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COMBINED NATURALLY OCCURRING SUBSTANCES IN TOPICAL FORMULATIONS FOR WOUND HEALING: IN VITRO AND IN VIVO EVALUATION

REEM M. ALANAZI[1], SAYED AUDA[2], LUBNA Y. ASHRI[3], AHLAM ALHUSAINY[4], ABEER ALANAZI[5], RIHAB AHMED[6], AMANY MAHMOUD[7]

1: Master degree, Department of Pharmaceutics, as a Head of pharmacy at Imam Medical Services Centre, Imam Muhammad Ibn Saud Islamic University, Riyadh, Saudi Arabia

2: Ph.D., Associate Professor, Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

3: Associate Professor, Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

4: Associate Professor, Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

5: Master degree, Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

6: Master degree, Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

7: B. Pharm, Institute for drug development & innovation research, Assiut University, Assiut, Egypt

***Corresponding Author: Dr. Ph. Reem Alanazi: E Mail: rmalanazi@imamu.edu.sa**

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ABSTRACT

Background: Herbal remedies are agents that are known to hasten the healing process of a variety of wounds where plant products efficacy in wound healing was confirmed. Some of the most important healing plants and herbal products are Aloe Vera, sesame oil and shea butter. Thus, this study aimed to prepare ointments and creams that contain natural extracts of Aloe Vera, sesame oil and/or shea butter or a mixture thereof, characterize and investigate their efficacy in wound healing process and compare their effect to that of Mebo® ointment and Betika® cream.

Methodology: The formulations were prepared, and their physicochemical properties were characterized. These properties include the measurement of pH, homogeneity, appearance, rubout, spreadable, after-feel, smear type, product removal, Draize test of skin sensitivity. Additionally, the test of rancidity, rheological properties, stability, and antimicrobial activity of different formulations were investigated. The in vivo potential for wound healing of all formulations was also investigated using different groups of rats.

Results: Ointment formulations were homogeneous, smooth, greasy, and showed easy spreadability and difficult removal after application. They did not produce any skin reactions, i.e., erythema and edema. The pH of all ointments ranged between 4.2 to 6.7, which lie in the normal pH range of the skin. Additionally, the rheological behavior of all ointments was quite close to the behavior of mebo® and blank ointment base. Regarding the rancidity test, all formulations showed no rancidity at the beginning, i.e., oils used in the formulations were free from oxidation. All preparations were monitored for changes in physical properties at different temperatures (4, 25 and 37°C) for 90 days. The results indicated that all ointment formulations were stable at 4 and 25°C for 3 months. However, at 37°C few formulations showed some changes in their physical properties. Finally, in vivo studies indicated that all formulations showed positive effects on wound healing in rats.

Conclusion: The present study demonstrated that a blend ointment containing herbal mixture of 15% of Aloe Vera, sesame oil and shea butter have a potent, synergistic, and accelerative wound healing activity in rats compared with other groups. This mixture may be considered a new therapeutic strategy that is effective in wound management and treatment.

Keywords: Herbal Remedies, Healing Process, Chronic wounds, Ayurvedic plants

INTRODUCTION:

The skin is the largest organ in the human body, protecting underlying muscles, bones, ligaments, and internal organs **[1]**. It plays a crucial role in maintaining physiological homeostasis and protecting against various factors such as microorganisms, electromagnetic radiations, allergens, and the external environment **[2]**. Wounds, caused by various sources such as inflammatory skin diseases, skin cancer or burns, are widespread and impact patients' lives **[3]**. Acute wounds heal within 8-12 weeks, while chronic wounds are caused by defects in the healing process, resulting in slow healing or recurrence **[3]**. Chronic wounds are classified into four categories: diabetic foot ulcers, pressure ulcers, venous ulcers and arterial deficiency ulcers **[3]**.

Burns are wounds or injuries to the skin caused by thermal trauma, that when untreated they significantly impact the quality of human lives, public health, and economies. The World Health Organization (WHO) reported 300,000 deaths in 2012 due to burns **[4]**. Therefore, researchers are exploring materials and products to promote wound healing and reduce complications. These materials include antimicrobial and antiseptic agents, which can inhibit or prevent infection and reduce the host defense system's burden.

