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To Study the Efficacy of Different Formulations of Aloe Vera (Spp. *Aloe barbadensis*) On Wound Healing in Rats.

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ABSTRACT

The aim of the study was to evaluate the effect of different formulations of *Aloe vera* on wound healing using Burn model in rats and to compare these different formulations with *Framycetin sulphate*.The study was approved by the Institutional Animal Ethics Committee. 50 Sprague-Dawley rats, of either sex, weighing 150-200g, were selected randomly divided into the following groups: (n=10 each) Group I-Untreated**;** Group II-Aloe gel treated **;** Group III-Aloe pulp treated **;** Group IV-Aloe powder treated **;** Group V-Framycetin cream treated**.** The partial thickness burn wounds were inflicted upon animals under ketamine anaesthesia, by pouring boiling water (above 100 degree C). The study formulations were applied topically daily till the complete healing of the wound. The following parameters were measured:1.)Measurement of Wound Contracture 2.) Period of re-epithelization. In burn model, Aloe pulp and Framycetin treated groups showed maximum wound contracture on day 21 as compared to Untreated groups and shorter period of re-epithelization in Aloe pulp and Framycetin treated groups as compared to Untreated groups. In household burns, Aloe pulp obtained directly from the plant which is comparable to Framycetin cream can be recommended as a faster remedy, safe and readily available alternative.

Keywords: *Aloe vera*, burns, Framycetin, Aloe pulp

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INTRODUCTION

"Wound can be defined as disruption of cellular and anatomical or functional continuity of living tissues". Wounds are perhaps, inescapable events in the life of an organism and at times they are dangerous or even life threatening [1]. There are several types of wounds including surgical, traumatic and chronic wounds with chronic wounds being non healing wounds [2]. Currently the treatment options for majority of wounds include supportive measures like maintenance of hygiene, proper wound dressing, and topical use of antiseptics. These measures do not inherently improve the healing process, but promote healing of the wound by preventing infections[3]. There is a need to find products which show good wound healing activity and are cost-effective for better wound care.

The Limitations of topical antiseptics and some bioengineered agents upon wound Healing led the investigators to study the wound Healing effects of Aloe vera. Aloe vera (syn: *Aloe Barbadensis Miller*) belongs to the Lilaceal Family, a cactus like plant which is most widely used commercially and for its therapeutic properties. Aloe vera is reputed for its use in treatment of wounds. Evidence on the effects of its sap on wound healing, however, is limited and contradictory [4]. Some studies, for example, show that aloe vera promotes the rates of healing [5,6], while, in contrast, other studies show that wounds to which aloe vera gel was applied were significantly slower to heal than those treated with conventional medical preparations [7]. A 2007 review concluded that the cumulative evidence supports the use of aloe vera for the healing of first to second degree burns [8]. Topical application of aloe vera may also be effective for genital herpes and psoriasis [5,6]. However, it is not effective for the prevention of radiation-induced injuries. Although anecdotally useful, it has not been proven to offer protection from sunburn or suntan [9].

Gels from *Aloe vera* have been compared to those derived from other aloe species and with other plants, is used widely for burns and a host of skin afflictions. *Aloe vera* extracts might have antibacterial and antifungal activities, which possibly could help treat minor skin infections, such as boils and benign skin cysts and may inhibit growth of fungi causing tinea [10].

Healing may differ with different formulations of aloe vera depending upon its preparation. Hence this study was taken to evaluate the efficacy of different formulations (gel, powder and pulp) of *Aloe vera* and to determine their healing properties.

Aim and Objectives

- To evaluate the effect of different formulations (gel, powder and pulp) of *Aloe vera* on wound healing using Burn model in rats.
- To study these different formulations (gel, powder and pulp) by comparing their effect on wound healing using Burn model in rats.
- To compare these different formulations (gel, powder and pulp) with *Framycetin sulphate* on wound healing using Burn model in rats.

MATERIALS AND METHODS

Fifty Sprague-Dawley rats, of either sex, weighing 150-200g, were selected at random, and bred in the Animal House, Dr. D. Y. Patil Medical College, Pimpri, Pune, and were divided into the following groups, (n = 10 each):

Group I-Untreated
Group II-Aloe gel treated (clear, thin gelatinous material obtained by crushing the inner tissues of the leaves. (90% w/w) Obtained from Brihans Natural Products Ltd, Pune)
Group III-Aloe pulp treated (obtained from the aloe plant itself. It is 96%water.The pulp contains mucilaginous portion yielding pure aloe gel).
Group IV-Aloe powder treated (produced by drying the inner fillet of the leaf of the plant and milling it into a powder which is green-brown in color. Obtained from Research Lab Fine Chem Industries, Mumbai-02.)
Group V-Framycetin cream treated.(1% w/w framycetin) skin cream-20g cream(AVENTIS)

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The study was approved by the Institutional Animal Ethics Committee.

