (60.54%)	95.83±1.537[a] (80.83%)	44.33±1.563[a] (91.13%)	17.67±0.2108[a]
III Formulation 1	320.8±2.386[a] (35.84%)	241.3±1.022[a] (51.74%)	182.8±1.014[a] (63.44%)	82.33±1.054[a] (83.53%)	34.67±1.667[a] (93.06%)	17.17±0.1667[a]
IV Formulation 2	375.8±1.537[a] (24.84%)	263.3±1.308[a] (47.34%)	209.7±2.261[a] (58.06%)	110.0±1.826[a] (78%)	65.00±1.826[a] (87%)	19.00±0.2582[a]

Values are mean±S.E.M. of six readings each.
[a] Comparing treatment group with control at $P < 0.05$ as compared to control

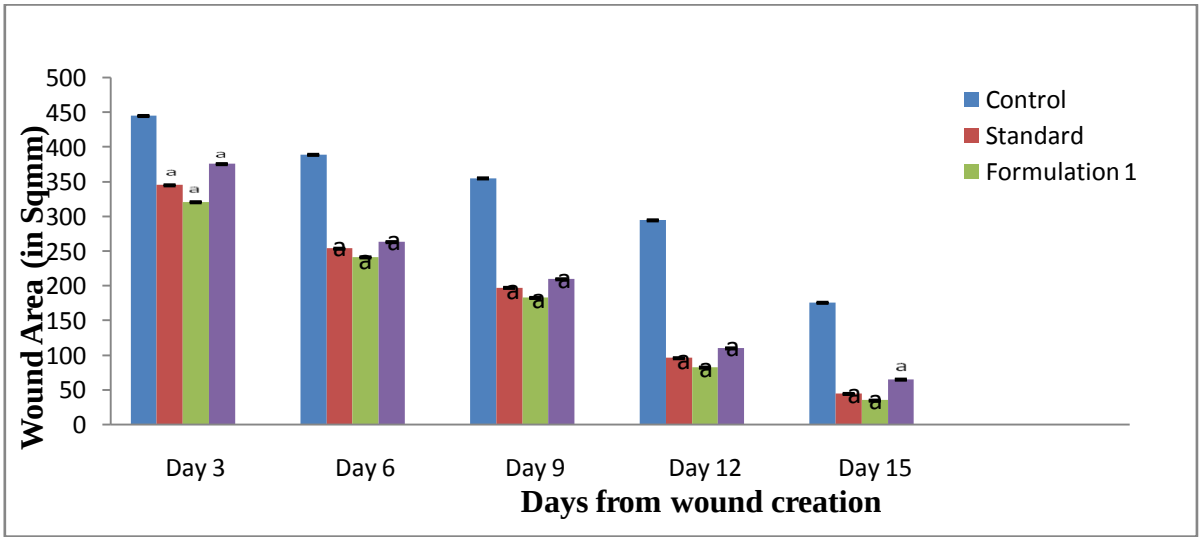

Figure 1: Effect of polyherbal formulations on Excision wound area in rats
[a] Comparing treatment group with control at $P < 0.05$ as compared to control

