



## Development and screening of topical herbal cream formulations for wound healing activity

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### ABSTRACT

The aim of the present work is to develop and standardize the topical herbal cream formulations using well documented herbs. Topical herbal cream formulations were prepared by using ethanol extract of *Tridax procumbens* and petroleum ether extract of *Cassia occidentalis* and mixed extracts of both the plants. The above plants are traditionally used for healing of cuts and wounds. Crude raw materials of *Tridax procumbens* (leaf) and of *Cassia occidentalis* (leaf) were collected, shade dried, grinded separately and the moisture free materials were sieved through 100 mesh. The physiochemical constants like ash values and extractive values were carried out for both the crude drugs. The aqueous extracts were prepared by maceration method and the extracts were formulated into a topical cream formulations individually and combination of both. The prepared cream formulations were screened for their wound healing activity by excision. The wound healing activity was carried out for the formulations using betadine 5% w/w cream as standard drug. The present study was observed that the prepared topical herbal cream formulations showed significant wound healing activity.

**Keywords:** *Tridax procumbens*, *Cassia occidentalis*, Wound healing; Topical cream formulation.

### INTRODUCTION

Ayurvedic medicine is a time-tested system of medicine which has been in clinical use for centuries in India. Being a time-tested system, it has an edge over other existing systems of health management. When two or more herbs are used in formulations, they are known as polyherbal formulations. Ayurveda and herbal medicine has

roots in medicinal herbs and they have been practiced for centuries. Herbal medicine is making dramatic comeback and increasing number of patients are visiting alternative medicine clinics. Side effects of synthetic medicine are alarming and recent time has seen risk of herbal and herbal-synthetic drug interactions.

In India, from ancient times, different parts of medicinal plants have been used to cure specific

ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants. To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore. The herbal drugs are boon to our society. These herbal drugs are considered as a therapeutic weapon to fight against various diseases in birds humans and animals, without having any side effects Under the prevailing circumstances further investigations into the concept of polyherbal formulations should be undertaken .so in the present work, we formulated a polyherbal ointment with better wound healing activity. In recent years, there has been a great demand for plant derived products in developed countries. The literatures have reported that the usage of the traditional medicines brought a great benefit in skin related diseases. Hence the plant entities derived from the natural source need to be identified and formulated in to suitable dosage form for the management and treatment of wound.

#### Preparation of cream: vanishing cream base Composition of cream base 100g.

S.no.	Ingredients	Quantity (g)
1	Stearic acid	18
2	Isopropyl myristate	3 ml
3	Triethanolamine	0.7 ml
4	Glycerin	5-6 ml
5	Water up to	100 ml
6	Methyl paraben	0.15
7	propel paraben	0.15

#### Procedure

Required quantities of stearic acid and isopropyl myristate were taken in a beaker heated to 60-70° C (oily phase). Triethanolamine, glycerine and distilled water were taken in another beaker and heated to 60-70°c (aqueous phase). Both phases were cooled to 40°c and the oily phase was added to the aqueous phase with continuous

## MATERIALS AND METHODS

The leaves of *Tridax procumbens* and *Cassia occidentalis* were collected from pakal region in Warangal district of Telangana state, India.

### Chemicals and Reagents

Betadine powder (Win-Medicare Pvt Ltd), white petroleum jelly and white bees wax (LobaChemie)

### Extraction

The collected plants (*Tridax procumbens* and *Cassia occidentalis*) were extracted by continuous hot percolation (Soxhletation). 50g of powdered leaves of the plants were defatted using petroleum ether and ethanol. The marc obtained from each of the powdered plant parts were successfully extracted separately with 250 ml of ethanol by using sox let apparatus. The extraction was carried out for 24 hours. After extraction, the solvents were distilled out; the concentrated residues were analyzed by chemical tests <sup>1,2</sup>

### Phytochemical analysis <sup>3,4</sup>

The methanolic extract obtained after soxhletation was subjected to various photochemical screening as per the standard procedure to reveals the presence of various active phytoconstituents.<sup>5</sup>

stirring. Finally the preservatives were added, volume make up to 100 ml with distilled water with continuous stirring.

### Evaluation of the Polyherbal Formulation

The polyherbal formulation was evaluated by the following physicochemical parameters

### Colour and odour

Color and odor was examined by visual examination.

### Loss on drying

Loss on drying was determined by placing the cream in a petridish on a water bath and dried until constant weight was obtained.

### pH

The pH of the formulation was recorded using a digital pH meter. Weighed quantity of the sample was dissolved in distilled water and stored for two hours. The measurement of pH was done in triplicate and average values were considered.