Plants play a significant role in treating wounds, burns, and skin disorders, with many used globally. Ayurvedic plants, such as Septilin, Acaiypha Wilkesiana, and pomegranate peel, have been used to treat skin infections and burns **[5]**. The present study aims to formulate, and study wound healing preparations containing Aloe Vera extract, sesame oil, and shea butter, or mixtures thereof focusing on their high effectiveness in wound healing.

MATERIALS AND METHODS:

Materials:

White soft paraffin, emulsifying wax, beeswax, sesame oil, and paraffin oil were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Shea butter and powdered Aloe Vera were obtained from Arab Chemists Company for cosmetics and medical industries Ltd. (Riyadh, Saudi Arabia). Mueller-Hinton agar medium was purchased from Merck®, Darmstadt, Germany. Mebo® ointment (contains sesame oil, beta-sitosterol, berberine, and beeswax) was purchased from al-dawaa pharmacies. Betika® cream (contains olive oil, sesame oil, Calendula officinalis flower extract, and Allantoin) was purchased from al-dawaa pharmacies. Other materials were of pharmaceutical grade and used as received.

Methods:

Preparation of Aloe Vera extract:

Approximately 200 g of coarse powdered Aloe Vera was added to 1000 mL of water. The extraction process was conducted in a Soxhlet apparatus for 24 h. The contents were then filtered by Whatman® No. 41 filter paper using a Buchner funnel to remove any undissolved solid particles. Then the solvent was evaporated at 50-65 °C using a rotary evaporator to give a dry extract. The extract was subjected to further drying in a desiccator until a constant weight was reached **[6]**.

Formulation of hydrophilic ointments containing the natural products:

In this study, five different ointment formulations were prepared, namely F1, F2, F3, F4, and F9. The ointments were prepared by mixing all components that are shown in (**Table1**) for each formulation, in a 250 mL-beaker with paraffin oil, except for Aloe Vera extract in F3 and F4. Each mixture was heated in a water bath at 70 °C until complete melting. The beaker was then removed from the heat source and allowed to cool. In case of F3 and F4, Aloe Vera extract and paraffin oil were levigated with the melted mixture at 40-45 °C. The components were stirred well until complete homogeneity **[7]**.

Table 1: Composition of different ointments formulations

Components % (w/w)		F1	F2	F3	F4	F9
Ointments	White soft paraffin	45	45	45	45	50
	Emulsifying wax	10	10	10	10	15
	Paraffin oil	30	30	30	30	35
	Sesame oil	15			5	
	Shea butter		15		5	
	Aloe Vera Extract			15	5	
	Total weight	100	100	100	100	100

Formulation of creams containing the natural products:

Different cream formulations were prepared by heating the oil phase that consists of beeswax and emulsifying wax in addition to the selected active component (shea butter and/or selected oil(s)) on a water bath at 70 °C until complete melting (phase 1). The water was then heated in a water bath at 70 °C (phase 2). Phase 2 was then added to phase 1 with vigorous stirring until a homogeneous cream is formed. In case of Aloe Vera containing cream, Aloe Vera extract was dissolved in the specified amount of water in phase 2 before mixing with phase 1 **(Table 2)** **[8]**.

Table 2: Composition of different creams formulations

Components % (w/w)		F5	F6	F7	F8	F10
Creams	Distilled water	50	50	50	50	55
	Emulsifying wax	12	12	12	12	17
	Paraffin oil	20	20	20	20	25
	Bees wax	3	3	3	3	3
	Sesame oil	15			5	
	Shea butter		15		5	
	Aloe Vera Extract			15	5	
	Total weight	100	100	100	100	100

In vitro evaluation of ointments and creams:

