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Original Research Article

Potential of Polyherbal Formulation in Burn Wound Model

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Abstract

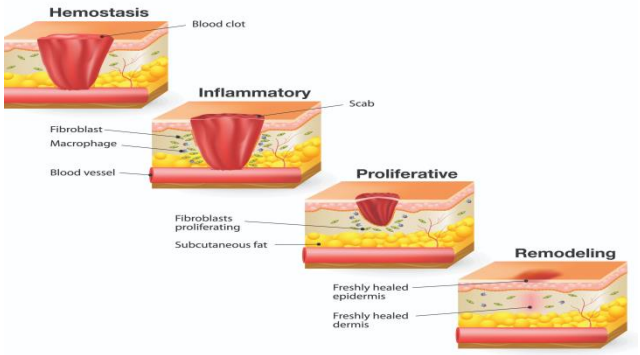
Polyherbal formula has been used everywhere in the global because of its medicinal and healing application. It has additionally referred to as polyherbal therapy or herb-herb combination. In an age of integrated healthcare where Ayush therapies are being accepted along side the fashionable medicine, we see that these are separate compartments in theory and practice. Wound healing begins at the time of injury and can last for a variety of times depending on the severity of the wound. The wound healing process can be divided into three stages: the inflammatory phase, the proliferative phase, and finally the remodelling phase, which determines the strength and appearance of the healed tissues. In the present work an attempt had been made to evaluate wound healing potential of Polyherbal formulation contains *Punica granatum* and *Coleus aromaticus* extract in burn wound model.

Keywords: Wound healing**,** Polyherbal formulation, *P.granatum* & *C.aromaticus*.

INTRODUCTION

Herbal medicines are extremely important in health-care systems, particularly in developing nations. In ancient Indian literature, medicinal plants are defined as possible sources of therapeutic ingredients in a very broad logic [1]. The number of patients seeking alternative and herbal treatments is rapidly increasing. For hundreds of years, herbal medicines have been the synthesis of therapeutic experiences of generations of practising physicians of indigenous systems of medicine. Herbal medications are in high demand for basic health care in the poor countries, not only because they are less expensive, but also because they are more culturally acceptable, have greater compatibility with the human body, and have less adverse effects [2]. Wound healing begins at the time of injury and can last for a variety of times depending on the severity of the wound. The wound healing process can be divided into three stages: the inflammatory phase, the proliferative phase, and finally the remodeling phase, which determines the strength and appearance of the healed tissues [3].

Phase of wound healing [4]



Alternative reasserts of drug treatments like natural drug treatments may also quickly come to be key additives within side the healthcare provision enterprise for each people and animals mainly in growing countries. These natural drug treatments will fill an opening resulting from a lower with inside the quantity of latest cutting-edge drug treatments being evolved within side the previous few decades (mainly with the case with anti-infective), growing costs, drug resistance, and aspect outcomes of cutting-edge pharmaceuticals [5]. Most herbs or components thereof incorporate Phytoconstituent which have huge ranging medicinal value like antimicrobial, anti-inflammatory, antioxidant, antipruritic, hypotensive, proliferative, hypoglycemic, and analgesic which are regularly key role in wound control or healing[6]. In the present work an attempt had been made to evaluate wound healing potential of Polyherbal formulation contains *Punica granatum* and *Coleus aromaticus* extract in burn wound model.

Punica granatum	***Coleus aromaticus***
	
Vernacular name: Eng: Pomegranate Hindi: Anar**,** Sanskrit: Dadima **Part used :** Peel (Rind)	**Vernacular name:** Eng: Country borage, Indian borage **Hindi:** Patta ajavayin, Patharcur Sanskrit:Karpuravalli, sugandhavalakam **Habitat:** Throughout India, cultivated in gardens **Part used:** Leaves
Chemical Constituients Punicalagin (high mol. Wt. Polyphenolic compound), vitamin C, Anthocyanins, Sterol, oleanolic, ursolic and gallic acid. It also contains ellagitannins, elagic acid, gallagic acid and punicalins.	**Chemical constituent** Leaves contains Carvacrol and Camphor is nearly equal amount. The aerial part yield an essential oil (0.1%) which contain thymol the chief constituent (79.6%),limonene (10.58%) Thymol(8.12%), Eugenol (7.35%), ethyl salicylate (5.50%), and also contain oxalacetic acid.
Uses A decoction of seed is used to treat syphilis. Juice used to treat jaundice and Diarrhea, Juice of flower is used to treat nosebleeds. The fruit pulp and the seed are stomachic traditionally it is used in itching, pyorrhea, gum and teeth disorder. Steam and root bark is an effective anthelmintic and teaniacide. Seeds & pulp possess antibacterial activity. The food rind powder has appreciable immuno-stimulatory activity and hepato protective activitity. It is also used as astringent [7].	**Therapectic uses** The leaves are given to children in Colic and also are prescribed in vaginal discharge urinary disease. A leaf infusion decoction or syrup is pronounced to be beneficial in case of epilepsy, convulsion, Asthma, bronchitis, persistent coughs, sore throats and congestive coronary heart failure. The leaves are bitter, aromatic, anodyne, digestive carminative, stomachic, anathematic, deodorant diuretic and liner tonic [8].

