APJNP 2016; Vol.1 (01): pp.55-62 **Original Research Article**

ASIAN PACIFIC JOURNAL OF NATURAL PRODUCTS

Available online at http://ainstin.com

Received: 20-07-2016
Revised: 09-08-2016
Accepted: 19-08-2016

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DEVELOPMENT AND EVALUATION OF POLYHERBAL FORMULATION CONTAINING METHANOLIC EXTRACTS OF *TRICHODESMA INDICUM, ALLIUM SATIVUM* AND *ACORUS CALAMUS*

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ABSTRACT

A polyherbal ointment containing methanolic extracts of whole plant of *Trichodesma indicum*, rhizomes of *Acorus calamus* and *Allium sativum* were prepared at 5% and 10% concentrations and subjected physical and pharmacological evaluations. The dermal toxicity studies revealed that the ointments were non toxic to the skin. The wound healing potential of the ointments was good. The polyherbal ointment though showing lesser rate of contraction (96.93 ± 1.15**)** of the wound but healing of the wound was complete up to the final stage at 10% Polyherbal ointment. The antimicrobial activity shows that the zone of inhibition was more than that of the standard Neosporin (14.5 ± 0.5)when compared with the polyherbal formulation at 10 % (18.8 ± 0.3). The scientific validation of the polyherbal ointment has proved the folklore claim of using the paste of the leaves of *T. indicum,* rhizomes of *A. calamus* and *A.sativum*.

Keywords*:* Polyherbal formulation*,* antimicrobial*,* wound healing activity.

INTRODUCTION

Wound is most commonly used when referring to injury to the skin or underlying tissues or organs by a blow, cut, missile, or stab. Wound also includes injury to the skin caused by chemicals, cold, friction, heat, pressure and rays, and manifestation in the skin of internal conditions, for example, pressure sores and ulcers [1]. Wounds have a tremendous impact on the healing healthcare economy. Chronic wounds represent a major health burden and drain on the healthcare

resources in the world including Ghana [2]. In folklore medicine the paste of the leaves Trichodesma indicum (family: Boraginaceae) along with bulbs of Allium sativum and rhizomes of Acorus calamus were used for wound healing [3].

The present study was undertaken to explore the effects of polyherbal ointment containing methanolic extracts of *Trichodesma indicum, Allium sativum* and *Acorus calamus.*

MATERIALS AND METHODS

Collection of plant materials and extract preparation

The whole plant of *Trichodesma indicum* Linn R.Br. was collected and authenticated by taxonomist of the American college of arts and science, Madurai. The whole plant of *Trichodesma indicum* bulbs of *Allium sativum* and rhizomes of *Acorus calamus,* were cut into small pieces and dried under shade and then pulverized to get a coarse powder of particle 40 mesh size. The plant materials were first macerated with petroleum ether for 72h. Then it was filtered and the marc dried. The dried marc was then immersed in methanol for 72h. It was then filtered and the filtrate was evaporated by rota vacuum evaporator.

Preparation of ointment

Simple ointment base was prepared as per IP method and the polyherbal ointment prepared by triturating the methanolic extracts of *T. indicum, A. calamus* and *A. sativum* at 5% (2:1.5:1.5 respectively) and 10% (4:3:3 respectively) to the simple ointment base.

Evaluation of the prepared formulation

CAMAG TLC Scanner 3 "Scanner3-070408"S/N 070408(1.41.21) was used for detection and CAMAG Linomat 5 sample applicator was used for the application of the track. Twin trough plate development chamber was used for development of chromatogram. Software used was winCATS 1.4.3. Toluene: Ethyl acetate: 100% formic acid (7 : 3 : 0.2) was used as the mobile phase [4].

Methanolic extract of *T. indicum,* polyherbal ointment along with the methanolic extracts of *T. indicum, A. sativum* and *A. calamus* were evaluated. The TLC visualization, 3D display of the finger print profile of the extracts of *T. indicum, A. sativum* and *A. calamus* and the polyherbal ointment at 254 and 366nm are presented in **Fig.1 to 4**.

PHARMACOLOGICAL EVALUATION

Dermal Toxicity Study

The prepared ointments at 10% concentration were applied over depilated Wistar albino rats after anesthetized by ether.The skin was observed for erythema, edema and necrosis once a day for 14 days [5,6].