### Spreadability

The spreadability was expressed in terms of time in seconds taken by two slides to slip off from cream placed in between the slides under the direction of certain load. Spreadability was calculated by using the formula.

$$S = (M.L/T)$$

Where, S = Spreadability, M = Weight tied to upper slide, L = Length of glass slides and

T = Time taken to separate the slides

### Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of known concentration. It was poured into a petridish and allowed to set. A hole was bored at the centre of the petridish and the prepared formulation was placed in it. The time taken for the cream to get diffused was noted.

### Stability studies

The stability studies were carried out for the prepared Polyherbal formulation at different temperature conditions (4°C, 27° C and 37°C) for 3 months.

### Development of topical herbal formulation

In the present investigation, petroleum ether extract of *Cassia occidentalis* and ethanolic extract of *Tridax procumbens* were used for the development of topical formulations. Accelerated stability studies of formulation were performed for nature, color, odour, texture, pH, phase separation, consistency and spreadability and trace of gritty particles.

Formulation I- ethanolic extract of *Tridax procumbens*

Formulation II-petroleum ether extract of *Cassia occidentalis*

Formulation III-Standard

Formulation IV-Herbal drug combination

## Results and Discussion

### STUDY DESIGN:

#### Excision method:

Screening of wound healing activity was performed by excision wound model. Adult albino rats of either sex weighing between 130-180 g were used in this study. Animals are divided into 15 groups, each containing 18 animals. They are depilated at the desired site and wound was performed light ether anaesthesia. A circular wound of approximately 2.5 cm diameter was impressed on the skin from the demarked area. The skin was excised to get a wound measuring approximately 500 mm<sup>2</sup>. After achieving full haemostasis by blotting the wound with cotton swabs soaked in saline, the animals were placed in their individual cages.

The animals were treated daily as follows, from 1-16<sup>th</sup> post- wound day.

Group I- treated with control (cream)

Group-II- treated with standard (betadine powder)

Group III- treated with 5% cream formulation of petroleum ether extract of *Cassiaoccidentalis*.

Group IV- treated with 5% cream formulation of ethanolic extract of *Tridax procumbens*.

Group V- treated with 5% cream formulation (herbal drug combination) of petroleum ether extract of *Cassiaoccidentalis* and ethanolic extract of *Tridax procumbens*.

The wound contraction rate was monitored by planimetric measurement of wound area of each animal on 1<sup>st</sup>, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, and 16<sup>th</sup> post wounding day. This was achieving by tracing the wound area on a graph paper. Reduction in wound area was expressed as a percentage of the original wound size. The results were expressed by calculating the mean and standard error using student's *t* test.

### Physical characteristics of extracts by soxhletion

Crude drugs	Extract	Natur e	Col our	Odour	Taste
<i>Cassia occidentalis</i>	Petroleum ether	Semi solid	Bla ck	Characteri stic	Mucilagenous,bi tter
	Ethyl	Semi	Bla	Characteri	Mucilagenous,B

	acetate	solid	ck	stic	itter
	Chloroform	Solid	Black	Characteristic	Mucilaginous, Bitter
	Ethanol	Solid	Brown	Characteristic	Mucilaginous, Bitter
	Aqueous	Solid	Brown	Characteristic	Mucilaginous, Bitter
<i>Tridax procumbens</i>	Petroleum ether	Solid	Green	Pungent	Bitter
	Ethyl acetate	Semi solid	Black	Pungent	Bitter
	Chloroform	Semi solid	Black	Pungent	Bitter
	Ethanol	Semi solid	Black	Pungent	Bitter
	Aqueous	Semi solid	Black	Pungent	Bitter

### Physicochemical parameters<sup>7</sup>

Parameter	cream formulation			
	Formulation I-ethanolic extract of <i>Tridax procumbens</i>	Formulation II-petroleum ether extract of <i>Cassia occidentalis</i>	Formulation III-Standard	Formulation IV Herbal drug combination
Nature	Semi solid	Semi solid	Semi solid	Semi solid
Color	Green	Black	white	black
Odour	Characteristic	Pungent	Characteristic	Characteristic
pH	6.32	6.02	6.32	6.75
Phase separation	No	No	No	No
Spreadability(dynes/cm <sup>2</sup> )	176	176	178	184
Trace of gritty particles	No	No	No	No

