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Wound healing activity of medicinal herbs of Cauvery Delta region

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Abstract

The herbal ointment formulations were prepared and their wound healing potentiality was evaluated in animal model experiments. Three different ointment formulation were prepared using different proportion of four medicinal plants namely *Andrographic echioides, Alpinia galanga*, *Borreria hispida* and *Curcuma longa*. The alcohol extract of the plants were used in the formulation, and white petroleum jelly and glycerine were used as base in the preparation of ointment. The ointments were applied topically over the artificiality created wounds of the experimental animals for 25 days. The wound contraction was measured on 4th, 8th, 12th and 16th day after treatement. The blood samples were analysed and the parameters including protein, cholesterol, platelets, WBC and haemoglobin contents were estimated. Group II animals treated with ointment (Formulation II) showed high percentage of wound contraction and low epithelization time. This study suggests that, the herbal preparations could prove to be boon for mankind as it is purely based on the combination of herbals.

Key words: *Alpinia galanga, Andrographis echioides, Borreria hispida, Curcuma longa,* Excision wound, Wound healing

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INTRODUCTION

Wounds of different kinds are developed due to various causes and reasons. They are categorized into acute and chronic wounds on the basis of their etiology. Acute wounds are most commonly due to accidents such as trauma or burns or some time due to deliberate, chronic wound developed due to ulcer for prolonged infection take long time to get cured. However, the acute wounds are cured with medication relatively in short duration of time.

When treating chronic wounds, it is important to note that biofilms play an important role in the prevention of wound healing. These biofilms harbour various microorganisms which delay the wound healing process (Lusby *et al*., 2005) due to the emergence of multi drug resistant organisms and the scarcity of newer antibiotics, wound care professionals have revisited the ancient and traditional healing methods and practice. People's perception towards traditional medicine has also changed and is very encouraging. The concept of moist wound healing has been well accepted and traditional medicine has also incorporated this method to fasten the healing process. Several studies using herbal and traditional medicine from different continents have been documented in wound care management. Recent scientific evidences and clinical trials conducted using traditional and alternative medicine in wound therapy hold good promise in the future (Ananda Dorai, 2012). The scientific validation of traditional or folk medicine and evolving scientific methods for formulating the various indigenious medicinal plants have after been emphasized.

The Cauvery delta region of Tamil Nadu, India, harbours several medicinal herbs with curative potentials. These medicinal plants have been used traditionally by the prevailing people of this area for treating various ailments and successful solutions are also available in the traditional folk medicine to cure wounds. Various plants have been used either individually or in combination to cure wounds. In the present study an attempt was made to formulate an ointment using four plants namely, *Alpinia galanga, Borreria hispida, Curcuma longa* and *Andrographis paniculata.* The plants were selected on the basis of the chemical constituents and their pharmacological potentialities.

The rhizome of *A. galanga* (Family : Zingiberaceae) has been reported to have antimicrobial (Chudiwal *et al*., 2010), antitumour (Itokawa *et al.,* 1987), antiulcer (Al Yahya *et al*., 1990), anti allergic (Matsuda *et al*., 2003), anti-inflammatory and analgesic (Phitak *et al*., 2009), antioxidant (Juntachote and Berghofer, 2005), hypolipidemic and hepatoprotective (Achuthan and Padikkala, 1997) activities.

Different extracts of *A.echiodies* (Family : Acanthaceae) are proved to be antimicrobial (Sermakkani *et al*., 2011), anthelmintic (Padma *et al*., 2012), hepato protective and antioxidant (Basu *et al*., 2009), antinociceptive, anti-

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inflammatory and anti-pyretic (Basu *et al*., 2009), anti-ulcer (Ramasubramania Raja 2014) potentials.

B.hispida (Family:Rubiaceae) showed free radical scavenging and antioxidant activities (Kaviarasan *et al*., 2008; Surveswaran *et al*., 2007), anti-inflammatory (Parthasarathy, 2010), analgesic (Sundhararajan and Ravichandiran, 2012), antihyperlipidic (Sivaelango and Senthil Kumaran, 2012), hypoglycaemic (Kaviarasan *et al*., 2008), anti hepatotoxicity (Orwa, 2009; Karthikeyan *et al*., 2011) and antifungal (Mahalingam *et al*., 2011) activities.

