



EVALUATING WOUND HEALING ACTIVITY OF POYHERBAL OINTMENTS

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ABSTRACT

Natural products have been an important resource for the maintenance of life since ages. Natural products are becoming increasingly important as alternative medicines. Side effects associated with allopathic drugs and synthetic compounds have prompted research into herbal and natural products. In India there has been interest in the potential of natural products obtained from plants and animals for development of drugs with wound healing properties as taught in a popular form of Indian medicine known as Ayurveda. Along with other dosage forms herbal drugs are also formulated in the form of ointment. The present work aims to formulate polyherbal ointment and evaluate whether combination of plant extracts produces any synergistic effect on wound healing. Two different formulations were made with *Lantana camara*, *Curcuma longa*, *Tamarindus indica* and *Psidium guajava*. Both formulations showed significant effect which was comparable with standard. Standard Soframycin 2% showed 91.13% protection. Among the two prepared formulations formulation 1 exhibited 93.03% protection which is significant to that of standard ($p < 0.05$). This may be due to the synergistic effect of phytochemicals like alkaloids and saponins present in the plant extracts.

Keywords: *Lantana camara*, *Curcuma longa*, *Tamarindus indica*, *Psidium guajava*,
alkaloids and saponins

INTRODUCTION

Medicinal plants are resource for healing in local communities and presently as a source of new drugs. It plays a major role in the

development of health care system. Many drugs commonly used today are of herbal origin. Medicinal plants are important for

pharmacological research and drug development. Phytoconstituents are used directly as therapeutic agents and also as starting materials for the synthesis of drug [1-2]. In this study a comprehensive work on the wound healing activity of prepared polyherbal ointment is under taken. Different plants like *Curcuma longa*, *Tamarindusindica*, *Lantana camara* and *Psidiumguajava* are used in the present study to make wound healing ointments. *Curcuma longa* is very important medicinal plant used in traditional systems as well as in modern medicine which belongs to Zingiberaceae family. It is commonly known as Turmeric. Traditionally it is used against biliary disorders, anorexia, cough, diabetic wound, hepatic disorders, rheumatic disorders, sprains and swelling caused by injury and sinusitis. Externally the dried rhizome has been applied to fresh wounds. It is also applied topically for ulcers, wounds, eczema and inflammation. The main constituent curcumin is reported to possess antibacterial activity which is very helpful for wound healing [3]. *Tamarindus indica* commonly called as Tamarind belongs to Fabaceae family. It is reported to possess flavonoids, tannins, saponins, alkaloid and sesquiterpenes. Medicinally it is used as antimicrobial, anthelmintic, antiulcer, antiseptic, antiviral, astringent, for asthma, bacterial infections, boils, diabetes etc. [4-5]. *Lantana camara*

is a shrub belongs to Verbenaceae family. Traditionally it is used as tonic, in abdominal pains, as anthelmintic and insecticide. In Asian countries leaves are used to treat cuts, rheumatism and ulcers. It contains various groups of chemical components such as terpenes, flavonoids, essential oil and iridoid glycosides [6]. *Psidium guajava* is commonly called as Guava belongs to the family Myrtaceae. Fruit of guava is rich in vitamin C and Vitamin A. Leaves of this plant contain tannins, resins, volatile oil etc. Various parts and extracts of plant are reported to possess antibacterial, antidiarrheal, anti-inflammatory and antispasmodic activities. It is also used to treat cough, diabetes and kidney problems [7-8].

MATERIALS AND METHODS

Collection of plant material

The plant material was collected from the surroundings of S. V University, Tirupathi, situated in Chittoor district of Andhra Pradesh. The whole plant portions were cut and deposited in a polythene bag. Formalin (2%) was poured into this bag to keep the specimen in fresh condition. The field data of plant like height, flower colour and soil condition were noted.

Identification and authentication

Plant material was identified and deposited at the herbarium of the Department of Pharmacognosy, Sree Vidyanikethan College of pharmacy, Tirupathi and

authenticated by Dr. Jayaraman, Taxonomist and Research co-ordinator, PARC, Chennai. Taxonomic identification was carried out following Henry et al (1983).

Preparation of plant extracts

The plant materials were collected, washed and dried at room temperature. After complete drying it was powdered and passed through a 60 mesh sieve and stored in air tight container. Dried powdered drug was used to prepare extract. A weighed quantity of air dried powdered drug of *Lantana camera*, *Tamarindus indica* and

Psidium guajava were taken and extracted with methanol in Soxhlet apparatus by continuous hot extraction for 72 hours. Acetone was used for the extraction of *Curcuma longa*. The extracts were evaporated to dryness in a rotary flash evaporator at a temperature not exceeding 60°C. Phytochemical tests were carried out following Kokate (1999) and Harbone (1998) [9-10].