### Viscosity

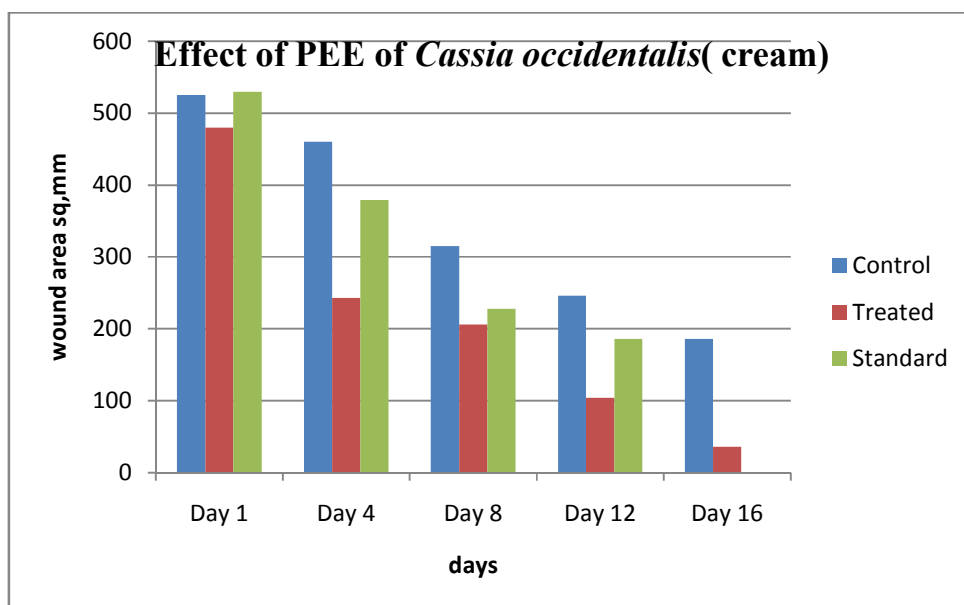
Crude drug	Dosage form	10 rpm	20 rpm	50 rpm	100 rpm
<i>Tridax procumbens</i>	cream	230	211	79	52
<i>Cassia occidentalis</i>	cream	370	308	180	72
Standard	cream	335	190	146	75

### Effect of cream formulation of 5% (w/w) petroleum ether extract of *Cassia occidentalis* on percentage reduction in wound size.

cream formulation		Day 1	Day 4	Day 8	Day 12	Day 16
	Control	525 ± 22.4	460 ± 10.6	315 ± 36.5	246 ± 15.4	186 ± 0.75

Treat ed	480 ± 0.9	243± 33.1	206 ± 3.7	104 ± 0.21	36 ± 1.86
Stan dard	530± 11.6	379± 22.8	228 ± 36.1	186 ± 8.2	0 ± 0.0

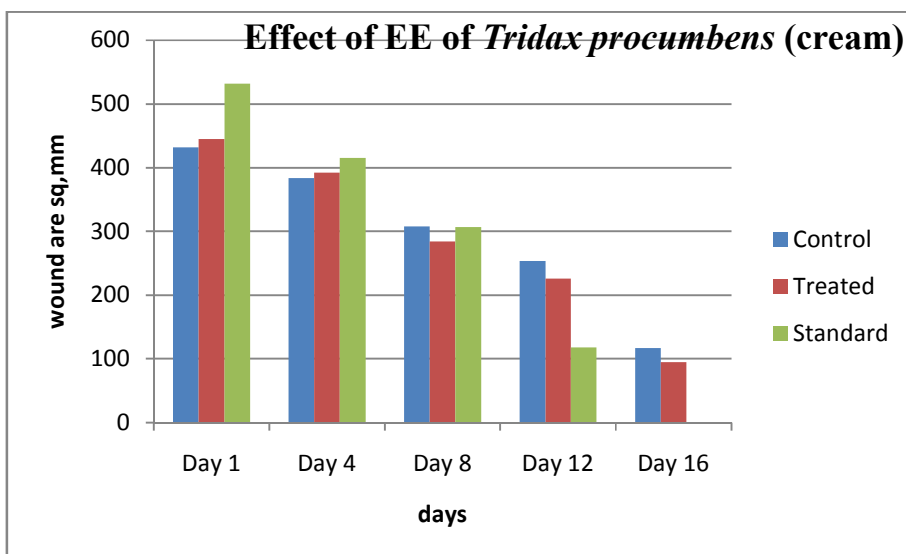
(Values are mean + SD from 6 readings each).