Wound Healing Activity

Wistar rats of either sex were used in the study. The range of the weight of the animals was between 200–250 g. they were housed individually in standardized environmental condition. All the animals were provided with water food *ad libitum*. Ethical clearance for the animal study was obtained *from the* Animal Ethical Committee, Madurai Medical College vide reference No. 10196/E2/4/2010 dated 14.12.2010.In the experiment, the rats were divided into six groups (n = 4): group 1 was the control (untreated), group 2 received standard Neosporin ointment [7] group 3 received 5%polyherbal ointment, group 4 received 10% polyherbal ointment. All treatments were applied topically once a day

for 16 days or till the wound healed completely [8].

Excision wound model

The rats were anaesthetized using light ether anaesthesia [6], hairs were shaved from the dorsal thoracic region of the animals using an electric clipper and the area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless stencil. The skin of the impressed area was excised using a sharp surgical blade to the full thickness to obtain a circular wound of diameter 2-2.5 cm and an area of 450-500mm^2 and 2mm depth. The wounds were blotted with a cotton swab soaked in normal saline for achieving hemostats.

Rate of contraction of wounds

Wound healing potential was expressed by the rate of wound contraction and wound closure time [9]. The progressive change in wound area were monitored by tracing the wounds on to a tracing paper and thereafter on a graph paper on 0th, 4th, 8th, 12th and 16th day or till complete epithelization occurred (**Tables 2 & 3.)**

Histology

The On the 16th day regenerated tissues were removed and transferred in to10% formalin, dehydration is carried out. Then were stained with haemotoxylin and eosin and studied under light microscope for the extent of re-eipthelization, fribrosis, inflammation and the organization and thickness of epidermal squamous cells; the thickness of the granular cell layer and the degree of tissue formation [10].

Bacterial isolation [11]

On day 5 of the experiment, bacterial culture determination was done on the wounds. The swabs were taken from the surface of the wound before application of ointment. The above procedure was repeated on day 10 of the experiment. The swabs were cultured on Brain Heat Infusion (BHI) agar overnight at 37^{o}C for any bacterial growth [11].

Anti bacterial Activity

The antibacterial activity of the prepared ointments was evaluated by agar well diffusion method [12]. The pathogenic strains were seeded on the MH agar media in a petri dish by streaking the plate with the help of a sterile swab. The test ointments and standard neosporin ointment (100mg) were introduced into the wells and then incubated at 37^{o}C for 24 hours. The presence or absence of zone of inhibition was then measured and the results are presented in **table 4.**

STATISTICAL ANALYSIS

In biological experiments, the data (results) obtained at the end of the experiment was subjected to statistical analysis to determine whether the effect produced by a compound under study is genuine and not due to chance. Hence, it is analyised by using arithmetic mean, standard deviation, standard error of mean, tests of significance, regression analysis. [13-15].

RESULTS

Evaluation of the prepared formulations

Table 1: Evaluation of physical parameters of the prepared ointment

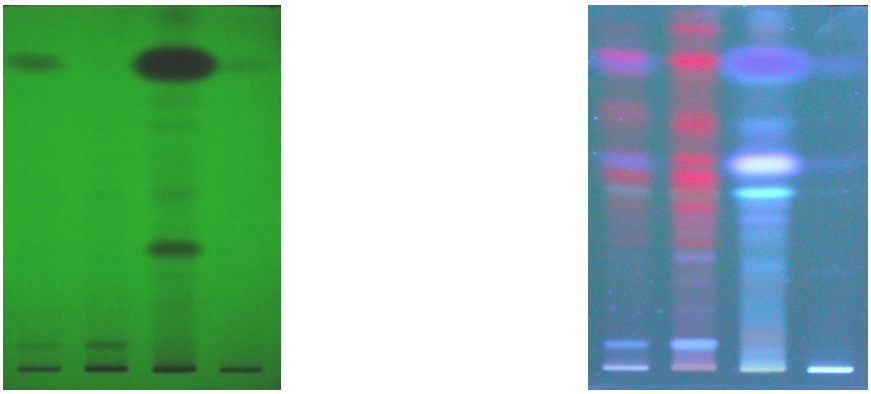
S. No.	Parameters	Observations
1	Colour	Brown
2	Odour	Garlic odour
3	Consistency	Semisolid