Ointments and creams under investigation were evaluated for homogeneity, appearance, rubout, after feeling, type of smear, product removal, and Draize skin sensitivity test by visual inspection. In addition, pH measurements, rheological properties, stability, and in vitro antimicrobial activity were also evaluated **[9]**. The homogeneity of different formulations was tested through visual inspection and physical touch with the bare hands **[10]**. The appearance, texture and color of different ointments and creams formulations were tested and evaluated visually **[11]**. The rubout study includes testing the spreadability and wetness of different formulations was also performed. Spreadability may be expressed by the extent of the area to which the topical application spreads when applied to the affected parts on the skin and wetness was determined by applying creams or ointments on skin surface of rats **[11]**. To evaluate the after feeling of the preparations, fixed amounts of ointments and creams were applied to the skin and the emollience and slipperiness were tested **[9]**. It was determined by applying ointments and creams on the skin surface of rats. After application, the type of film or smear formed on the skin was evaluated **[9]**. Ointments and creams were applied on shaved intact skin of Wistar albino rats and examined for any changes on the skin after 24 h. These changes include redness, burning and edema **[12]**. As far as the rancidity test is concerned, 10 mL of concentrated hydrochloric acid was added to 10 mL of cream or ointment and 10 mL of Phloroglucinol solution and shaken for one minute. The material was taken to have passed the rancidity test if no pink or red color is developed **[12]**. To measure the preparations' pH values, 1 g of ointments or creams was diluted with 100 mL of distilled water and stored for 2 h. The pH was then measured using a digital pH meter (Mettler Toledo, Switzerland) and the average of three reading was taken **[13]**. The viscosity of different formulations was determined at 25°C using cone and plate Brookfield viscometer with spindle 25 (model Hadi-II, Middleboro, MA). Specific quantity of each formulation to be examined was placed on the plate and the rate of shear was 6 rpm. All measurements were made in triplicate and then the average was taken **[12]**. Finally, all formulations were monitored for pH, odor, color, viscosity and homogeneity by changing temperature (4, 25 and 37 °C) for 90 days. The formulations were monitored every two weeks and the changes were recorded **[11]**.

In vivo evaluation of wound healing activity:

Animals:

Adult female Wistar rats of weight ranged from 180 to 200 g were obtained from the animal house of King Saud University, Riyadh, Saudi Arabia. The animals were housed in plastic cages under standard environmental conditions of temperature, humidity, and light/dark cycle. They were provided with standard laboratory chow and water ad libitum. The institutional animal ethics committee (KSAU-HSREC-20-278) approved the experimental protocol.

Excision wound model

The excision wound study included the use of female Wistar albino rats (180 - 200g). Animals were chosen and divided into 13 groups of five animals per group. Intramuscular (IM) injection of xylazine 5 mg/kg, and ketamine 40 mg/kg **[14]** were used to anaesthetize the rats, which were depilated before wounding at the predetermined site **[7]**. An excision circular wound was induced by removing away approximately 572 mm2 full thickness of the assigned area on the anterior-dorsal side of each rat **[15]**. Each group of rats, namely G1 to G10, was treated with one of the 10 preparations and holds a number corresponding to the used formulation number. Additionally, G11 and G12 groups were treated with Malibo and …, respectively while G13 was the control non-treated group. All formulations were applied to corresponding groups twice daily for 18 days, which began from the day of wounding. The wound area was measured every other day by placing a transparent paper over the wound and tracing it out, the area of this impression was calculated using graph sheet, and wound contraction was expressed as percentage of contraction. Wound closure time was recorded for total wound healing **[16]**.

Histopathological evaluation:

On day 14, after euthanasia, the skin tissues were collected from the wound area and fixed in 10% neutral buffered formalin. The tissues were then processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histopathological examination.

Percentage wound recovery:

Wound areas were measured on different days and the recovery percentage was evaluated with the following formula. Wound size area in mm^2 and it measured area of a circle by $A = \pi r^2$

Where r = radius and π = 3.14

$$\textbf{Recovery percentage} = \frac{\text{Wound surface on the first day} - \text{wound surface on day X}}{\text{Wound surface on the first day}} * 100$$

Where X= day of wound surface measurement [17].

Statistical analysis:

The data obtained from the in vivo study were analyzed using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA).

RESULTS:

Evaluation of prepared ointments and creams:

The results showed that ointments were homogeneous, spread easily, soft, smooth, greasy, and non-water washable. On the other hand, creams were homogeneous, spread easily, soft, smooth, non-greasy, and water washable. Both ointments and creams did not produce any skin irritation when applied to the skin for a 24 h period.

The rancidity test was performed using phloroglucinol solution, which indicates that none of the tested formulations showed pink or red color, indicating that the tested formulations are not rancid. Furthermore, the pH of all formulations ranged between 4 to 6.7 (**Table 3),** making them suitable for use on the skin. The viscosity of the tested formulations was almost similar to the corresponding blank bases and marketed formulas.