**Effect of cream formulation of 5% (w/w) ethanol extract of *Tridax procumbens* on percentage reduction in wound size.**

cream formulation	Day 1	Day 4	Day 8	Day 12	Day 16
Contr ol	432 ±26.6	384± 13.8	308 ± 10.8	254 ± 10.10	117 ± 09.80
Treat ed	445±20.10	392 ±19.7	284 ± 6.2	226 ± 14.00	95 ±11.60
Stand ard	532± 31.6	415 ± 16.2	307± 2.8	118± 02.80	00 ± 0.0

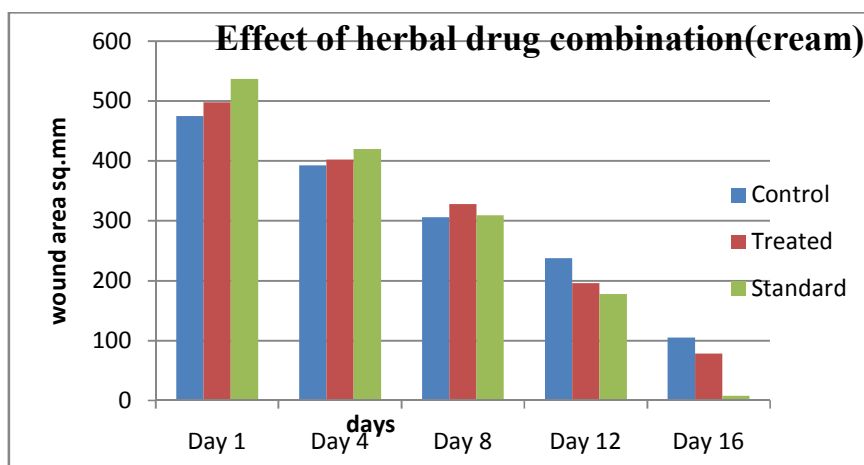
(Values are mean + SD from 6 readings each).



**Effect of cream formulation of 5% (w/w) herbal drug combination (ethanol extract of *Tridax procumbens* and petroleum ether extract of *Cassia occidentalis*) on percentage reduction in wound size.**

Cream formulation herbal drug combination	Day 1	Day 4	Day 8	Day 12	Day 16
Control	475± 29.3	393 ± 26.10	306 ± 22.80	238 ± 10.80	105 ± 08.92
Treated	498± 32.4	402± 18.40	328 ± 25.30	196 ± 11.00	78.40 ± 10.60
Standard	537± 36.2	420 ± 25.30	309 ± 13.40	178 ± 04.30	8 ± 0.0

(Values are mean + SD from 6 readings each).



**Excision wound model**

Formulation: 5% (w/w) petroleum ether extract of *Cassia occidentalis* cream

The percentage reduction in wound was 525 ± 22.4, 460 ± 10.6, 315 ± 36.5, 246 ± 15.4, 186 ±

0.75mm<sup>2</sup> as measured on the 1<sup>st</sup>, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> day respectively in the control group 480 ± 0.9, 243 ± 33.1, 206 ± 3.7, 104 ± 0.21, 36 ± 1.86 mm<sup>2</sup> respectively in the treated group. The percentage reduction in wound size was

significantly increased in 5% w/w ointment of petroleum ether extract of *Cassia occidentalis* treated group compared to the control group on the 16<sup>th</sup> day.

Formulation: 5% (w/w) ethanolic extract of *Tridax procumbens* cream

The percentage reduction in wound was  $432 \pm 26.6$ ,  $384 \pm 13.83$ ,  $308 \pm 10.8$ ,  $254 \pm 10.10$ ,  $117 \pm 09.80$  mm<sup>2</sup> as measured on the 1<sup>st</sup>, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> respectively in the control group and  $445 \pm 20.10$ ,  $392 \pm 19.7$ ,  $284 \pm 6.2$ ,  $226 \pm 14.00$ ,  $95 \pm 11.60$  mm<sup>2</sup> respectively in the treated group. The percentage reduction in wound size was significantly increased in 5% w/w ointment ethanolic extract of *Tridax procumbens* treated group compared to the control group on the 16<sup>th</sup> day.

Formulation: 5% (w/w) herbal drug combination (ethanolic extract of *Tridax procumbens* and petroleum ether extract of *Cassia occidentalis*)

The percentage reduction in wound are was  $475 \pm 29.3$ ,  $393 \pm 26.10$ ,  $306 \pm 22.80$ ,  $238 \pm$

$10.80$ ,  $105 \pm 08.92$  mm<sup>2</sup> as measured on the 1,4,8,12 and 16 day respectively in the control group and  $498 \pm 32.4$ ,  $402 \pm 18.40$ ,  $328 \pm 25.30$ ,  $196 \pm 11.00$ ,  $78.40 \pm 10.60$  mm<sup>2</sup> respectively in the treated group. The percentage reduction in wound size was significantly increased in 5% w/w ointment (ethanol extract of *Tridax procumbens* and petroleum ether extract of *Cassia occidentalis*) treated group compared to the control group on the 16<sup>th</sup> day.

## CONCLUSION

The formulation promotes wound-healing activity. It showed remarkable wound healing activity and it may be suggested for treating various types of wounds in human beings. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity.

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