Rhizome of *C.longa,* (Family : Zingiberaceae) is bestowed with anthelmintic (Dhiman, 2004), anti-asthamatic (Mali and Dhake, 2011), anti- inflammatory (Chainani, 2003), immunomodulatory (Jagetia and Aggarwal, 2007), antidiabetic (Acharya, 1994), antidiabetic, hypolipidaemic and hepatoprotective (Rai *et al*., 2010) , neuroprotective (Dohare *et al*., 2008) potentials. On the basis of these background information the rhizome of *Alpinia galanga* and *Curcuma longa* and the whole plant of *Borreria hispida* and *Andrographis echiodies* were used in the ointment formulation, tested in the animal model and the results are discussed in this article.

MATERIALS AND METHODS

Plant Material

The plants, *Andrographis echioides, Borreria hispida, Alpinia galanga* and *Curcuma longa,* were collected in and around Mannargudi and the identity was confirmed. The herbarium voucher specimens are being deposited in the Department of Botany, STET Women's College, Mannargudi.

Preparation of extracts

The whole plants of *A.echioides* and *B.hispida*; and rhizome of *A. galanga* and *C. longa* were powdered . The powdered materials were loaded in soxhlet's apparatus (Plate-3) and defatted with 70% ethanol (80 p C) in 20 batches (50mg each batch). The alcoholic extract was concentrated in hot plate to a semisolid mass. The residue was stored in desiccators until use.

Ointment Preparation

Three different herbal ointment formulations such as formulation I (*B.hispida* 70%, *A. echioides* 20%, *A. galanga* 5%, *C. longa* 5%), formulation II (*B. hispida* 20%, *A. echioides* 70%, *A. galanga* 5%, *C. longa* 5%), and formulation III (*B.hispida* 45%, *A.echioides* 45%, *A. galanga* 5%, *C. longa* 5%) were separately taken in porcelain and thoroughly mixed with 7 g of white petroleum jelly and 1 ml of glycerin. These formulations were collected in glazed paper and applied over wounds artificially created on the experimental animals..

Animal

Healthy rabbits of either sex (750g to 1000 g / b.w) were selected. Animals were divided individually, and free access to food and water was established. Animals were divided into seven groups and each group contained three animals.

Experimental Design

Group I: Wounded animals were dressed with formulation I

Group II : Wounded animals were dressed with formulation II

Group III: Wounded animals were dressed with formulation III

Group IV: Mupirocin of 2% w / w (T- bact of Glaxosmithkline Pharmaceuticals Ltd).

Group V: Sisomycin Sulfate of 1mg with Chlorocresol preservative of 1mg (Ensamycin of fulford (India)Ltd).

Group VI: Neomycin and Polymyxin Sulfates, and Bacitracin Zinc ointment USP (Neosporin of Glaxo Smith Kline).

Group VII: Ointment base (White petroleum jelly and Glycerin)

Creation of wound

The rear portion of the rabbit was chosen for creating wound. All the hairs on the relevant area were completely removed by using a razor. Shaving facilitated wound management. Care was taken to remove all the removed hairs from the wound area. Then the animals were anaesthetized with Zylocaine by injecting into the sub-cutaneous layer of the selected are for the creation of wounds. Wound was made with hot iron piece. After three days the burn wound of about 1.5 sq.cm^2 was formed on the selected area.

Application of the ointment

Wounded animals of all the experimental groups were given the respective treatment for twenty five days. The area of the wound was traced everyday. Wound contraction rate was monitored by tracing the raw wound on a transparent paper on 4th, 8th, 12th and 16th post wound days. Reduction in wound area was expressed as % of original wound area (150mm^2). Period of epithelization was monitored by recording

the number of days required for the scab to fall away leaving no raw wound behind. Finally the measurements were calculated and tabulated.

WC(%) =Initial wound size – Specific day wound size / Initial wound size x 100

Estimation of Biochemical Parameters

Blood samples were collected from the experimental animals at definite time intervals (prewound, postwound) and parameters such as haemoglobin, WBC count and platelet count were estimated according to the procedure of Samuel (1992). Estimation of cholesterol was carried out as per the method described by Allain (1974). Serum protein was estimated following the method of Gornall *et al.* (1949).