Concerning stability studies, they were conducted on all prepared formulations at different temperatures (4, 25, and 37 °C) for 90 days. In general, the viscosity of ointment preparations increased when cooled to 4 °C with no significant changes in their pH, homogeneity, color, and odor. Besides, no changes in viscosity, pH, homogeneity, color, and odor were recorded when ointment formulations were maintained at 25 °C for 90 days. additionally, at 37 °C the instability of F1 has been shown as changes in its color and odor after only 45 days. Change in its homogeneity was also observed over time, particularly after 60 days with a noticeable decrease in viscosity and pH values over the period of 90 days. Moreover, accelerated stability studies of ointment preparations F2, F3 and F4 at 37 °C with time interval of 0, 15, 30, 45, 60 days gave only slight changes in pH and viscosity but at 75- and 90-days remarkable changes were observed in pH, viscosity, homogeneity, and physical characters. It is worth noting that at 37 °C the physical properties of F1 have changed faster than F2, F3 and F4.

The same stability studies were performed on cream preparations where, when incubated at 4 °C, they showed stability in pH, odor, color, homogeneity, with a slight increase in viscosity over 90 days. However, when the same study was conducted on cream formulas at 25 °C, the physical properties (color and odor) of F5 and F6 changed over time, particularly after only 45 days. Whereas F7 and F8 have changed in their physical properties (color and odor) after 60 days. On the other hand, at 37 °C, the pH and viscosity of F5 and F6 did not change for up to 30 days, after which the preparations became non-homogeneous, lost their odor, color, decreased in pH as well as viscosity. Other cream formulations, namely F7 and F8, when stored at 37 °C, turned to non-homogeneous, lost their odor, color, decreased viscosity, and pH over time particularly after 45 days.

It is worth mentioning that blank ointment and cream bases showed high stability at 4, 25 and 37 °C at different time intervals of 0, 15, 30, 45, 60, 75 and 90 days. They showed no change in pH, viscosity, homogeneity, color and odor.

Generally, creams and ointments are sensitive to temperature changes, but there is a negative non-linear relationship between temperature and viscosity. This can be explained by the fact that thermal fluctuations reduce intermolecular cohesion, and changes in temperature cause changes in interfacial tension, viscosity, and nature of surfactants. For ointments with a white base, the viscosity is reduced by a factor of 0.5 for every 5 °C rise in temperature.

As far as the microbiological activity is concerned, it was found that all the tested herbal ointment and cream formulations achieved antimicrobial activities against several species of microorganisms with inhibition zones ranging from 13 to 38 mm **(Table 4)**.

F2 and F4 showed good and similar antibacterial activities against *Escherichia coli* (*E. coli*) (17 ± 0.01 and 17 ± 0.06 mm, respectively). Their antimicrobial effect against E. coli is greater than F1 (13 ± 0.02 mm) and F3 (15 ± 0.03mm). It is worth mentioning that the commercially available ointment formula (Mebo®) and F9 did not report any antibacterial activity against E. coli. However, the antibacterial activities of ointment formulations (F1, F2, F3, F4, F9 and Mebo®) showed that only F3 has a significant activity against pseudomonas aeruginosa (*P. aeruginosa*) (27 ± 0.02 mm), while other formulations reported no zones of inhibition.

The antimicrobial screening of ointment formulations (F1, F2, F3, F4, F9, and Mebo®) against Bacillus subtilis (*B. subtilis*) exhibited that F1 and F4 had similar antibacterial activities (25 ± 0.01 mm and 25 ± 0.02 mm, respectively). Whereas F2 and F3 were less potent (20 ± 0.14 mm and 19 ± 0.00 mm, respectively), while F9 and Mebo® had no effect against *B. subtilis*.

When the same antimicrobial screening test was applied against Staphylococcus aureus (S. aureus), F4 showed the highest zone of inhibition (35 ± 0.01 mm), which was greater than F1, F2, and F3. Additionally, it was also found that F9 and Mebo® have no effect against S. aureus.

On the other hand, when the anti-fungal activity of these formulated ointments was studied, it has been proven that F4 (13 ± 0.012 mm) was less effective against *Candida albicans* (*C. albicans*) than F3 (15 ± 0.02 mm) and more potent than F2 (10 ± 0.07 mm). This study ruled out the antifungal effect of F1, F9 and Mebo®.