Preparation of polyherbal wound healing ointment

Formulation 1

S. No.	Ingredients	Quantity (100g)
1	<i>Lantana camera</i>	2%
2	<i>Curcuma longa</i>	1%
3	<i>Tamarindus indica</i>	1%
4	<i>Psidium guajava</i>	1%
5	Methyl paraben	0.01%
6	Ointment base	95%

Formulation 2

S. No.	Ingredients	Quantity (100g)
1	<i>Lantana camera</i>	2%
2	<i>Curcuma longa</i>	2%
3	<i>Psidium guajava</i>	1%
4	Methyl paraben	0.01%
5	Ointment base	95%

Preparation of ointment base

Polyethylene Glycol (PEG) was used for the preparation of ointments base. A mixture of PEG 4000 and PEG 600 in the ratio of 3:7 was used in the formulation as it has sufficient consistency [11].

Preparation of polyherbal ointment

Two formulations were prepared by fusion method. Specified quantities of the above mentioned extracts were incorporated into the ointment base and it is triturated well to obtain uniform distribution of extracts. The

prepared formulations were then evaluated by various parameters.

Evaluation of polyherbal ointment

Stability of formulation

Stability of prepared formulations was evaluated in terms of the change in physical and chemical parameters, which were likely to affect the acceptability of the formulation [12].

Physical stability

Physical changes like phase separation, changes in colour, odour, and consistency

were evaluated which will affect their stability and other desired properties of formulation. Formulations were kept at different temperature conditions like 40°C, 37°C and room temperature for 45 days. They were periodically observed for physical changes like phase separation and development of objectionable colour and odour [12].

Centrifugation

Prepared ointments were centrifuged at 10000 rpm for 10 min in 10ml graduated cylinder using Remi centrifuge. It is an excellent tool for the evaluation of accelerated deterioration of ointments [12].

Spreadability and Homogeneity

Formulated ointments were applied on the skin and tested for the spreadability. The formulated ointment showed good spreading ability. Homogeneity was tested by visual inspection.

Pharmacological studies

Healthy Wistar albino rats of either sex weighing between 160g and 200g were used in the experiments and were procured from animal house of Sree Vidyanikethan College of Pharmacy. The rats were housed in polypropylene cages on normal food and water ad libitum. Animals were periodically weighed before and after experiments. The rats anaesthetized prior to infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ether

anesthesia. Animals were closely observed for any infection; those which show signs of infection were separated and excluded from the study. The study was approved by the Institutional Animal Ethics Committee (SVCPIAEC/I-001/2011-12).

Wound healing activity

Excision wound model

The rats were inflicted with excision wounds as described by Shivananda Nayak *et al.* Rats were anaesthetized prior to creation of the wound, with anesthetic ether. The dorsal fur of the animal was shaved with an electric clipper and the area of the wound to be created was outlined on the back of the animal with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 2.5 cm in width (circular area=5.0 cm²) and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open. Animals were categorized into 4 different groups with 6 animals each. Group 1 is considered as control which received PEG ointment base. Group 2 was served as reference standard and treated with Soframycin 2% w/w. Group 3 and 4 were the test groups which received Formulation 1 and formulation 2 respectively for 15 days. The parameters studied were wound contraction and epithelialization time. The measurements of the wound areas of the excision wound

model were taken on 3rd, 6th, 9th, 12th and 15th day following the initial wound using transparent paper and permanent marker. The recorded wound areas were measured with graph paper. The period of epithelialization was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound [13].

The percentage protection was calculated on the 15th day by using the following formula and tabulated in **Table 2**.

Percentage protection= 100-(Final X100)/ Initial
Statistical analysis

The data was statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple comparison test with equal sample size. The difference was considered significant when P-values <0.05. All the values were expressed as mean \pm SEM.

RESULTS

All the prepared extracts were analyzed for the phytoconstituents and results are presented in the **Table 1**.

Two polyherbal wound healing ointments were made and found to be stable after physical stability testing. Both the formulations were stable at various temperatures. There was no phase separation, colour change, odour or change in consistency. The spreadability of the formulation was also excellent when

compared to marketed formulation. No lumps were noticed while testing.

