
Research Article



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**Formulation and Evaluation of Poly herbal Ointment for Wound Healing
activity**

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ABSTRACT

Herbal therapy and herbal drugs predominates in traditional medicine as well as in alternative medicine practiced in the developed world. Among the various indications where traditional herbal medicines are used, skin and skin related disorders is ranked top. Thus, the main objective of the present study is to formulate and evaluate a poly herbal ointment with wound healing activity. Ointments were formulated using ethanolic extracts (by continuous hot Percolation-Soxhletation) of *Tridax procumbens* and petroleum ether extract of *Cassia occidentalis* were evaluated for its wound healing activity. Formulations were then tested for its physicochemical properties like loss of drying, pH, and spreadability and diffusion study and gave satisfactory results. The prepared formulations were also stable at 4°C, 25°C and 37°C. Hence an attempt was made to formulate a polyherbal ointment, and to evaluate for its physical parameter, wound healing activity by excision model and to compare its activity with a marketed formulation (5% w/w betadine). Overall result of this study reveals that this is an effective polyherbal wound healing ointment.

Keywords: *Tridax procumbens*, *Cassia occidentalis*, wound healing activity, poly herbal formulation, excision wound method.

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 management¹. 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

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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. 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 folklore². 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.

MATERIAL AND METHODS

Collection of plants

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 soxhlet apparatus. The extraction was carried out for 24 hours. After extraction, the solvents were distilled out; the concentrated residues were analyzed by chemical tests^{2,6}.

Phytochemical analysis^{3,4}

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.⁵

Formulation of ointment

Procedure

The hydrocarbon base selected for this study contains 95%w/w white petroleum jelly and 5%w/w of white bees wax. Required quantities of white petroleum jelly, white bee's wax were melted together in a beaker. The melted ingredients were stirred until cooled. The medicated ointment prepared by incorporating 5% extract in prepared hydrocarbon base. The fusion method was employed in the preparation of the medicated ointment. The required quantity of the ointment base was weighed and melted at a temperature of about 70° C in a hot water bath. The designated quantity of the extracts were added to the melted base at 40° C and stirred gently and continuously until a homogenous dispersion is obtained.

Physicochemical parameters⁷

Preliminary evaluation of formulations at different concentrations was carried out as follows:

- **Color and odour** :Color and odour was examined by visual examination.
- **Loss on drying** :Loss on drying was determined by placing ointment in petridish on water bath and dried for 105⁰C.
- **pH** :The pH of various formulations was determined by using digital pH meter. One gram of ointment was dissolved in 100 ml of distilled water and stored for two hours.
- **Viscosity**: Viscosity of formulation was measured by using brookfield viscometer.

Determination of viscosity by using brookfield viscometer (DV-II + pro)

Procedure: Required formulation was taken in a beaker around (50-200g/ml).suitable spindle (No.61, 62, 63 or 64) was fixed to the below spring of the viscometer. Set the speed from (3,6,9,12,16,30,45 and 60) note the obtained Cps value every time set the speed and on the motor, note the Cps value with increase in speed of rotation we will observed the decrease in viscosity i.e,Cps values.

Spreadability: Spreadability is a term expressed to denote the extent of area to which the ointments readily spreads on application to skin or

affected part. A special apparatus has been designed by multimer to study the spreadability of formulations. The spreadability was expressed in terms of time in seconds taken by two slides to slip off from ointment and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, result the better spreadability. 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

Stability studies

The stability studies were carried out for the prepared formulations at different temperature conditions (4° C, 25° C, 37° C and 45°C) for 3 months.

Standard used: 5% w/w betadine ointment.

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 mm². 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 1-16th post- wound day.

- Group I- treated with control (ointment)
- Group-II- treated with standard (betadine powder)
- Group III- treated with 5% ointment formulation of petroleum ether extract of *Cassiaoccidentalis*.
- Group IV- treated with 5% ointment formulation of ethanolic extract of *Tridax procumbens*.
- Group V- treated with 5% ointment 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 1st, 4th, 8th, 12th, and 16th 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 soxhlation

Crude drugs	Extract	Nature	Colour	Odour	Taste
<i>Cassia occidentalis</i>	Petroleum ether	Semi solid	Black	Characteristic	Mucilagenous,bitter
	Ethyl acetate	Semi solid	Black	Characteristic	Mucilagenous,Bitter
	Chloroform	Solid	Black	Characteristic	Mucilagenous,Bitter
	Ethanol	Solid	Brown	Characteristic	Mucilagenous,Bitter
	Aqueous	Solid	Brown	Characteristic	Mucilagenous,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 parameters⁷