Unlike the ointment preparations, the blend cream formula F8 was less effective against E. coli (25 ± 0.01 mm) when compared with F7 (30 ± 0.01mm), while greater than F5 (16 ± 0.01 mm) and F6 (16 ± 0.01mm). F10 and F12 showed no antibacterial activity against the same bacteria. However, cup plate agar diffusion test for (F5, F6, F7, F8, F10 and betika®) against *P. aeruginosa* showed that only F7 has a significant antibacterial activity (27±0.02 mm), while the other formulations reported no effect. F5 and F8 were effective against S. aureus and they reported almost similar zones of inhibitions (35 ± 0.05 mm and 35 ± 0.01 mm, respectively), which were less than F7 (38 ± 0.01 mm), while F6 reported 32 ± 0.02 mm as a zone of inhibition. Similarly, the antimicrobial superiority of F7 (30 ± 0.02 mm) over other cream preparations was observed again against *B. subtilis* whereas the inhibition zones of F5, F6 and F8 were (25 ± 0.01 mm, 25 ± 0.01 mm and 29 ± 0.01 mm, respectively). F10 and betika® showed no antibacterial activity against S, aureus and *B. subtilis*. Furthermore, the formulated creams were evaluated for their antifungal activities. The results showed that the antifungal effect of F8 (25 ± 0.02 mm) against *C. albicans* was less than F7 (30 ± 0.01 mm) and greater than F6 (15 ± 0.00 mm). However, F5, F10 and betika® have no effect on *C. albicans*.

Wound healing activity

The current study evaluated wound healing effects of various formulations on experimentally induced wounds in rats for 18 days. In the case of ointments, results showed that F4 and Mebo® had the best wound healing activity on the 15th day, which is significantly ($P < 0.001$) greater than that of other groups. The results of this study showed that in F4 and Mebo® the percentage of wound contraction ranged from 19.59 % to 79.55 % and 15.36 % to 78.51%, respectively in the period from 3 to 9 days and from 97% and 96.35% to 100 % for F4 and Mebo®, respectively in the period from 12 to 15 days. The percentage of wound contraction in rats, which were treated externally with F1, F3, and F8 ranged from 11.47 % to 62.66 %, 12.53 % to 64.57%, and 11.94 % to 63.34%, respectively in the period from 3 to 9 days. While this percentage improved to 100% from 78.74%, 81.37%, and 80.49%, respectively in the period from 12 to 18 days. Furthermore, the percentage of wound contraction for F2, F5 and F7 ranged from 9.14 % to 50.88%, 7.04% to 45.20% and 10.43% to 48.75%, respectively in the period from 3 to 9 days and from 75.28 % to 99.74%, 72.83% to 99.41% and 75.18% to 99.64%, respectively in the period from 12 to 18 days.

As far as cream formulations are concerned, F6 and Betika® showed a percentage of wound contraction that ranged from 5.53% to 38.68 % and 7.17% to 40.84%, respectively in the period from 3 to 9 and 67.38% to 99.21% and 72.23% to 99.30%, respectively in the period from 12 to 18 days. As noted, for F9 and F10, the percentage of wound contraction ranged from 3.72 % to 30.20 % and 3.71 % to 28.88 %, respectively in the period from 3 to 9 days and from 55.83% to 98.84% and 53.96% to 98.56%, respectively in the period from 12 to 18 days.

The lowest rate of wound healing was observed in the untreated group, where the percentage of wound contraction ranged from 1.11 % to 25.25% in the period from 3 to 9 days and from 50.75% to 98.13% in the period from 12 to 18 days. In general, this study showed that ointment formulations had better wound-healing activity than cream formulas and it showed that the blend ointment formulation had the most potent wound healing activity in rats compared to other formulations. In general, the excision wound model study results showed that the extent of wound healing properties of different formulations is in the following order: G4 > G 11 > G 3 > G 8 > G 1 > G 2 > G 7 > G 5 > G 12 > G 6 > G 9 > G 10 >G 13 **(Table 5)**