High Performance Thin Layer Chromatography

The visualization of the polyherbal ointment, methanolic extract of *T. indicum,* methanolic extract of *A. calamus* and *A. sativum* TLC plate at 254nm and 366nm is presented in **Fig. 1.** The polyherbal ointment shows the presence of 8 spots at 254nm and 11 spots at 366nm. The methanolic extract of *T. indicum* showed the presence 8 spots at 254nm and 16 spots at 366nm. The methanolic extract of *A. calamus* showed the presence of 9 spots at 254nm and 13 spots at 366nm. The methanolic extract of *A. sativum* showed the presence 3 spots at 254nm and 2 spots at 366nm.

Fig. 1: Visualization at 254nm and 366nm

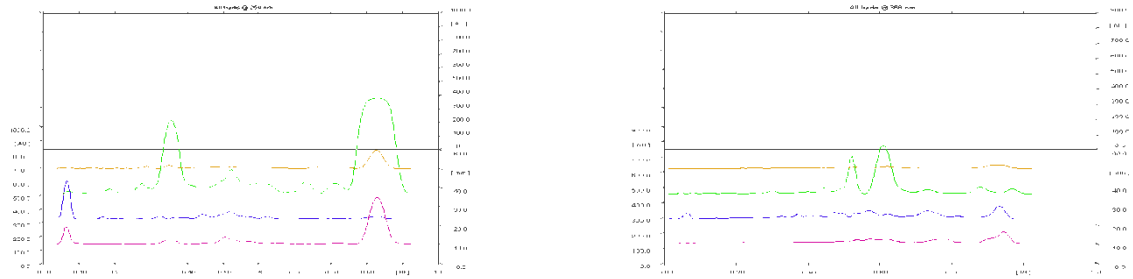
@ 254nm **@366nm**



Note: Track 1 - Polyherbal ointment; Track 2 - Methanolic extract of *Trichodesma indicum*; Track 3 - Methanolic extract *Acorus calamus*; Tract 4 - Methanolic extract of *Allium sativum*

Fig. 2: 3D Display of the fingerprint profile at 254nm and 366nm

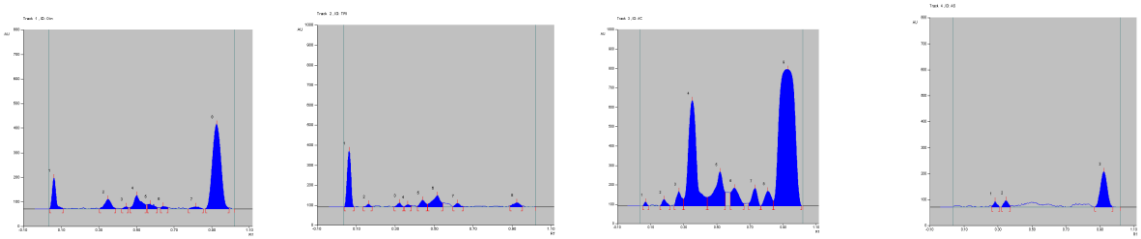
@ 254nm **@366nm**



The 3D display of the fingerprint profile and the peak display at 254nm and 366nm are presented in **Figs. 3 & 4.**

Fig. 3: Peak display of at 254nm

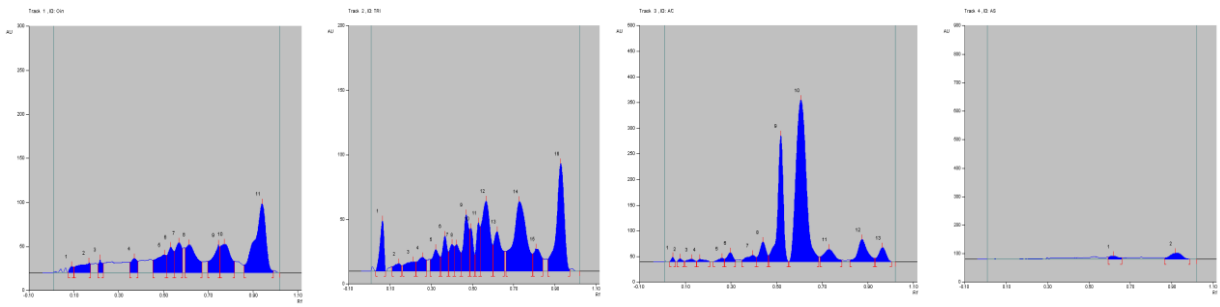
Track 1 **Track 2** **Track 3** **Track 4**