Parameter	Ointment 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	Green	Green
Odour	Characteristic	Pungent	Characteristic	Characteristic
pH	6.22	7.02	6.37	6.52
Phase separation	No	No	No	No
Spreadability(dynes/cm ²)	252	232	236	209
Trace of gritty particles	No	No	No	No

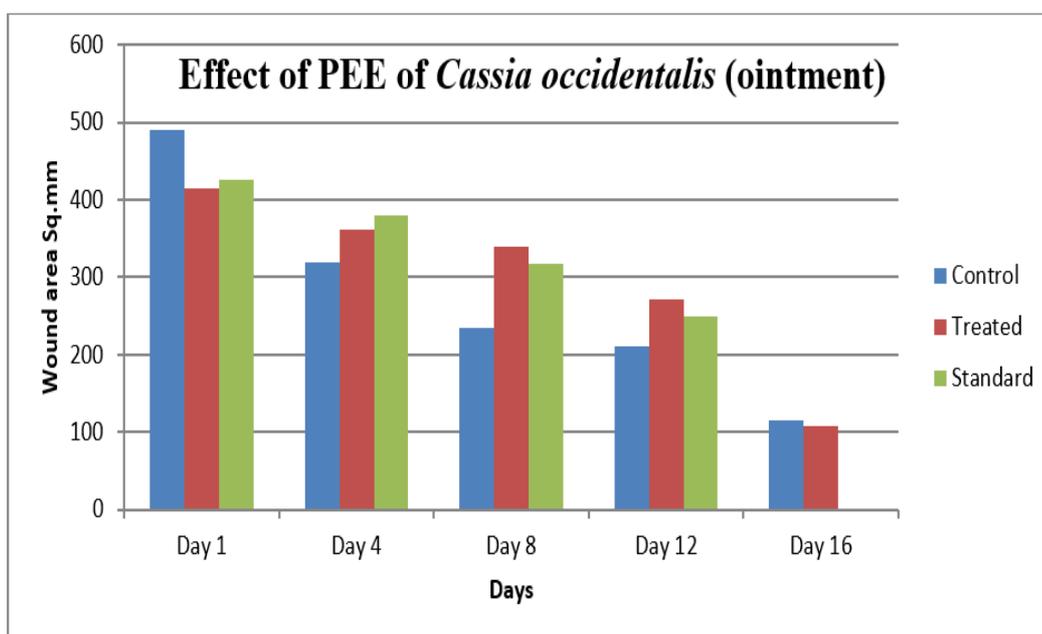
Viscosity

Crude drug	Dosage form	10 rpm	20 rpm	50 rpm	100rpm
<i>Tridax procumbens</i>	Ointment	230	211	79	52
<i>Cassia occidentalis</i>	Ointment	338	190	146	75
Standard	Ointment	376	324	167	128

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

Ointment formulation		Day 1	Day 4	Day 8	Day 12	Day 16
		Control	490 ± 34.0	320 ± 21.8	235 ± 11.2	210 ± 2.10
Treated		414 ± 18.0	362 ± 32.4	339 ± 9.5	271 ± 0.7	108 ± 0.86
Standard		425 ± 36.7	379 ± 28	317 ± 16.8	250 ± 2.5	0 ± 0.0

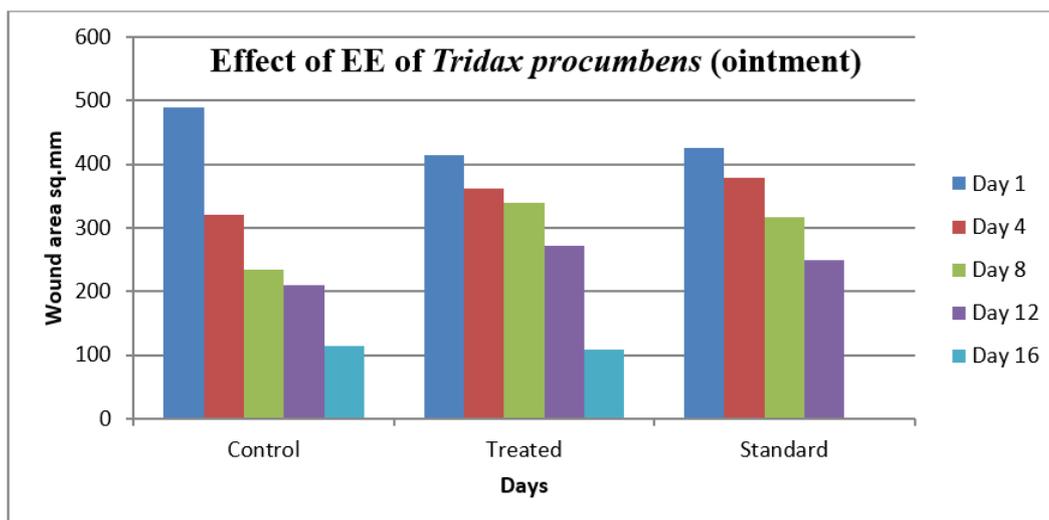
(Values are mean + SD from 6 readings each).