Procedure: (Modified Bairy KL,Somayaji SN,Rao CM method) **:**The partial thickness burn wounds are inflicted upon animals under ketamine anaesthesia, by pouring boiling water (above 100 degree C) instead of molten wax used in the original method [11].

A metal cylinder was made using a can which is split longitudinally. On the outer surface of this can a circular opening was made. To this circular opening a syrup bottle cap was glued which was made open on both sides. Thus the lower end of the syrup cap was attached to the cylinder which was covered by a water resistant substance. This procedure helped in maintaining the boiling water inside the required circumference area without causing leakage of water. Thus by pouring hot boiling water from above the cylindrical opening and maintaining a contact time of 1 minute to the skin surface, a burn wound was produced on all the rats in this model.

The study formulations were applied topically daily till the complete healing of the wound. The following parameters were evaluated.

Measurement of Wound Contracture: The reduction in the area of the excision wound on the days mentioned above, following infliction of the wound, is known as wound contracture. The faster wound contracture rates indicate a better wound healing activity by the formulation. The wound contracture was calculated by the following formula:

% closure=1-AD x100 { AO=wound area on day 0; AD=wound area on corresponding day}

The mean and S.D values were calculated.

Period of re-epithelization: The period of re-epithelization is defined as the number of days required for falling off of the dead tissue remnants without any residual raw wound. It gives a measure of the time taken of any particular treatment group to completely recover from the inflicted wound and again resemble the original skin structure and function. Shorter duration period indicates better wound healing activity of the study formulation.

RESULTS

In burn model, Aloe pulp (24.3±9.15 sq.mm) and Framycetin (14.6±10.99 sq.mm) treated groups showed maximum wound contracture on day 21 as compared to Untreated (148.5±39.67 sq.mm) group. Shorter period of re-epithelization was observed in Aloe pulp (16.6±1.24 days) and Framycetin (16.9±1.24 days) treated groups as compared to Untreated groups (29.8±1.46 days). The percentage of closure was also maximal with Aloe pulp (93%) and Framycetin (96%) treated groups as compared to Untreated groups (57%).

Figure 1: Comparison of burn wound area in study groups:

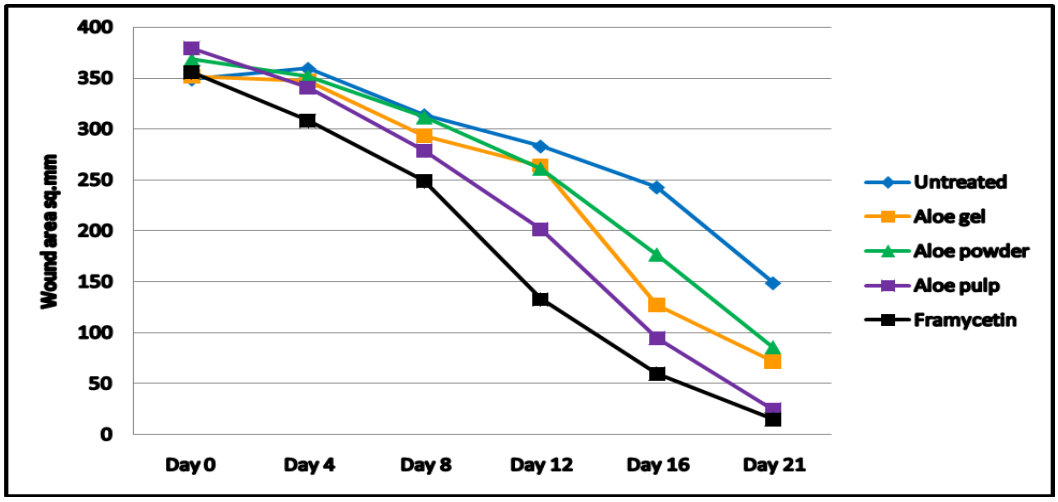

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Table 1: Comparison of wound area of burn model on following days: (Mean±SD)