Effect of polyherbal ointment on Excision wound model in rats

Wound contraction and epithelialization time was considered for the evaluation of wound healing capacity of prepared ointments. Wound contraction is a factor which indicates rate of reduction of unhealed area during the course of treatment. Results showed the reduction of wound area of different groups over the period of 15 days. The fastest healing of wound took place in case of animals which received formulation 1 and complete healing was obtained within 17 days. The least rate of wound healing was seen in control group. It was further found that all four groups showed a decrease in wound area from day to day. However on 15th day the group I showed 64.84% (**Table 2**) protection which may be due to self-immunity of animals whereas the group II (standard) showed 91.13% (**Table 2**) protection. Group III showed 93.03% (**Table 2**) protection which is significant to that of standard Soframycin 2% indicating significant wound healing activity (P<0.05), whereas group IV exhibited appreciable wound healing activity (P<0.05) of 87% (**Table 2**) protection as compared to control and the results were comparable with standard. Among two ointments formulation 1 showed better results which was very well comparable with standard.

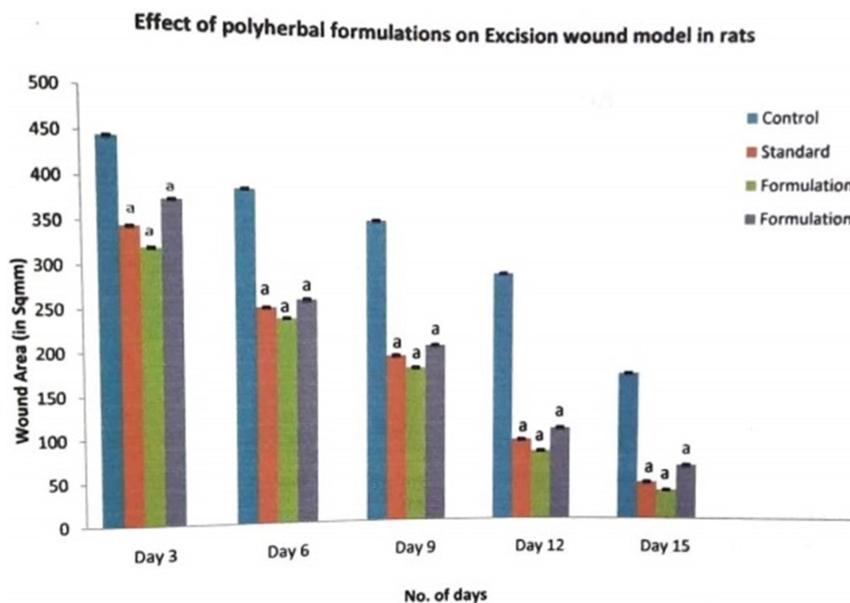
Table 1: Table showing results of phytochemical analysis

S. No.	Phytoconstituents	<i>C.longa</i> (Acetone)	<i>L.camara</i> (Methanol)	<i>T.indica</i> (Methanol)	<i>P.guajava</i> (Methanol)
1	Alkaloids	-	+	+	-
2	Glycosides	-	+	-	+
3	Carbohydrates	+	+	-	+
4	Triterpenoids	+	-	-	+
5	Fats	-	-	-	-
6	Saponins	-	+	+	-
7	Tannins	-	+	+	+
8	Proteins	-	-	-	-
9	Gums & Mucilage	-	-	-	-
10	Flavonoids	-	+	+	+
11	Volatile oils	+	-	-	-

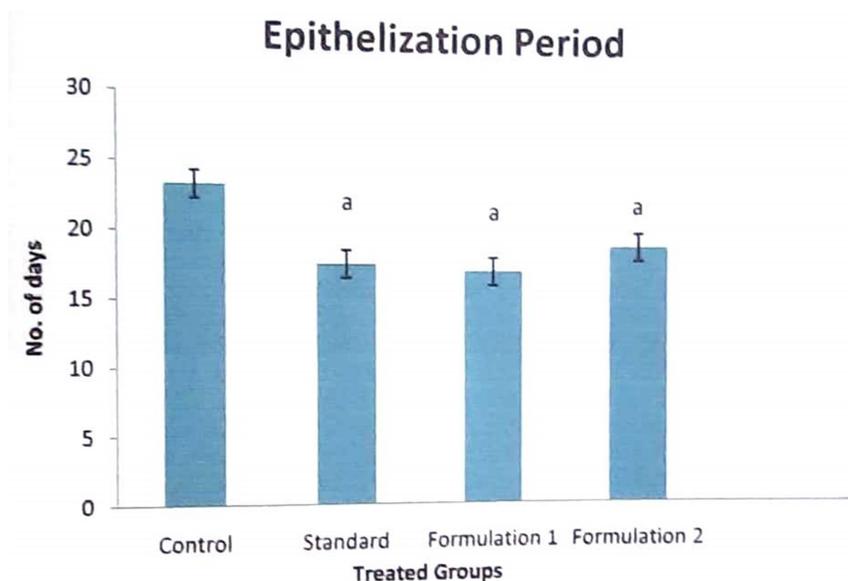
Table 2: Effect of topical application of polyherbal formulation on excision wound model