Wound healing is a complex process. The disruption of this process can lead to morbidity. They include chronic wounds, infection and scarring. It has been well established that the natural products have been used to cure wounds of various kinds since time immemorial. It is strongly believed that the natural products are viable alternative to the current practice of treatement of wounds using synthetic chemicals, and it is also widely accepted that they are safe and hence it becomes necessary to understand the current knowledge of their efficacy.

The studies on excision wound healing model revealed that all the seven groups showed decreased wound area from day to day. However on 20th post wounding day, Group I animals showed 60.3±0.88% of wound healing, where as Group II and Group – III

RESULTS

Table 1. Effect of ployherbal formulation on Percentage of wound healing and period of epithelialization in excision wound model in rabbits by topical application.

S. No.	Formulations[a]	Days after burn wound				
		4th Day	8th Day	12th Day	16th Day	Period of epithelialization (Days)
1	I	11.3± 1.76	25.7± 2.18	49.3 ± 2.33	60.3± 0.88	17.7± 0.88
2	II	20.0± 1.15	33.3± 1.85	64.3± 2.60	79.3±1.76	16.0±0.58
3	III	17.7± 1.45	27.0± 3.6	53.3±2.73	63.3± 1.20	17.7±0.33
4	IV	12.0±2.3	19.0±0.67	30.0± 0.57	57.3±2.33	18.7± 0.33
5	V	6.8± 0.93	11.0±0.57	21.7± 2.03	37.3±1.76	20.0± 0.57
6	VI	5.3± 0.88	10.0± 0.57	20.0±0.57	29.7±0.88	22.3±0.88
7	VII	5.2±0.14	8.5±0.28	13.7±0.88	26.3±1.20	21.3± 0.88
	F	17.84	29.75	105.33	168.79	10.92
	P	<0.001	<0.001	<0.001	<0.001	<0.001

The values are expressed as mean± S.E; n=3 animals; P< 0.001 as compared with control

[a]Formulation
I *Borreria hispida-70%, Andrographis echioides-20%, Alpinia galangal- 5%, Curcuma longa- 5%*
II *Borreria hispida-20%, Andrographis echioides-70%, Alpinia galangal- 5%, Curcuma longa- 5%*
III *Borreria hispida-45%, Andrographis echioides-45%, Alpinia galangal- 5%, Curcuma longa- 5%*
IV Mupirocin 2%
V Sisomycin sulfate
VI Neomycin & Polymyxin sulfates
VII Ointment base (white petroleum jelly and glycerin)

animals showed 79.3±1.76 and 63.3±1.20% of healing respectively. Group IV animals showed 57.3± 2.33%. of wound healing, while Group V and Group VI animals showed 37.3±1.76 and 29.7±0.88% of wound healing respectively. All readings are found to be statistically significant and comparable with control. On the basis of the present investigation and results it is concluded that the Group II animals treated with herbal formulation showed significant wound healing activity and it was found to be better than the standards (Group IV,V,VII and VII) (Table 1)

The haemoglobin level in the treated Groups on 8th day, slightly increased on all the tested groups. At the end of 16th day. Group I showed 9.23±0.74 gm% of haemoglobin, Group II and Group III animals showed 10.4±0.115 and 9.87±0.58 gm% respectively and Group IV animals showed 10.7±0.15gm%. While Group V and VI animals showed 10.2±0.26 and 9.87±0.17 gm% respectively. On the other hand Group VII animals showed 8.4±0.11 gm% of haemoglobin. There was no significant change observed in haemoglobin level between treated groups on 16th day (Table 2).

White blood cells help the process of wound healing by removing tissue debris and dead cells from the site of injury. The significant increase of WBC and platelets was observed in all the test group of animals on 8th day. On the contrary Group II animals treated formulation II attenuated the increased level of WBC and platelet count effectively when compared to control and standards on 16th day. This is due to the phytoconstituent present in the poly herbal ointment. (Table 3)

There was a significant reduction in the protein content in all the test groups of animals on 8th day. Among the three (Group I, II and III) formulations tested group II animals exhibited significant decrease (PÂ0.001) in protein content on 16th day when compared to control (Group I) and standards (Group IV, V, VI and VII). But in contrast, the cholesterol level was significantly decreased on 8th day in all the test animals. Group II herbal formulations treated animals showed that the level was increased and moreover less equal to the normal group. When compared to control and standards Group II formulation was found to be more effective than the other groups.