Group	Day 0	Day 4	Day 8	Day 12	Day 16	Day 21
Untreated	348.9±23.97	359.4±31.37	313.7±27.54	283±34.42	242.7±31.83	148.5±39.67
Aloe gel	351.5±41.53	346.8± 32.02	293.1±31.37	263.7±23.16	127±22.37	71.3±28.52
Aloe powder	368.3± 35.12	351.5±41.89	311.6±42.40	261±43.29	176.5±47.23	85.2±32.94
Aloe pulp	379.5±24.93	340.5±29.26	278.6±30.47	201.5±24.24	94.8±25.93	24.3±9.15
Framycetin	355.7±28.69	308.1±53.07	248.6±29.07	132.8±32.21	59.5±19.33	14.6±10.99
F value	1.66	2.63	6.77	36.73	53.68	39.25
p value	>0.05	<0.05	<0.0001	<0.0001	<0.0001	<0.0001

Figure 2: Comparison between different groups on day 0 on burn wound area:

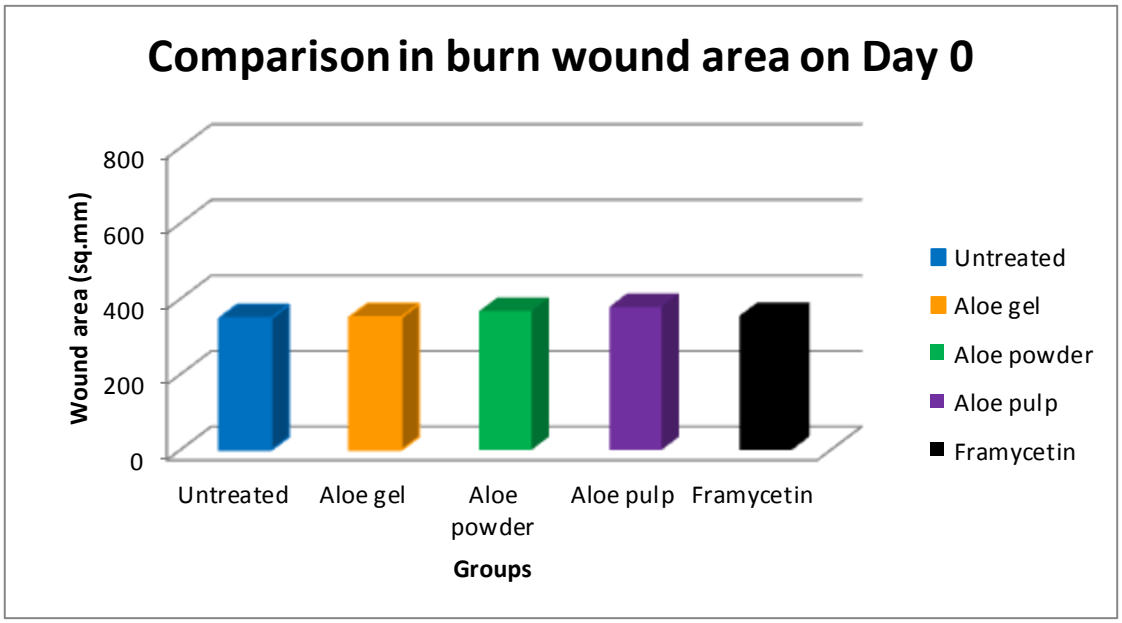

Figure 3: Comparison between different groups on day 4 on burn wound area:

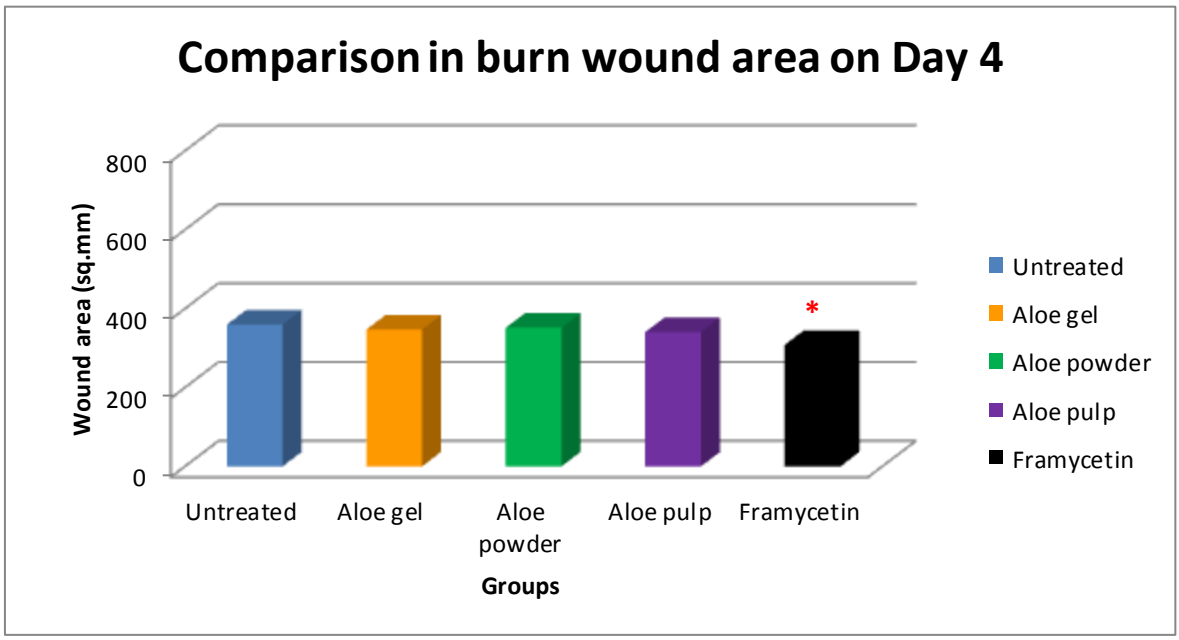

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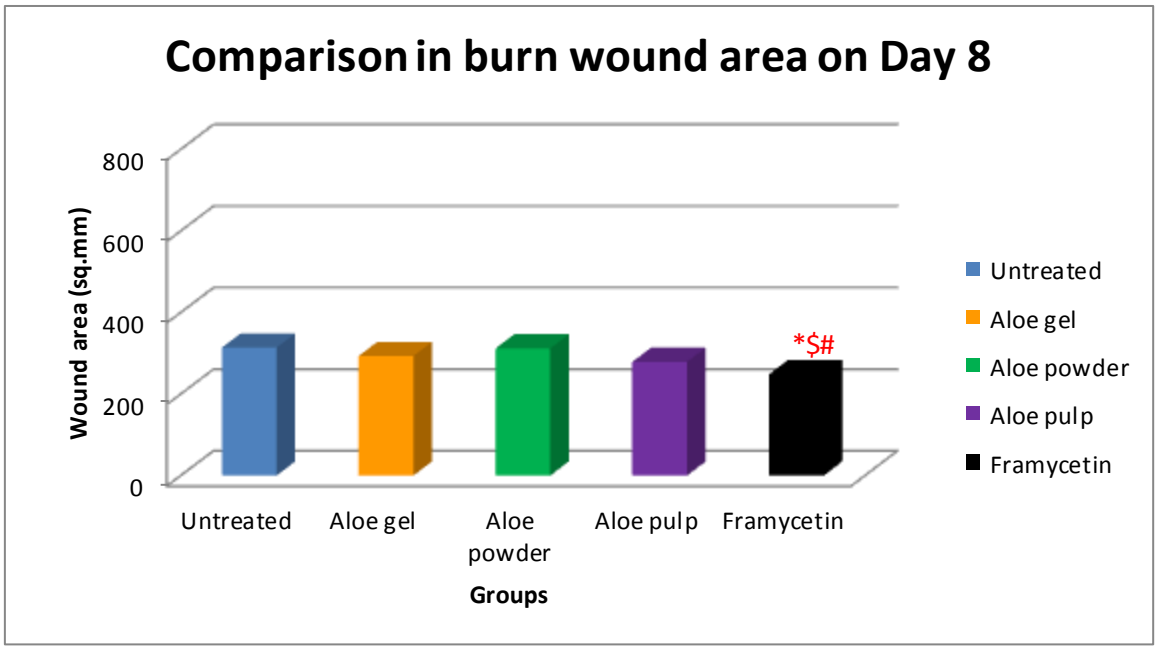
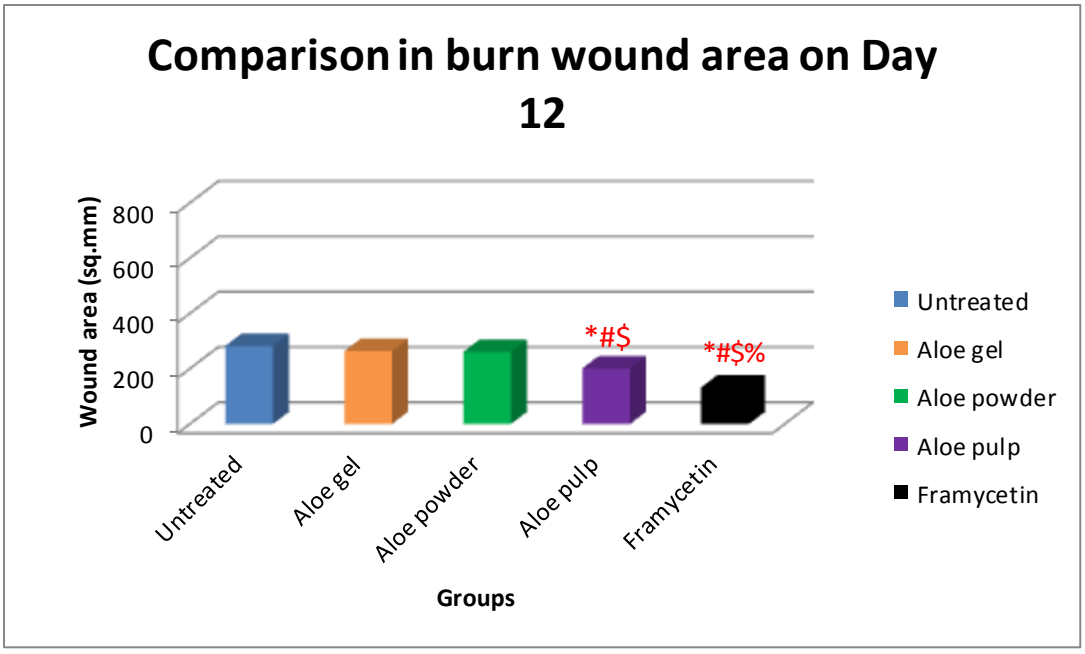
Figure 4: Comparison between different groups on day 8 on burn wound area:



*-p<0 05, significant as compared to Untreated, $- significant compared to Aloe gel, #- significant compared

Figure 5: Comparison between different groups on day 12 on burn wound area:



*-p<0 05, significant as compared to Untreated, #-significant compared to Aloe gel, $-significant compared to Aloe powder, %-significant compared to Aloe pulp

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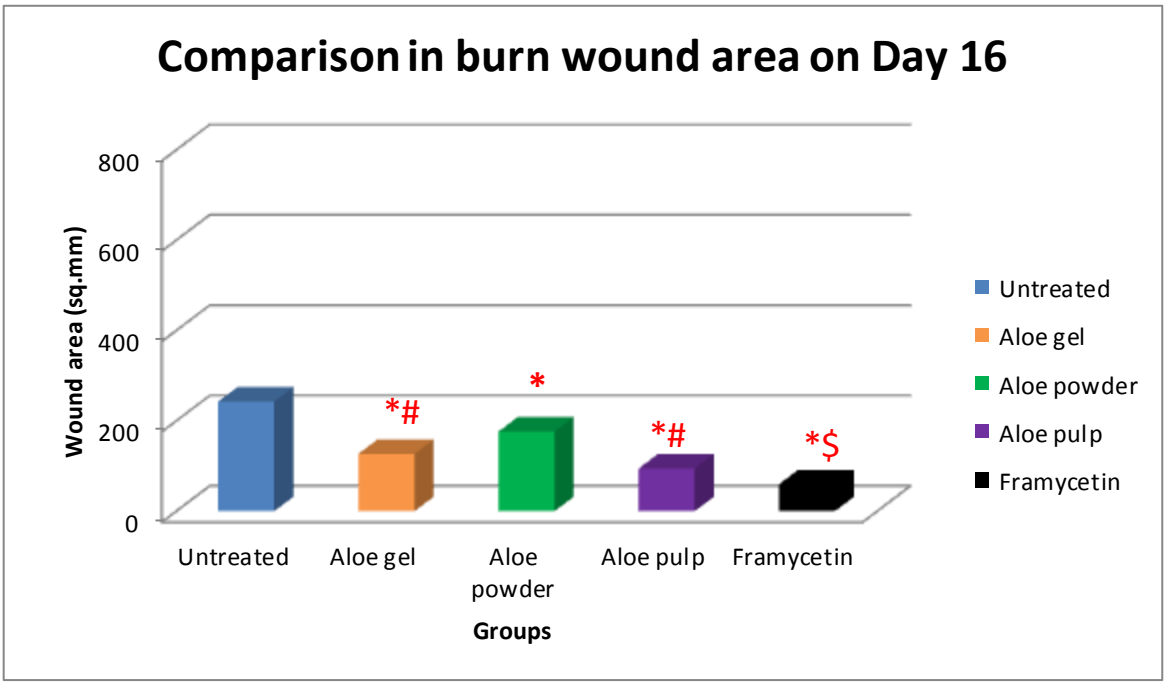
Figure 6: Comparison between different groups on day 16 on burn wound area:



*p<0 05, significant as compared to Untreated, #-significant compared to Aloe powder, $-significant

Figure 7: Comparison between different groups on day 21 on burn wound area:

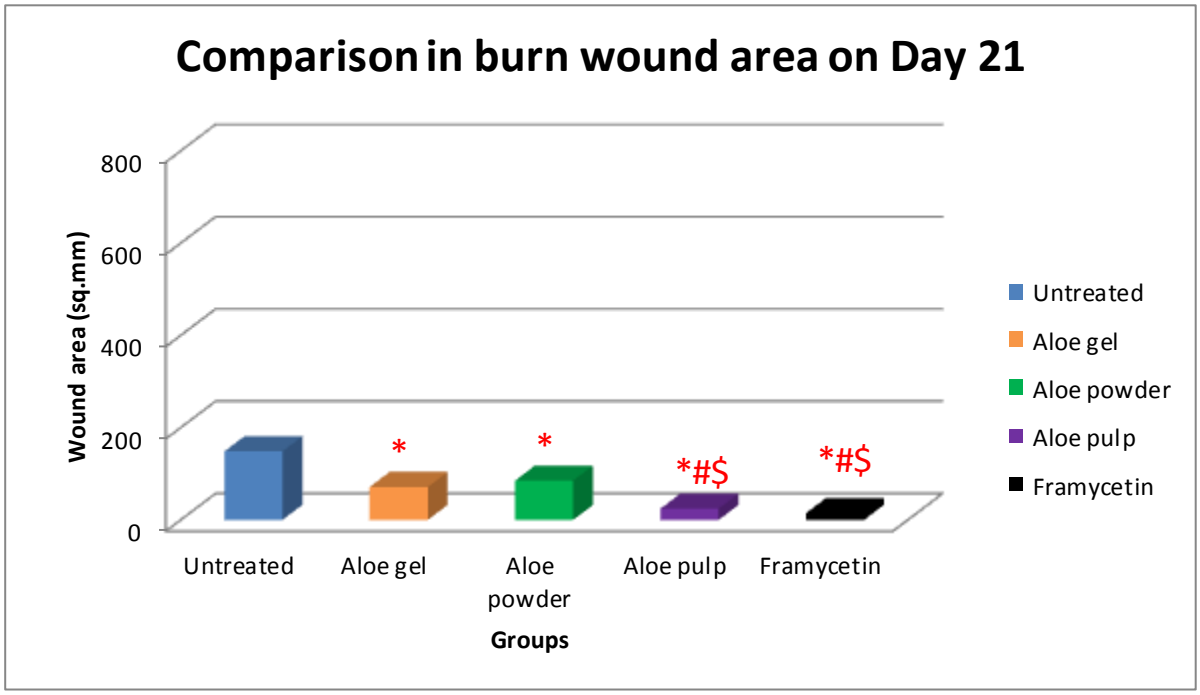


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Figure 8: Percentage of closure in Burn wound area in study groups:

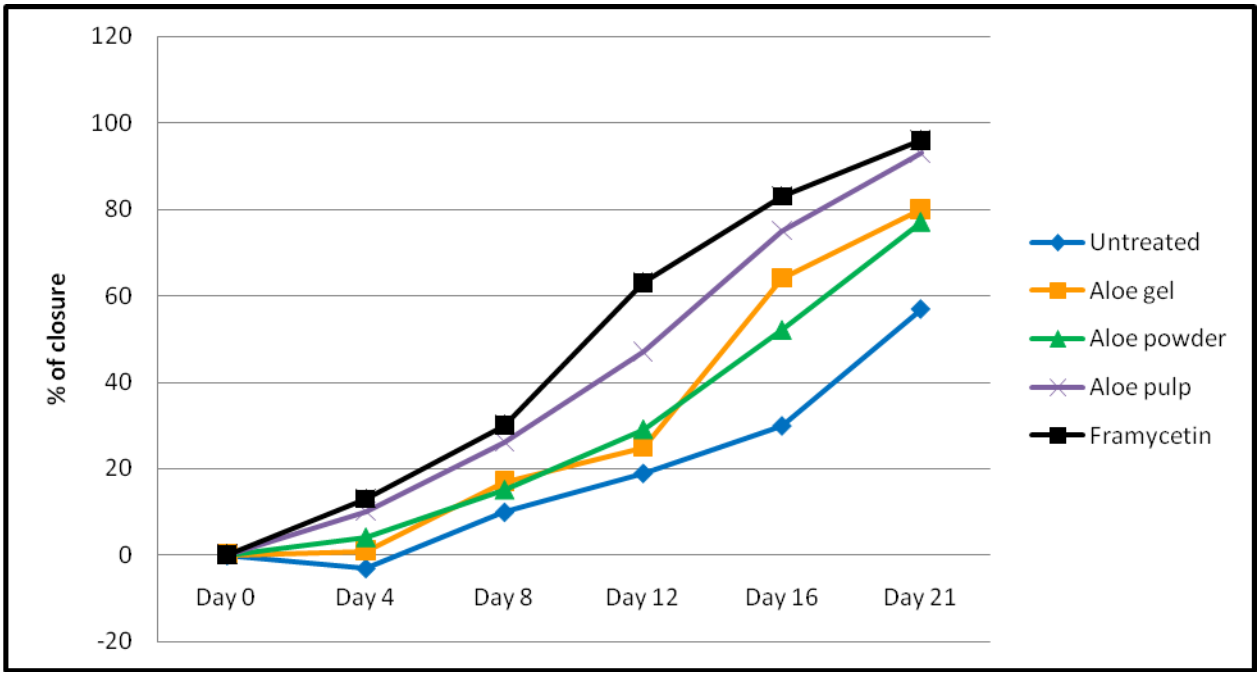
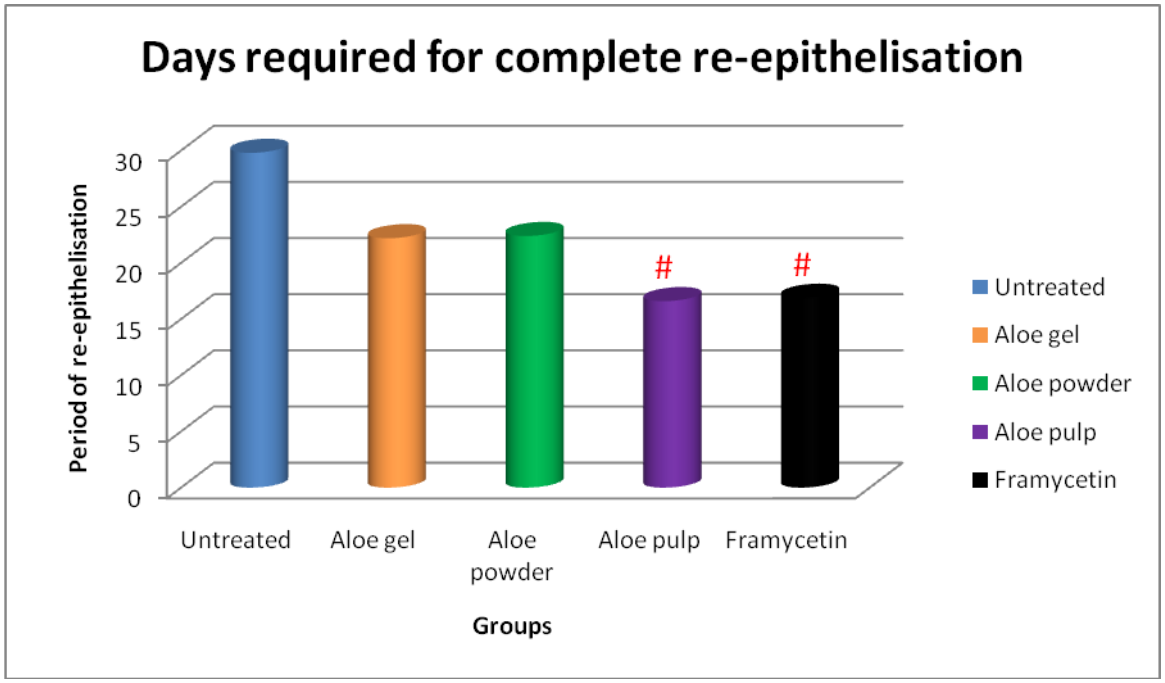


Figure 9: Days required for complete re-epithelisation



p<0 05, #- non significant

Significant figures :