MATERIAL AND METHODS

SELECTION OF PLANTS

The plants were selected on the basis of their anti inflammatory activities, chemical constituent and wide medicinal uses in the traditional literatures. The ease of availability of plant is also taken into consideration during selection.

Rind (Peel) of Punica granatum (Anar)
Leaves of Coleus aromaticus (Patta Ajavayin)

Collection and Authentication

The plant work collected in the month of September – October 2021 locally from Bhopal (M.P.). and authenticated.

Extraction

Coleus aromaticus

Approximately 200g of powder crude drug were separately extracted with Hydroalcoholic solvent [Ethanol: water (70:30)] by double maceration and

recovered the solvent by distillation and combined extract of each drug were concentrated under reduced pressure and air dried.

Punica granatum: The powder crude drug were separately extracted with methanol by soxhelation method.

FORMULATION PREPARATION

After preparation of extract and phytochemical studies, the next step was to formulate a polyherbal preparation. An ointment with water soluble base was of first choice due to their ease of preparation and also eases of cleaning after application [9].

Three formulations were prepared by Fusion Method
1-***Punica granatum*** *extract* (10% w/w)
- **TEST I**
2- ***Coleus aromaticus*** *extract* (10% w/w)
- **TEST II**
3- Polyherbal (*Punica granatum : Coleus aromaticus* 5:5%) - **TEST III**

OINTMENT BASE – Hydrophilic ointment USP
Methyl paraben - 0.25 g
Propyl paraben - 0.15 g
Sodum lauryl sulphate - 10 g
Propylene glycol - 120 g
Stearyl alchol - 250g
White petrolatum - 250g
Purified water - 370 g to make about 1000g.

Procedure

Melted the stearyl alchol & white petrolatum on a steam bath ,& warmed to abut 75^{0c} .Added the other ingredients with drug extracts, previously dissolved in the water & warmed to 75^{0} C & stir the mixture until it congeals .

Evaluation of prepared formulation

Ointment should be stable and uniform so that will make it easy to use under different conditions and will also be stable for a sufficient period of time. Thus, the prepared formulations were subjected to various tests to determine the following parameters.

- Physical appearance
- Stability
- pH
- Irritant effect

Physical appearance:-

- Hydrophilic USP ointment base - White to cream
- **Test-I** formulation - Reddish brown
- **Test-II** formulation - Green brown
- **Test-III** formulation - Greenish brown

Physical stability

The prepared formulations were stored at room temperature and at elevated temperatures 40 ± 2^{0}C for 30 days. After two weeks interval, the formulation was tested for its physical characteristics like color and odor.

pH

The pH of the formulation was determined using pH-meter. The pH of the all formulations was found between 6.5 to 7.5.

Irritant effect

Healthy albino rats, weighing 150-200gm were selected for the study. Rats were caged individually. 24 hours prior to the test, rats depilated by removing hairs at the dorsal thoracic region. The test sites were cleaned with surgical spirit. Measured quantity of the formulation was applied over the respective test sites. The tested animals were observed for any irritation for 48 hr after application.

Controlled partial skin thickness burn model

Partial-thickness burn wound (2 by 2.5 cm) was created on a shaved area of the animals dorsal skin, modifying a method described by Smahel.8 Briefly, the animals were anaesthetized and shaved. A steel plate 2 mm thick was heated in boiling water for 7 min and applied firmly to the skin for 12 sec. After creating the wound the animals were housed individually and wound contraction was measured on 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th}, 12^{th}, 14^{th} days [10].

Wound Contraction

Studied by tracing the raw wound area on transparent polythene paper on each alternate days. Later the area assessed using graph paper. The wound contraction was measured as the percentage decrease of original wound size $300mm^2$ for each animal group.