Table 2. Haematological parameters of excision wound model on topical application on 8th and 16th post wounding day

S. No.	Formulations [a]	Haemoglobin (gm%)		
		Normal	8 th day	16 th day
1	I	9.25± 0.17	11.0± 0.22 ns	9.23±0.74 ns
			(-18.92)	-0.22
2	II	9.25±0.17	10.87±0.18 ns	10.4±0.115 *
			(-17.51)	(-12.43)
3	III	9.25±0.17	10.87±0.94 ns	9.87±0.58 ns
			(-17.51)	(-6.70)
4	IV	9.25±0.17	11.2±0.61 ns	10.7±0.153*
			(-21.08)	(-15.68)
5	V	9.25±0.17	9.8±0.23 ns	10.2±0.265*
			(-5.95)	(-10.27)
6	VI	9.25±0.17	10.13±0.18 ns	9.87±0.176*
			(-9.51)	(-6.70)
7	VII	9.25±0.17	9.8±0.50	8.4±0.115
			(-5.95)	-9.19
	F	1.51	1.508	4.018
	P		0.246	0.015

The values are expressed as mean± S.E; n=3 animals; P< 0.05 as compared with control; ns=the difference from control value is not significant

Table 3. Haematological parameters of excision wound model on topical application on 8th and 16th post wounding day

S. No.	Formulations[a]	WBC (Cells/cumm)			Platelets (lakhs/cumm)		
		Normal	8 th day	16 th day	Normal	8 th day	16 th day
1	I	6257.14± 99.66	8200±568.62 ns	8233±185.59 ns	13857.4±5084.3	140000±11547.0 ns	150000±5773.5 ns
			(-31.05)	(-31.58)		-1.03	(-8.25)
2	II	6257.14±99.66	8466.7±145.2 ns	8366.7±202.75 ns	138571.4±5084.3	156666.7±6666.67 ns	153333.3±5773.5 ns
			(-35.31)	(-33.71)		(-13.06)	(-10.65)
3	III	6257.14±99.66	8666.7±643.7 ns	8500±288.67 ns	138571.4±5084.3	150000±5773.5 ns	140000±100000 ns
			(-38.51)	(-35.84)		(-8.25)	(-1.03)
4	IV	6257.14±99.66	8000±577.35 ns	82000±173.20 ns	138571.4±5084.3	153333.3±8819.17 ns	160000±5773.5 ns
			(-27.85)	(-31.05)		(-10.65)	(-15.46)
5	V	6257.14±99..66	8033.3±260.3 ns (-	7900±206.17 ns	138571.4±5084.3	150000±5773.5 ns	150000±5773.5 ns
			28.39)	(-26.26)		(-8.25)	(-8.25)
6	VI	6257.14±99.66	8100±305.5 ns	8133.3±176.38 ns	138571.4±5084.3	150000±5773.5 ns	140000±5773.5 ns
			(-29.45	(-29.98)		(-8.25)	(-1.03)
7	VII	6257.14±99.66	8100±378.59	8033.3±88.19	138571.4±5084.3	140000±10000	136666.7±18559.2
			(-29.45)	(-28.39)		(-1.03)	-1.37
	F		0.311	1.037		0.618	0.855
	P		0.921	0.443		0.713	0.55

The values are expressed as mean± S.E; n=3 animals; P< 0.05 as compared with control; ns=the difference from control value is not significant

Table 4. Biochemical parameters of excision wound model on topical application on 8th and 16th post wounding day