+ Suggestive significance (P value: 0.05<P<0.10)

*** Moderately significant (P value:0.01<P ≤ 0.05)**

**** Strongly significant/highly significant (P value : P≤0.01)**

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Table 2: Percentage of closure in Burn wound area on day 0 to day 21 in study groups

Groups	% of closure in Burn wound area					
	Day 0	Day 4	Day 8	Day 12	Day 16	Day 21
Untreated	0	-3	10	19	30	57*
Aloe gel	0	1	17	25	64**	80**
Aloe powder	0	4	15	29	52*	77*
Aloe pulp	0	10	26	47**	75***	93***
Framycetin	0	13	30	63***	83***	96***

- p< 0.05, *- p<0.001, significant as compared to untreated

Table 3: Days required for Complete Re-epithelization:

Groups	**Period of re-epithelization** (Mean±SEM)
Untreated	29.8±1.46
Aloe gel	22.2±0.8#
Aloe powder	22.4±1.21#
Aloe pulp	16.6±1.24#*
Framycetin	16.9±1.24#*

Statistical Analysis

Results were expressed as Mean ± SD and statistical significance between means was analyzed using one-way analysis of covariance (ANOVA) followed by Tukey multiple comparison test. Significance was assessed at 5 % level of significance. Value of $p < 0.05$ was considered as statistically significant.

DISCUSSION

Wound healing involves different phases such as contraction, epithelization, granulation, collagenation which are concurrent but independent to each other. Any agent that promotes any of the above processes, will promote wound healing. Currently, the treatment options for majority of wounds include more of supportive measures rather than agents that actually enhance the process of wound healing. There is a need to find products which show good wound healing activity and which are cost-effective for better wound care.

One of the most effective means to achieve a microbial balance in a colonized or infected wound is the proper use of prophylactic topical agents. Maintaining wounds at low colonization levels diminishes the frequency and duration of septic episodes caused by wound flora [12]. The introduction of topical antimicrobial agents has resulted in a significant reduction in burn mortality to date [13,14]. The most common topical antimicrobial agent used is 1% silver sulfadiazine (SSD) cream and Framycetin[15]. Its side effects are delayed wound healing [16], resistance to 1% silver sulfadiazine cream, renal toxicity, and leukopenia: several studies confirmed that this topical cream should not be used for long periods on extensive wounds [17,18].

In this study, scalding burn wounds were produced by using boiling water where Framycetin was used as the active standard. The area of wound was measured as indication of wound contracture. There was significant decrease in the wound area with Aloe pulp (24.3±9.15) sq.mm as compared to Untreated group (148.5±39.67) sq.mm and shorter period of re-epithelization with Aloe pulp (16.6±1.24) days and Framycetin (16.9±1.24) treated groups. Although the present study did not explore the exact mechanism of prohealing of Aloe pulp, it could be attributed to both anti-inflammatory and antiseptic properties. Aloe Vera contains 6 antiseptic agents: Lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulfur. They all have inhibitory action on fungi, bacteria and viruses. An interesting theory was suggested by Morton, "It is possible that the seeming efficacy of Aloe pulp maybe attributable not to any miracle ingredient but merely to the fact that is 96% water and provides a means of making water available to injured tissues without sealing it off from the air"[19]. A clinical study showed that in the treatment of superficial and deep second-degree burns, addition of another prohealing agent like hyaluronic acid significantly overcame the disadvantages of topical

antiseptics normally used for burns [20]. The Aloe plant contains anthraquinones; glycosides, polysaccharides, aloeresins, glycomannans and b-sitosterol especially in the pulp which is different from the gel [21]. An increased synthesis of hyaluronic acid and dermatan sulfate in the granulation tissue of a healing wound following oral or topical treatment has been reported[22]. Based on these, we propose that Aloe vera pulp could significantly enrich the assortment of topical medications available for the treatment of burns. The burn wound inflicted in this study were superficial and the affected area was less than 15% and therefore these results cannot be directly extrapolated to humans. However in household burns involving upto 15%, Aloe pulp obtained directly from the plant can be recommended.

Formulations obtained from the plant directly or fresh leaves cut shows great promise by promoting wound healing in burns. More research is needed in terms of evaluating more formulations that can be obtained from the plant like Aloe ointment and Aloe cream too. Better formulations need to be designed for the use in different types of wound.

CONCLUSION

In household burns, Aloe pulp obtained directly from the plant which is comparable to Framycetin cream can be recommended as a faster remedy, safe and readily available alternative.

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