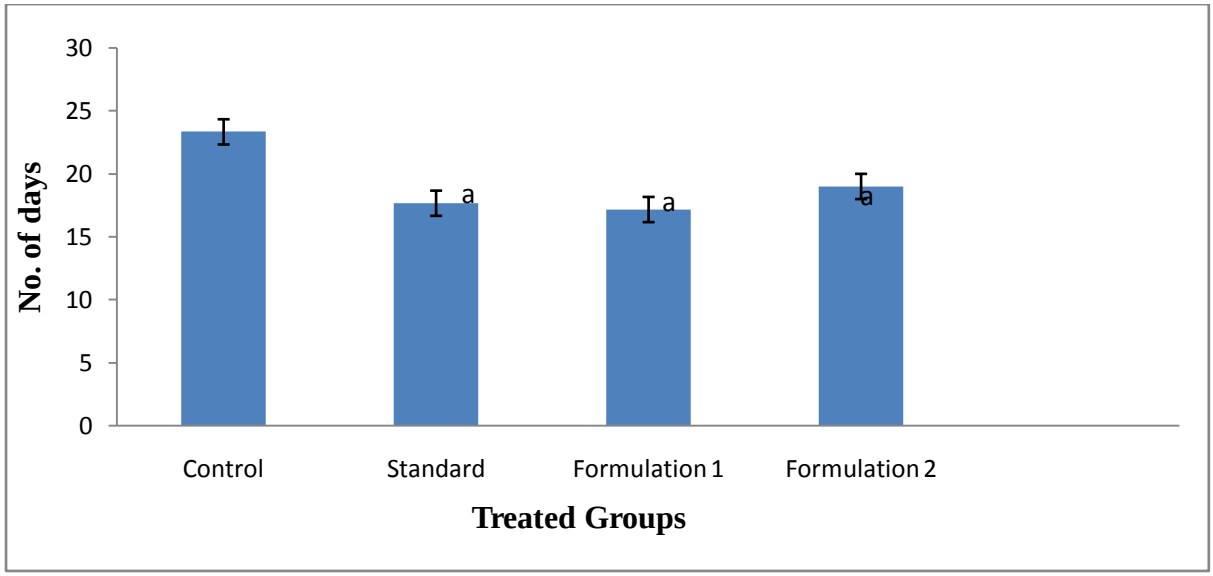

Figure 2: Effect of polyherbal formulations on epithelialization period in excision wound model

[a] Comparing treatment group with control at $P < 0.05$ as compared to control.

DISCUSSION

Wound healing is a very complex, multifactor sequence of events involving several cellular and biochemical processes which helps in restoration of functional and anatomical continuity [20]. Physiologically this process advanced in sequential of events like coagulation, inflammation, proliferation and remodeling [21]. Any agent which accelerates the above processes is a promoter of wound healing. Even though great advancements in the chemical drug industry, the availability of substances that are capable of stimulating the wound repair process is still limited [22]. In addition to this the clinical management of chronic wounds is limited due to increase in cost of therapy and presence of side effects [23]. In generally plants are reported to possess the wound healing activity by means of experimentally using different animal wound models which reveals the presence of most promising active components to promote the wound healing process [24]. In generally polyherbal ointments are used in folk medicine which have been reported to be beneficial in wound care, with promising wound healing devoid of pain, discomfort and scarring [25, 26]. Moreover it is an obvious choice of dosage form due to convenience of topical application.

Results in the present study revealed that the formulations were efficient in terms of acceptability of ointment by abiding the physical standards and skin irritation studies. Assessment of wound healing activity of the formulations was made by using parameters like wound contraction and epithelialization period. By the results of the present study, F1 and F2 have achieved the objective of study (Table 4). More over to this F1 showed more significant results which are comparable to that of standard formulation. Phytochemical investigation of used plants extracts revealed the presence of alkaloids, tannins, flavonoids, saponins and triterpenoids which were the important phytoconstituents responsible for wound healing activity in generally. In the wound healing management the flavonoids were believed to be one of the important components which are present in the extracts responsible for the free radical scavenging activity [27]. In epithelization of wound and chemotaxis in fibrosis alkaloids are known to play a pivotal role [28]. By modifying the balance of protease/protease inhibitor secretion in human endothelial vascular cells, Saponins promotes angiogenesis [29, 30]. By precipitating proteins to form vascular plugs tannins are believed to have haemostatic activity, arresting bleeding from damaged or injured

vessels [31]. Curcumin is a well-known antimicrobial agent which might have protected the wound from infections. Possible combination of these phyto-chemicals may explain the reduction in wound area and epithelialization period of the formulations (F1, F2) in excision wound model. But the results had shown that F1 was more significant than F2 due to the variation in composition of plant extracts in those formulations. In F1, the composition was in the ratio of 2:2:1:1 of *Lantana camara : Curcuma longa : Tamarindus indica : Psidium guajava*, while in F2 was in the ratio of 2:1:1:1 respectively which revealed that the presence of more amount of cucumin in the extract of *Curcuma longa* enhances wound healing process due to its anti-microbial and its anti-inflammatory properties. The wound healing activity to the formulations may be attributed due to the synergetic effect of phytoconstituents like alkaloids, saponins, tannins, flavonoids, curcumin, triterpinoids present in the used plant extracts.

CONCLUSION

By abiding the physical standards and wound healing properties, formulations (F1, F2) were considered to be effective in management of wound healing activity which was even comparable to that of standard soframycin ointment (2% w/w). But further studies are needed with the purified constituents in order to establish them in clinical setting.

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