S. No.	Formulations [a]	Protein (g/dl)			Cholesterol (mg/dl)		
		Normal	8 th day	16 th day	Normal	8 th day	16 th day
1	I	5.67±0.143	6±0.12 ns	5.8±0.18 ns	142.86±4.21	138.3±7.26 ns	115±2.88 ns
			(-5.82)	(-2.29)		-3.19	(19.50
2	II	5.67±0.143	6.2±0.23 ns	5.87±0.19 ns	142.86±4.21	130±5.77 ns	143.3±6.0 ns
			(-9.35)	(-3-53)		-9	(-0.31)
3	III	5.67±0.143	6.3±0.43 ns	5.17±0.09*	142.86±4.21	136.7±11.67 ns	125.6±2.96 ns
			(-11.11)	-8.82		4.31)	-12.08
4	IV	5.67±0.143	6.0±0.12 ns	5.6±0.23 ns	142.86±4.21	145.0±10.4 ns	136.67±8.82 ns
			(-5.82)	(.1.23)		(-1.50)	-4.33
5	V	5.67±0.143	5.67±0.24 ns	5.63±0.27 ns	142.86±4.21	131.67±6.01 ns	121.67±7.26 ns
			(0.00)	-0.71		-7.83	-14.83
6	VI	5.67±0.143	5.8±0.17 ns	6.07±0.14 ns	142.86±4.21	123.33±7.26 ns	116.6.01 ns
			(-2.29)	(-7.05)		-13.67	-18.33
7	VII	5.67±0.143	6.1±0.15	5.87±0.08	142.86±4.21	141.67±11.67	141.67±13.64
			(-7.58)	(-3.53)		-0.83	-0.83
	F		0.559	2.602		0.693	2.405
	P		0.755	0.066		0.659	0.083

The values are expressed as mean± S.E; n=3 animals; P< 0.05 as compared with control; ns=the difference from control value is not significant

