



## EVALUATION OF POLYHERBAL FORMULATION FOR WOUND HEALING ACTIVITY USING EXCISION MODEL

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### ABSTRACT

Wound can be described as a breach in the normal tissue continuum, which leads to mechanical separation of functional structures such as blood vessels, inflammation and at a later stage, infection. Wound healing is a complex multifactorial process that results in the contraction and closure of the wound and restoration of a functional barrier. Proper healing of wound is essential for the restoration of disrupted functional status of skin. It is a product of the integrated response of several cell types to injury. In the present study, polyherbal formulation of Hydroalcoholic extracts of the leaves of *Azadirachta*

*indica*, *Cassia fistula*, *Eucalyptus globulus* and bulbs of *Allium sativum* was investigated for their wound healing activity. The wound healing property appears to be due to the presence of active principles, which accelerates the healing process and confers breaking strength to the healed wound. Further, wound healing activity by polyherbal formulation may be attributed to the synergistic action of the active phyto-constituents like like Flavonoid, Tannis and Steroids.

**Keywords:** Wound healing, *Azadirachta indica*, *Cassia fistula*, *Eucalyptus globulus*, *Allium sativum*, Polyherbal formulation.

### INTRODUCTION

Wounds may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissue<sup>1</sup>. Wound healing is a process that is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and other extracellular macromolecules that are later remodeled to form

scar<sup>2,3</sup>. Wound healing studies are mainly aim to detect various means and factor influencing healing process, so they could be either used or avoid in clinical practice to favorably alter the healing process.<sup>4</sup> The human adult wound process can be divided into 3 distinct phases: the inflammatory phase, the proliferative phase and the remodeling phase. Within these 3 broad phases is a complex and coordinated series of events that includes chemo taxis, phagocytosis, neocollagenesis, collagen degradation, and collagen remodeling. In addition, angiogenesis, epithalization, and the production of new glycosaminoglycans (GAGs) and proteoglycans are vital to the wound-healing milieu. The culmination of these biological processes results in the replacement of normal skin fibroblastic mediated scar tissue.<sup>5</sup> Although many indigenous tribes around the world have long suspected that this ubiquitous, annual, herbaceous plant might have medicinal wound healing properties, it has not really got the attention of orthodox medical practitioners as a potential source of a healing agent which may prove to be useful in the treatment of wounds.<sup>6</sup>

## MATERIALS AND METHODS

**Plant material & Preparation of extract:** To study phytochemical constituents and physical parameters selected plant parts were collected from various sources e.g. *Azadirachta indica*, *Cassia fistula* leaves, *Eucalyptus globules leaves* & *Bulbs of Allium sativum* from, Bhopal, during the month of Sep-Oct and were authenticated by the department of Pharmacy, SIRT-P. Bhopal. Herbarium no. SIRTPH-no.1A (*Azadirachta indica*, SIRTPH no.2B(*Cassia fistula*), SIRTPH no.3C(*Eucalyptus globulus*), SIRTPH no.4D(*Allium sativum*).

### Extraction

Preparation of extract of various plant parts mentioned was done by maceration, using hydroalcoholic solvents (1:1). Extracts after filtration were concentrated at low pressure by distillation and finally air-dried.

### Preparation of and Application of test Sample

After preparation of extract the next step was to formulate a polyherbal preparation. Due to sticky nature of extracts, it was difficult to formulate solid and liquid dosage form and thus a semi- solid dosage form was preferred. An ointment with water soluble base was of first choice due to their ease of preparation and also eases of cleaning after application.<sup>7,8</sup>

Two formulations were prepared by Fusion Method e.g. one containing *A. indica* leaf extract (10% w/w), (Treated as main or TEST-I) and the other one containing all four extracts *A.*

*indica* leaf extract *C. fistula* leaf extract, *Eucalyptus globulus* leaf extract, *Allium sativum* Bulbs extract in Hydrophilic ointment USP base plants parts in equal ratios i.e. containing 2.5% w/w of each extract, equal to total 10% w/w in Hydrophilic ointment USP base (Treated as polyherbal or TEST-II).

Hydrophilic ointment USP contains methyl paraben 0.25 g, propyl paraben 0.15 g, sodium lauryl sulphate 10 g, propylene glycol 120 g, stearyl alcohol 250g, white petrolatum 250g, purified water 370 g to make about 1000g.

**Procedure:** Stearyl alcohol & white petrolatum were melted on a steam bath, & heated to about 75°C. The other ingredients with drug extracts, previously dissolved in the water were added & warmed to 75°C. The mixture was stirred until it congealed.

The extracts were incorporated into the Hydrophilic ointment USP base in concentration of 10% w/w. To perform the experiment, the albino rats were divided into four groups for topical dose consisting of six animal each. Group-I served as Control which received Hydrophilic ointment USP by topical route, once daily, GroupII served as STANDARD to which Soframycin (Framycetin sulphate I.P., 1% w/w) was applied topically once daily, Group-III served as Test-I, animals were treated with formulation containing the *Azadirchta indica* leaf extract (10% w/w) by topical route, once daily and Group IV served as TEST-II(Poly) receiving 10%(2.5% each drug extract w/w) w/w topically once daily. In Excision wound model topical route is given.

#### Wound Healing Activity Animals

Albino rats of either sex weighing 150-200 gm were fed on standard diet and water ad libitum. The animals were housed at room temperature on normal day-night cycle. All animals were adjusted in the environment before 10 days starting the experiment. Animal ethical clearance was obtained from Ethics Committee (1429/PO/a/11/CPCSEA) of Sagar Institute of Research & Technology-Pharmacy, Bhopal, India

**Excision Wound Model:-** For the excision wound study, each group containing six animals (150-200gm) were selected circular wound of about 2.5cm (500mm<sup>2</sup>) diameter were made on depilated dorsal thoracic region of rats under light ether anaesthesia in semi-aseptic condition and observed throughout the study. The animals were housed individually. The extracts (10%) were formulated as an ointment in emulsifying base and ointment was applied on the