Note: Track 1 - Polyherbal ointment, Track 2 - Methanolic extract of *Trichodesma indicum*, Track 3 - Methanolic extract *Acorus calamus*, Tract 4 - Methanolic extract of *Allium sativum*.

Fig. 4: Peak display of at 366nm

Track 1 **Track 2** **Track 3** **Track 4**



Pharmacological evaluation:

Dermal toxicity studies

The polyherbal ointments were evaluated for its toxicity on topical application. The ointments exhibited no toxic effects (ie) no erythema, edema and necrosis.

Wound healing potential

Rate of wound contraction and wound area

The results for the rate of contraction of the wounds are presented in **table 4** and the period of re-epithelization of the wound is presented in **table 5**.

Table 2: Rate of contraction of wound area in treated and untreated animals

S. No.	Treatment	Percentage rate of contraction of wound area			
		4^{th} day	8^{th} day	12^{th} day	16^{th} day
1	Control	29.14 ± 2.49	39.54 ± 1.52	64.55 ± 6.21	79.08 ± 4.38
2	Standard – Neosporin	42.30 ± 2.00[a]	72.30 ± 5.10[b]	90.77 ± 2.67[b]	96.37 ± 1.82*
3	Polyherbal ointment 5%	19.23 ± 3.93	58.33 ± 3.41[b]	79.64 ± 2.30	86.18 ± 3.03
4	Polyherbal ointment 10%	41.54 ± 1.71[a]	64.21 ± 1.91[b]	89.45 ± 2.08[a]	96.93 ± 1.15[b]

Note: [a]p< 0.05; [b]p< 0.01; [c]p< 0.001 Vs. control by Student's t test

Table 3: Period of re-epithelization of the wound

S. No.	Treatment	Period of re-epithelization
1	Control	21.67± 0.33
2	Standard – Neosporin	19.67 ± 0.39
3	Polyherbal ointment 5%	18.33 ± 0.41
4	Polyherbal ointment 10%	17.67 ± 0.35

Histopathological observations

Normal skin

The sections of the normal skin tissues showed the presence of thinned out epidermis, collegenisation, congested blood vessels and sparse inflammatory cell filtrates.

Animals untreated (Control)

The sections of skin tissues of animals untreated showed the presence of lining squamous epithelium with areas of ulceration. No granulation tissues seen.

Animals treated with standard drug Neosporin

The sections of skin tissues of animals treated with standard Neosporin ointment showed the presence of lining squamous epithelium with areas of ulceration, inflammatory cell infiltration and granulation tissues.

Animals treated with 10% Polyherbal ointment

The sections of the skin tissues of animals treated with 10% polyherbal ointment showed lining squamous epithelium with proliferating capillaries and fibroblasts.

Bacterial isolation

Bacterial culture of the swabs removed from the wounds of treated animals on 5^{th} & 10^{th} day showed no any bacterial growth in standard (Neosporin), Polyherbal ointment 5%, Polyherbal ointment 10%.

Antibacterial activity

The results obtained for the antibacterial activity of prepared ointments are presented in **table 6.**

Table 4: Antibacterial well diffusion assay against various microorganisms by polyherbal ointments

S. No	Name of the organism	Zone of inhibition (in mm)		
		Standard Neosporin ointment	Polyherbal ointment	
			5%	10%
1.	*Escherichia coli*	14.5 ± 0.5	15.8 ± 0.3	18.8 ± 0.3
2.	*Staphylococcus albus*	17.0 ± 1.0	15.5 ± 0.5	17.5 ± 0.5
3.	*P. aeurginosa*	20.0 ± 2.0	16.0 ± 0.0	18.0 ± 2.0
4.	*K.pneumoniae*	17.0 ± 1.0	17.5 ± 1.5	16.8 ± 0.8

***mean of three readings ± SEM**

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