Plate 1 : Post Wound of 8th day

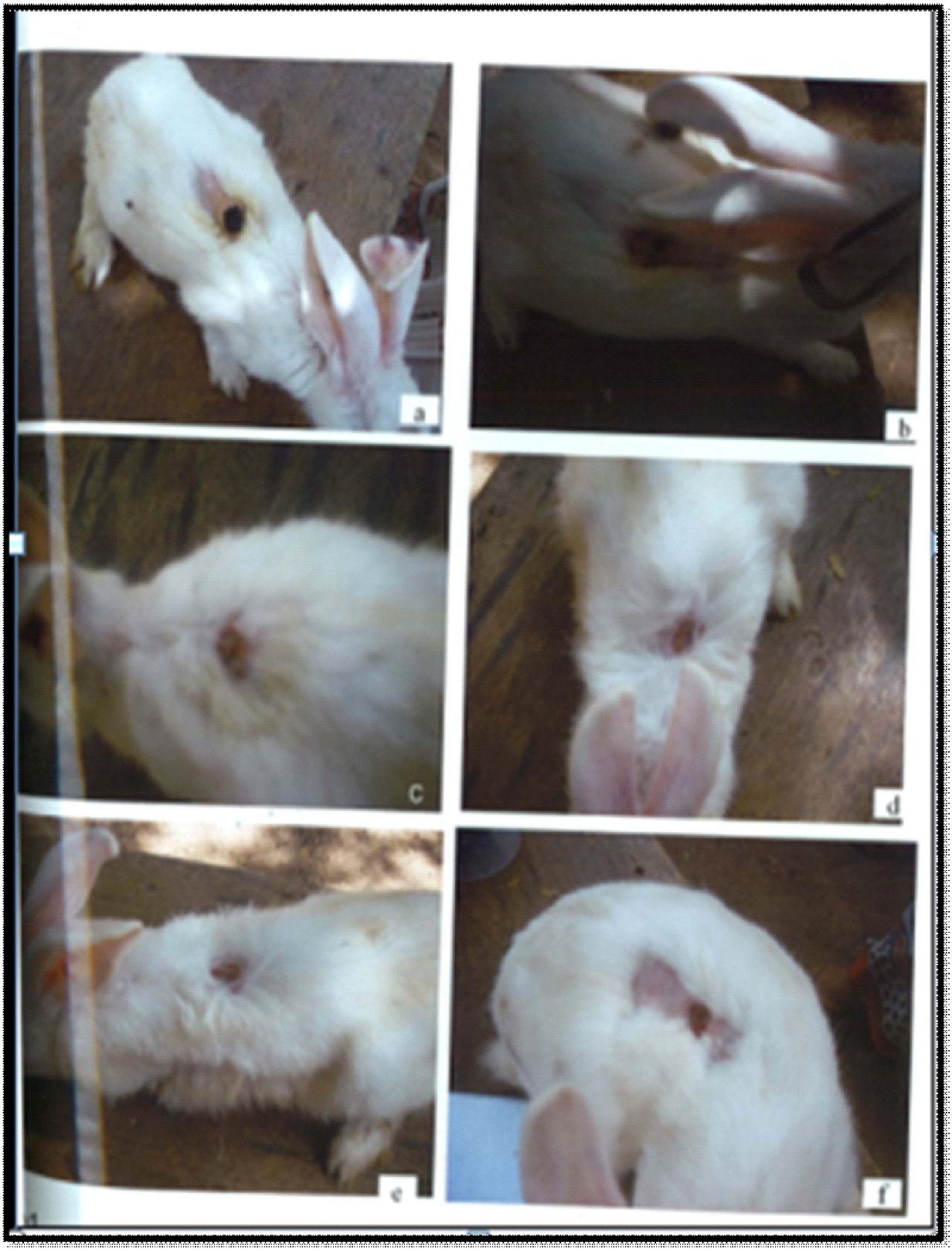

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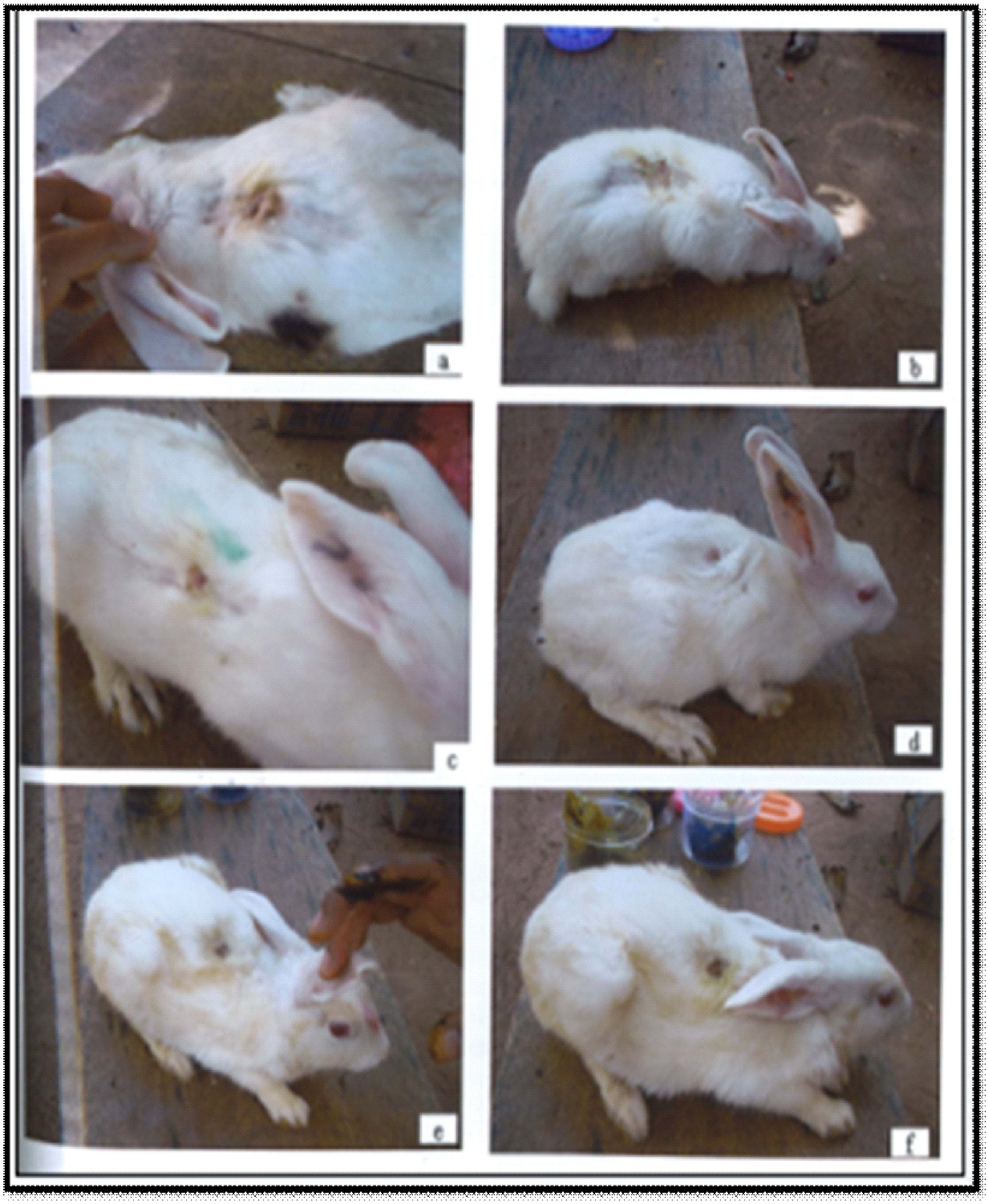
Plate 2: Post Wound of 16th day