Groups	Area of wound closure (Sq. mm ± SEM)					Epithelization period (Days)
	3 rd day	6 th day	9 th day	12 th day	15 th day	
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 (47.37%)	209.7±2.261 ^a (58.06%)	110.0±1.826 ^a (78%)	65.00±1.826 ^a (87%)	19.00±0.2582 ^a

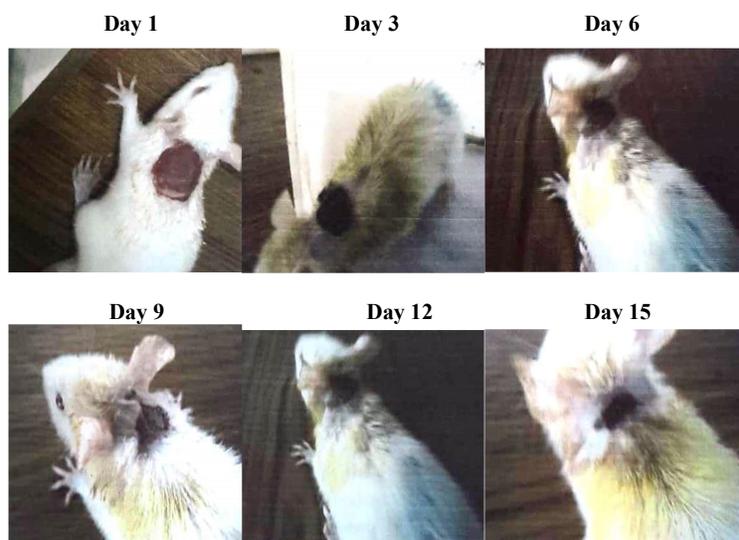
Values are mean±SEM of six readings each. ^acomparing treatment group with control at P<0.05 as compared to control. Figure in % indicate percent wound contraction. Initial wound area approx.500 Sq.mm



Graph 1: Effect of polyherbal formulation on Excision wound model in rats



Graph 2: Effect of polyherbal formulation on epithelization in experimental rats



Images showing wound contraction in different days

DISCUSSION

Phytochemical investigation of plants extract revealed the presence of alkaloids, tannins, flavonoids, saponins and triterpenoids which are reported to possess the wound healing activity. Prepared formulations were stable and no patches on rat skin were observed during skin irritant

test. No mortality was noticed amongst the animals in all the treated groups. Study on animal models showed enhanced rate of wound contraction and drastic reduction in healing time which might be due to enhanced epithelisation properties of phytoconstituents present in the extracts. The flavonoids which are present in the extracts

responsible for the free radical scavenging activity were believed to be one of the important components in wound healing. Alkaloid is known to assist in epithelization of wound and chemotaxis in fibrosis [11, 14]. Saponin on the other hand stimulates angiogenesis by modifying the balance of protease/protease inhibitor secretion in human endothelial vascular cells [15-16]. The presence of tannins could be associated with faster wound closure due to its antimicrobial property [17]. Curcumin is a well-known antimicrobial agent which might have protected the wound from infections. Possible combinations of these chemicals may explain the fluctuation of wound reduction. **Formulation 1** showed significant wound healing activity than the **Formulation 2**. This may be due to the synergistic effect of phytoconstituents like alkaloids and saponins which is present in the *Tamarindus indica* extract.

CONCLUSION

The process of wound healing has two components, first is formation of new tissue and other is protection from microbial invasion during the healing process. With this aim in mind two formulations were attempted in which different plant extracts were combined in single formulation. PEG was used as ointment base in both the formulations. Methyl paraben served as preservative in the formulation. Stability of the formulation was evaluated with

physical stability parameters. There was no evidence of phase separation, development of objectionable odour or any other evidence of physical instability and effect on storage at varying temperature. Spreadability of test formulations F-1 and F-2 were compared to that of marketed formulation. Storage even at accelerated stability conditions does not influence the stability of formulation. Thus it may be concluded that formulations are adequate and satisfactory as far as physical parameters are concerned. In-vivo study gave solid evidence for the synergistic activity of herbs and its utilization in making formulation.

Conflict of interest statement

We declare that we have no conflict of interest.

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