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

		Day 1	Day 4	Day 8	Day 12	Day 16
Ointment formulation	Control	540 ± 33.1	495 ± 36.10	408 ± 22.4	260 ± 10.10	115 ± 09.80
	Treated	485 ± 27.10	408 ± 19.80	320 ± 28.10	271 ± 14.00	138 ± 11.60
	Standard	526 ± 9.50	367 ± 14.60	245 ± 09.17	135 ± 02.80	0 0 ± 0.0

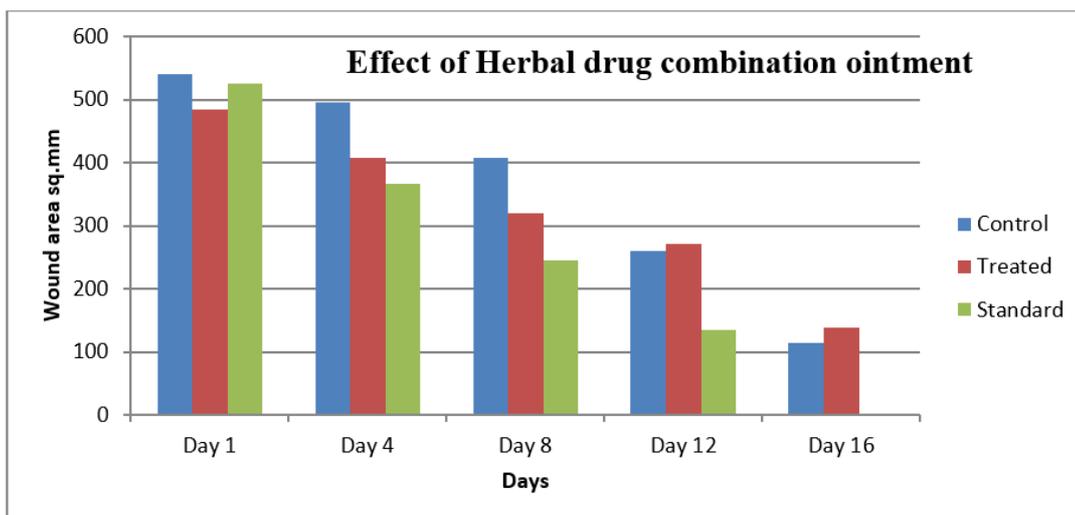
(Values are mean + SD from 6 readings each).



Effect of ointment 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.

		Day 1	Day 4	Day 8	Day 12	Day 16
Ointment formulation herbal drug combination	Control	522 ± 29.3	493 ± 26.10	406 ± 22.80	338 ± 10.80	135 ± 08.92
	Treated	460 ± 32.4	410 ± 18.40	308 ± 25.30	216 ± 11.00	92.40 ± 10.60
	Standard	438 ± 36.2	369 ± 25.30	242 ± 13.40	32.00 ± 04.30	0 0 ± 0.0

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



Excision wound model:

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

The percentage reduction in wound was 490 ± 34.01 , 320 ± 21.8 , 235 ± 11.2 , 210 ± 2.10 , 115 ± 16 mm² as measured on the 1st, 4th, 8th, 12th and 16th day respectively in the control group and 414 ± 18.02 , 362 ± 32.4 , 339 ± 9.5 , 271 ± 0.7 , 108 ± 0.86 mm² 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 16th day.

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

The percentage reduction in wound was 540 ± 33.10 , 495 ± 36.10 , 408 ± 22.4 , 260 ± 10.10 , 115 ± 09.80 mm² as measured on the 1st, 4th, 8th, 12th and 16th respectively in the control group and 485 ± 27.10 , 408 ± 19.80 , 320 ± 28.10 , 271 ± 14.00 , 138 ± 11.60 mm² 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 16th 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 522 ± 29.33 , 493 ± 26.10 , 406 ± 22.80 , 338 ± 10.80 , 135 ± 08.92 mm² as measured on the 1,4,8,12 and 16 day respectively in the control group and 460 ± 32.40 , 410 ± 18.40 , 308 ± 25.30 , 216 ± 11.00 , 92.40 ± 10.60 mm² 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 16th 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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