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DISCUSSION

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity (Choudary, 2008). Earlier reports showed that the leaves of herbal plants of *Kaempferia galanga* (Shanbang *et al*., 2006), *Buddleji globosa* (Mensah *et al*., 2001) *Catharanthusu roseus* (Nayak and pereira,2006). *Evolvulus numularius* (Saini *et al*., 2007) and *Thespesia populnea* (Nagappa and Cheryan,2001) had significant wound healing properties. In the present study, *Andrographis echioides, Borreria hispida, Alpinia galanga* and *Curcuma longa* showed significant effect on wound healing in rabbits. Percentage of wound healing was high in Group II animals treated with formulation II and on 16th day the wound contraction was found to be 79%. All the three formulated ointments had shown more than 55% of healing of wound and among them, formulation II proved to be highly potential in wound healing. Period of epithelization of Group II animals required 16 days. The results of the present study also showed that the herbal ointment formulations were highly effective to heal when compared to the commercial synthetic ointments. *Alpinia galanga* has been reported to have flavanoids (Kumar *et al*., 2007), that have neuroprotective (Shi *et al*., 2006) effects and antiulcer properties which may promote wound healing property (Mitsui *et al*., 1976). *Curcuma longa* has natural antibiotic and antioxidant activity so that it was used in skin care lotions (Ashawat *et al*., 2007). *Borreria hispida* seeds have flavanoids which might have resulted in antioxidant activity because the lipid peroxidation released free radicals which have antioxidant property that reduce the tissue damage (Kaviarasan *et al*., 2008). Results of the present study showed that among the combinations tested, treatment II (*Andrographis echioides* 70%, *Borreria hispida* 20%, *Alpinia galanga* 5% and *Curcuma longa* 5%), had the highest efficiency in wound healing so that this proved to be an useful new combination for wound healing, which could be due to the presence of specific active principle in each plant which both individually and in combination exhibit wound healing properties.

The phytochemical analysis of the four plants selected in the present study, namely *Andrographis echioides, Borreria hispida, Alpinia galanga* and *Curcuma longa* have been made by many workers (Abdullah *et al*., 2015; Jain *et al*., 2012; Jirovetz *et al*., 2003; Vinayak Meti*et al.,* 2013; Poonguzhali *et al*., 2016; Nelson *et al*., 2017; Tayyem *et al*., 2006; Hong *et al*., 2014; Agnel Ruba and Mohan, 2016). They revealed the presence of secondary metabolites such as alkaloids, flavanoids, terpenoids and saponins. They are the broad groups of phytochemicals present in most of the plants. The subclass of these chemicals constitute the active principles which are generally species specific and exhibit therapeutic properties. For example, *Borreria hispida* shows the presence of borreline , alpinin in *Alpinia galanga,* curcumin in *Curcuma longa* and flavonones and aconitin in *Andrographis echioides.*

Thus, though it has been proved that the ointment formulation of the four herbs could be effectively used for wound healing, obviously it is due to the presence of specific active principles in such plants. Hence it has been planned to thoroughly investigate the phytochemical characteristics of these four plants in order to identify the active principles of each plant involved in wound healing.

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