Formulation and Evaluation of Cashew Leaf Extract Ointment for Wound Healing Activity

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KEYWORDS	Abstract			
Anacardium	The present study was designing, formulating and evaluating a herbal ointment contain			
occidentale	ethanolic extracts of the Anacardium occidentale leaves. However, there were no scientific reports			
leaves, Soxhlet	scientific evaluation for the wound healing potenti	al of the crude extract of Anacardium		
extraction,	occidentale (Cashew Plant) leaves. The proposed here	bal ointment was evaluated with different		
Ointment,	parameters like pH, viscosity, spreadability and stability	were evaluated. The formulation showed		
Excision wounds,	n wounds, good spread ability, good consistency, homogeneity, there was no change in the appearance, j			
Inscision wounds	and no phase separation noticed at the end of the stabil	ity studies. There was no evidence of skin		
	irritation. This study has revealed that the herbal ointme	nt has shown the wound healing effect due		
	to the synergistic activity of the phytoconstituents pr	esent in the extracts and may be used a		
	potential herbal formulation for wound healing. The par	ameters evaluated for wound healing were		
	period of contraction and tensile strength using excision	n and incision models. The animals were		
	divided into groups and were treated with herbal oin	tment, standard group and one served as		
	control group. Nitrofurazone (0.2% w/w) was use	d as reference standard. These results		
	demonstrate that the crude extract of Anacardium occ	identale (Cashew Plant) leaves possesses		
	wound healing activities. This justifies the tradition	al claimed use of the plant for treating		
	uninfected and infected wounds.			

Introduction

Herbal therapy predominates in traditionalmedicine as well as in alternative medicine practiced in the developing and the developed world. The widespread interest in drugs derived from plants is because of the belief that plants are safe and dependable, and with lesser side effects. Review of literature reveals that traditional plant drugs are beneficial for several skin related problems and for wound healing [1]. It is known that plants have different phytoconstituents which are responsible for the various activities that are attributed to them and when a combination of plants with these constituents are combined together it may show better activity when compared to the individual extract [2]. Humankind needs to learn more from natural products and traditional medicines. In order to further promote the development of modern medical research on natural products, humans have to face up to various difficulties

and challenges. Valuable information on natural products and TMs is mixed in a large number of documents, data, and useless rumors [3]. Traditional medicines make use of natural products and are of great importance. Such forms of medicine as traditional Chinese medicine (TCM), Ayurveda, Kampo, traditional Korean medicine (TKM), and Unani employ natural products and have been practiced all over the world for hundreds or even thousands of years, and they have blossomed into orderly-regulated systems of medicine. In their various forms, they may have certain defects, but they are still a valuable repository of human knowledge [4]. Cashew is an evergreen perennial plant belonging to the family Anacardiaceae. This family consists of 400-600 species. Among the eight species in the genus Anacardium, the only cashew is valuable due to its nutritious kernel. Cashew is a tropical tree present in South America and Brazil [5]. Cashew nuts are used



for several medicinal purposes and have great importance related to health, as evidenced by research. These are used for obesity, diabetes, heart disease, urinary disorders, digestive disorders, and many other clinical applications like bone relaxation, cold and flow, etc. It also has importance in Cancer [6]. The purpose of this study was to evaluate the wound healing activities of the crude extracts of leaves of Anacardium occidentale (Cashew leaves), which can in turn provide basis for isolation and identification а of pharmacologically active compounds.

Materials and Methods

Preparation of the Extracts: Solvent extraction of selected plant i.e. Anacardium occidentale leaves was performed using soxhlet extractor. Air-dried powdered of Anacardium occidentale leaves was defatted with petroleum ether using maceration method. The marc was dried in an air every time prior to extracting with the ethanol; The marc was macerated with ethanol for 24 h (three times) to obtain the ehanol extract. The obtained extract concentrated using rotary vaccum evaporator. The consistency; color; appearance of the extracts and their percentage yield (% w/w) were noted [7].

Formulation of Ointment: The required quantity of the chemicals was weighed and the herbal ointment was formulated by fusion method using emulsifying ointment base. The herbal ointment formulation of isolated plant extract was prepared with mixing of ethanol extract of Anacardium occidentale leaves and isolated compound to ointment base mixture. First the ointment base was prepared by containing various waxes. The accurate amount of hard paraffin (0.5 g) weighed accurately, which was placed in evaporating dish on water bath. After melting of hard paraffin remaining ingredients (Wool fat (0.5 g), Cetostearyl alcohol (0.5 g) and Yellow soft paraffin (8.0 g) were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base [8].

Evaluation of the herbal Formulation: The herbal formulation was evaluated by the following physicochemical parameters.

Colour and odour: Color and odor was examined by visualexamination.