Table 3: pH measurement and viscosity results of different formulations

Abbreviation	Formulations	pH	Viscosity (pa.s)
F1	Sesame oil ointment	6.2 ± 0.05	1.5 ± 0.02
F2	Shea butter ointment	6.4 ± 0.02	2 ± 0.07
F3	Aloe Vera ointment	4.2 ± 0.01	1.8 ± 0.01
F4	Blend ointment	4.4 ± 0.03	1.7 ± 0.02
F9	Ointment base	6.1 ± 0.05	2.2 ± 0.03
Commercial formula	Mebo®	6.7 ± 0.00	2 ± 0.06
F5	Sesame oil cream	5.6 ± 0.03	1.3 ± 0.01
F6	Shea butter cream	5.5 ± 0.02	1.51 ± 0.0
F7	Aloe Vera cream	4.2 ± 0.03	1.5 ± 0.024
F8	Blend cream	4.3 ± 0.03	1.4 ± 0.02
F10	Cream base	6.2 ± 0.01	1.56 ± 0.03
Commercial formula	Betika ®	6.6 ± 0.03	1.34 ± 0.02

Table 4: Evaluation of antimicrobial activities of creams by Cup plate diffusion method						
Abbreviation	Formulation	Gram-negative bacteria		Gram-positive bacteria		Fungi
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*Yeast C. albicans*
F1	Sesame oil ointment	13 ± 0.02	NZ	34 ± 0.01	25 ± 0.01	NZ
F2	Shea butter ointment	17 ± 0.01	NZ	33 ± 0.02	20 ± 0.14	10 ± 0.07
F3	Aloe Vera ointment	15 ± 0.03	27 ± 0.02	30 ± 0.04	19 ± 0.00	15 ± 0.02
F4	Blend ointment	17 ± 0.06	NZ	35 ± 0.01	25 ± 0.02	13 ± 0.02
F9	Ointment base	NZ	NZ	NZ	NZ	NZ
Commercial formula	Mebo®	NZ	NZ	NZ	NZ	NZ
F5	Sesame oil cream	16 ± 0.01	NZ	35 ± 0.05	25 ± 0.01	NZ
F6	Shea butter cream	16 ± 0.01	NZ	32 ± 0.02	25 ± 0.01	15 ± 0.00
F7	Aloe Vera cream	30 ± 0.01	27 ± 0.02	38 ± 0.01	30 ± 0.02	30 ± 0.01
F8	Blend cream	25 ± 0.01	NZ	35 ± 0.01	29 ± 0.01	25 ± 0.02
F10	Cream base	NZ	NZ	NZ	NZ	NZ
Commercial formula	Betika ®	NZ	NZ	NZ	NZ	NZ
NZ: no inhibition zone						