RESULT AND DISCUSSION

In this work Polyherbal formulation containing *C.aromaticus* leave extract and *P.granatum* rind extract was taken in consideration to evaluate wound healing potential with reference to burn wound model. The hydrophilic ointment USP base was taken as drug delivery system and prepared formulation was evaluated for their stability. The result was tabulated in table.1. . The pH of the all formulations was found between 6.5 to 7.5. The irritant effect was tested in healthily albino rats and no sign of irritation were found. The % wound contractions of *C.aromaticus*, *P.granatum* and polyherbal were found to be 78.4%, 86% and 87.4% respectively at 14^{th} day. Studies proven that selected individual plants contained rich quantity of phenolics and flavonoids and their polyherbal combination. In Burn wound model wound contraction was measured in 2^{nd}, 4^{th}, 6^{th}, 8^{th} , 10^{th} , 12^{th}, 14^{th} days and it may be seen that the fastest healing of wound

took place in case of animals treated with Polyherbal extract than the control.

Table 1: Stability Parameters of Formulation

S. No	Parameters	Temperature			
		At Room		At 40°C	
		15 days	30 days	15 days	30 days
1.	Colour	No Change	No Change	No Change	No Change
2.	Odour	No Change	No Change	No Change	No Change

Table 2: Mean Percentage closure of Burn wound area by Topical route on following post wounding days

Post wounding days	Wounding Area (mm^2)			
	Control	*Punica granatum*	*Coleus aromaticus*	Polyherbal
0	181.40±2.22	185.67±0.42	186.50±0.67	188.50±0.67
2	170.91±1.2 (5.78%)	174.71 ± 0.39 (5.9%)	175.68± 0.63 (5.8 %)	177.15 ± 0.64 (6 %)
4	156.00 ± 0.63 (33.74%)	158.19 ± 0.35 (14.8%)	162.08± 2.79 (14.7 %)	157.95± 0.55 (16.2 %)
6	139.67 ± 0.94 (39.16%)	141.66 ± 0.32 (23.7%)	144.72± 0.51 (22.4 %)	144.48± 0.48 (23.5 %)
8	99.68 ± 0.93 (45.05%)	96.54 ± 0.21 (48%)	91.38± 0.32 (51 %)	98± 0.34 (48 %)
10	81.40 ± 2.22 (55.13%)	81.69 ± 0.18 (56%)	69.38± 0.25 (62.8 %)	81.05± 0.28 (57 %)
12	39.75 ± 1.33 (78.09%)	38.8 ± 0.88 (79.1%)	55.76 ± 0.20 (70.1 %)	37.7 ± 0.13 (80 %)
14	25.44 ± 1.01 (85.98%)	25.99 ± 0.059 (86%)	40.28 ± 0.14 (78.4 %)	23.75 ± 0.84 (87.4 %)

Value are expressed as the Mean ± SEM, n = 6 in each group P< 0.001 significance Vs control

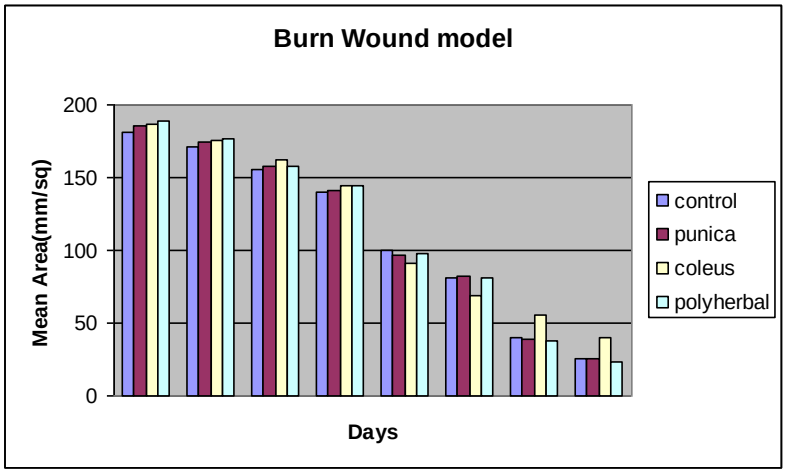


Fig 1: % wound contraction

CONCLUSION

On the basis of the results obtained in the present investigation, it is possible to conclude that the ointment of the *C.aromaticus*, *P.granatum* and polyherbal extract has significant wound healing potential by synergestic effect of phenolic and flavonoids present in the extract. The above findings justify the wound healing properties of these plants.

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