Loss on drying: Loss on drying was determined by placing the ointment 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 twohours. The measurement of pH was done in triplicate and average values were considered [9].

Spreadability: The spread ability was expressed in terms of times in seconds taken by two slides to slip off from ointment placed in between the slides under the direction of certain load. Spread ability 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 ointment to get diffused was noted.

Skin irritation study: Healthy rabbits were selected and were shaved in two different areas of the dorsal side, each about 500 mm2. The rabbit was kept in rabbit holder and the first area was kept as control, to which emulsifying ointment base was applied, the second area was treated with herbal ointment. After 4hrs the skin was observed and compared with the control [10].

Wound Healing Activity:

Excision wound model: Male and female healthy Sprague dawley rats weighing approximately 250 gm were used for the study. The animals were maintained under standard conditions during the course of the experiment. The animals were anesthetized using ether. An impression was made on the dorsal thoracic region 1 cm away from the vertebral column and 5 cm away from the ear of the anesthetized rat. Skin was excised to full thickness to obtain a wound area of about 500 mm2. The animals were divided into four groups and they were treated as follows: Group I: emulsifying base, Group II: standard nitrofurazone 0.2% (w/w) ointment, Group III: polyherbal formulation and Group IV: control. The ointments were applied once daily until complete healing of wound and the wound area was measured on a millimeter scale graph paper on alternate days. The percentage of wound healing was calculated. Falling of scar was taken as the endpoint for complete epithelization and the days taken for this was considered as periodof epithelization.

Incision wound model: The selection of the animals was done as mentioned above. Two paravertebral straight



incisions of 6 cm were made on either sides of the vertebral column. Homeostasis was achieved by blotting the wound with a cotton swab dipped in saline and the wound was closed by means of interrupted sutures at equidistance 1 cm apart. Animals were treated daily with formulations, as mentioned above under excision wound modelfrom 0 day to 9th post-wounding day. The tensile strength in each group is determined on the 10th day by continuous, constant water flow technique [11].

Results and Discussions

A wound may be defined as the loss or rupture of the cellular, anatomical or functional continuityof living tissue. Healing of skin wounds is a complex process which ultimately leads to therestoration of the injured skin. The aim of wound care is to promote wound healing in the shortest time possible. The process of wound healing is promoted by several natural products which have been reported and used in Ayurveda, Siddha and Unani systems of medicines. The results of the herbal ointment formulation have shown significant activity when compared to the control and the base. The period of epithelization in the excision wound mode was found to be 15.10 days in case of the formulation when compared to control which was 22.00 days. While in case of incision wound model the tensile strength was480 in case of formulation which is extremely significant (**P<0.0001). The formulation was evaluated for its physicochemical properties and was subjected to stability studies.

CONCLUSION

The results obtained in this study show that the cashew leaf extract formulated as herbal ointment. The different phases of wound repair, wound contraction, epithelialization, and tensile strength, were enhanced by the ethanolic crude extract ointment of the leaves of Anacardium occidentale leaves as compared to the negative control group. These results collectively demonstrate that the ethanolic extract possesses wound healing activity and this justifies the use of the leaves of Anacardium occidentale leaves for treatment of wounds as claimed in the folklore literature. This study also showed that the crude extract of Anacardium occidentale leaves was endowed with significant antibacterial activities that explain at least in part its wound healing activity. The prepared ointment accelerates the healing process by enhancing collagen formation and increasing

the breaking strength of the healed wounds. This potent activity can be attributed to the phyto-constituents present in the plants which may be acting synergistically to enhance the wound healing effect.

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Table 1: Physiochemical evaluation of the herbal formulation ointment

Parameters	Herbal Ointment	
Colour	Yellowish green	
Odour	Characteristic	
Loss on Drying	12.20%	
pH	6.1	
Spreadability (Sec)	15 sec	
Diffusion study	0.72cm	
Skin irritation study	No skin irritation was observed	

Table 2: Effect of the herbal formulation ointment in excision wound model

Treatment	Wound contraction (50%) (days)	Period of epithelization (days)
Control	11±0.04	20.00±0.48
Standard	7±0.00	15.00±0.40*
Ointment Base	8±0.04	17.00±0.00
Herbal Ointment	7±0.2	13.20±0.02**

All values are mean SEM $\pm n=6$, *P<0.05 indicates significant and**P<0.001 indicates extremely significant compared to the control.

Table 3: Effect	of the herbal	formulation	ointment in	incision	wound model
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Treatment	Tensile strength
Control	317±5.15
Standard	438±11.05
Ointment Base	352±11.21
Herbal Ointment	446±2.6*

All values are mean SEM \pm n=6, **P<0.001 indicates extremely significant compared to the control.



Figure 1: Effect of the herbal formulation ointment in excision wound model

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Figure 2: Effect of the herbal formulation ointment in incision wound model