Table 5: The measurements of wound areas during a period of 18 days with the percent of contraction using different formulations							
	0 day	3rd day	6th day	9th day	12th day	15th day	18th day
G 1	577.8±13.5 (0%)	511.5 ± 6.6 (11.47%)	340.5±6.4 (41%)	215.7±3.9 (62.66%)	122.8±4.0 (78.74%)	23.21±1.9 (95.98 %)	0 (100%)
G2	561.9 ±10.4 (0%)	510.5 ±8.17 (9.14 %)	359.5±1.94 (36 %)	276±4.24 (50.88%)	138.9±2.54 (75.28 %)	68.29±1.72 (87.84%)	1.445±0.20 (99.74%)
G 3	567.4±18.12 (0%)	496.3 ±3.57 (12.53 %)	334 ±4.07 (41.13 %)	201±4.31 (64.57%)	105.7±2.49 (81.37%)	21.26±1.01 (96.25%)	0 (100%)
G 4	567±5.25 (0%)	455.9±2.68 (19.59 %)	290.9± 4.36 (48.69%)	115.9±2.29 (79.55.%)	16.48±1.10 (97%)	0 (100%)	0 (100%)
G 5	583.1 ±10.8 (0%)	542±1.97 (7.04%)	395.6±1.76 (32.15%)	319.5±3.53 (45.20%)	158.4±3.18 (72.83%)	87.84±2.50 (84.93%)	3.383±0.15 (99.41%)
G 6	567.2±13.18 (0%)	535.8±1.96 (5.53%)	411.7±4.84 (27.41%)	347.8±0.95 (38.68 %)	185±2.84 (67.38%)	106.1±2.30 (81,29%)	4.435±0.27 (99.21%)
G 7	572.2 ± 0.02 (0%)	512.5±6.67 (10.43%)	375.7±4.27 (34.34%)	293.2±3.57 (48.75%)	142±1.04 (75.18%)	78.6±3.21 (86.26%)	2.048±0.37 (99.64%)
G 8	572.6±14.85 (0%)	504.2 ±4.69 (11.94 %)	330±6.57 (42.36%)	209.9±2.45 (63.34%)	111.7±2.66 (80.49%)	23.32±0.42 (95.92%)	0 (100%)
G 9	561.8 ± 6.06 (0%)	540.9 ±1.19 (3.72 %)	461.7±5.49 (17.81%)	392.1±3.35 (30.20 %)	248.1±4.47 (55.83%)	124.2±2.97 (77.89%)	6.483±1.00 (98.84%)
G 10	572.6±15.13 (0%)	551.3 ± 2.95 (3.71 %)	476±4.86 (16.87 %)	407.2±5.73 (28.88 %)	263.6±2.96 (53.96%)	132.7±4.15 (76.82%)	8.2±0.67 (98.56%)
G 11	583.1 ± 10.8 (0%)	493.5 ± 2.99 (15.36 %)	300.8±4.61 (48.41 %)	125.3±4.71 (78.51%)	21.26±1.01 (96.35%)	0 (100%)	0 (100%)
G 12	561.9 ± 10.4 (0%)	521.6 ± 7.63 (7.17%)	396.5±0.88 (29.43%)	332.4±4.85 (40.84%)	156±3 (72.23%)	89.08±2.54 (84.14%)	3.898±0.26 (99.30%)
G 13	572.6±14.85 (0%)	566.2±14.86 (1.11 %)	521.6±6.48 (8.90%)	428±3.50 (25.25%)	282±4.92 (50.75%)	147.9±3.45 (74.17%)	10.68±1.09 (98.13%)

Histopathological Studies

A study examining 12 treated groups showed well-formed normal epithelialization, restoration of adnexa, and fibrosis within the dermis. The treated group had a higher percentage of eosinophilic collagen tissue and neovascularization, suggesting healing by fibrosis. The total healing score was calculated, with lower scores indicating poorer wound healing. Risk factors in the study group were correlated with delayed wound healing. Maximum healing was reported in the treated group in the following order, the highest healing reported in F4 , F8 , Mebo®, F1, F3 , F2, F7, F5, F6 , Betika®, F9 , F10 , and finally the untreated groups.

DISCUSSION:

In the current study, ointments and creams containing Aloe Vera, sesame oil, shea butter or mixture thereof were successfully prepared. Physicochemical characterization, in vitro and in vivo studies were carried out to determine their properties and their activity as wound healing remedies. It was found that the faster wound contraction rate of F4 (blend ointment containing sesame oil, shea butter, and Aloe Vera extract), maybe due to motivated fibroblast activity and collagen proliferation and increasing the content of granulation tissue and tissue crosslinking **[18]**. The phytochemical constituents of the herbal extract of Aloe Vera contains protein factors such as growth factors, cell migration-related factors, matrix-forming factors, and matrix-degradation factors, enzymes, vitamins, minerals, which have anti-inflammatory, antioxidants, antiseptic, immunomodulatory effects and antimicrobial properties **[19]**. Sesame oil has endogenous antioxidants, as it contains gamma tocopherols along with sesaminol, sesamin and vitamin E which help its anti-inflammatory, antibacterial and antioxidants effects **[20, 21]**. Whereas shea butter contains mainly triterpene alcohols with some hydrocarbons, sterols, and other minor components such as vitamin E, A, D, B and F, minerals and α-amyrin with potent anti-inflammatory, antibacterial and antioxidant properties **[22]**.

Moreover , the physical characteristics of the prepared ointments and creams were consistent with what is reported previously in literatures **[9]** as the primary features **[11]**. In addition, in all formulations, pH ranged between 4 to 6.7, which make them suitable for use on the skin **[23]**. The viscosity test results of studied ointments and creams were almost similar to the corresponding blank bases and marketed formulas. The slight difference in the degree of viscosity may be attributed to the difference in active substance concentrations in the studied formulas **[24]**.

Generally, creams and ointments tend to be very sensitive to temperature changes and there is a negative non-linear relationship between temperature and viscosity. Thermal fluctuations reduce intermolecular cohesion and cause changes in interfacial tension, viscosity and nature of the surfactants (hydrophilicity-lipophilicity) **[25, 26]**. It was reported that for ointments whose base is predominately white ointment, the viscosity is reduced by a factor of 0.5 for every 5 °C rise in temperature **[27]**. Furthermore, change in pH of different formulations was significant at different levels of time and temperature **[25]**. This can be explained by the fact that under the influence of temperature, fats and oils are susceptible to oxidation primarily leading to the formation of hydroperoxides. These hydroperoxides are highly reactive and rapidly react with secondary oxidative products especially at high temperatures causing undesirable rancid odors **[28, 29]**. The oxidation mechanism and the formation of oxidation products are enhanced by the presence of double bonds, which leads to a decrease of unsaturation index, an increase of carbonyl index and a broadening of the carbonyl band, which consequently changes the pH of products **[30]**. According to Naveed *et al*., (2011) the odors of several products disappeared with the passage of time and elevated temperature which may be due to volatilization of related oils **[25]**. Overall, ointment formulations were more physically stable over time than cream formulations at 25 and 37 °C. At increase temperatures, pharmaceutical creams that form “Oil in water” (O/W) or “water in oil” (W/O) are unstable and usually divide into two phases **[25]**.

As far as the antimicrobial studies are concerned, the study showed that herbal ointment and cream formulations effectively combat various microorganisms, with inhibition zones ranging from 13 to 38 mm. F2 and F4 ointments showed similar antibacterial effects against E. coli, with F2 showing better results than F1 and F3. Commercially available formulas, Mebo® and F9, did not show any antibacterial activity against E. coli. Similar results were found in a study conducted by Olutayo *et al* (2019), who evaluated the antibacterial effects of several cream formulations containing aqueous and methanolic extract of andrographis paniculate and shea butter. They found that adding refined or unrefined shea butter to the formulas enhanced their antimicrobial activity **[22]**. In addition, it was found that only F3 has significant activity against *P. aeruginosa* (27 ± 0.0186 mm), while the other formulations reported no zones of inhibition. This result is in agreement with Mehdi Goudarzi *et al*. study, that showed that Aloe Vera was effective against *P. aeruginosa* and other several microbial pathogens **[31]**. Moreover, F4, the ointment containing equal concentrations of sesame oil, shea butter, and Aloe Vera extract showed the highest zone of inhibition (35 ± 0.0133 mm) and it was greater than other preparations against S. aureus. These results are in agreement with different studies conducted using herbs under investigation. For example, in a study conducted by Sheikuduman (2011), he found that, sesame oil exhibited potent antimicrobial activity against the tested microbial strains including *S. aureus* and *B. subtilis* **[32]**. Moreover, based on research carried out by Adamu *et al*. (2013), the results of its antibacterial assay indicated that petroleum extract of shea butter has antibacterial activity against S. aureus **[33]**. In addition, an antimicrobial susceptibility test of a study conducted by Mian Khizar Hayat *et al*. (2016), showed that Aloe Vera extracts inhibited the growth of E. carotovora, *E. coli*, *B. subtilis* and S. aureus **[34]**. Finally, it can be concluded from our study that shea butter, Aloe Vera and sesame oil ointments have good antibacterial activity against Gram-positive and negative bacteria.

Considering the antifungal effects, this study showed that an ointment containing sesame oil, shea butter, and Aloe Vera extract was less effective against *Candida albicans* than Aleo vera ointment but more effective than shea butter ointment. Many researches proved the effect of Aloe Vera extract and shea butter as antifungal herbs **[35, 36]**. For example, in 2017, Jain, S. *et al*., studied the anti-fungal effect of Aloe Vera; the study showed further promising antifungal properties **[37]**. Moreover, in the present study, no antifungal activity was observed for the sesame oil formulations and in literature, there are many controversies about the antifungal activity of sesame extracts. According to the literature, sesame antifungal activity depends on extraction type whether oil, ethanol, methanol, hexane and or aqueous extract, as well as the plant part used in the study: leaves, seeds, stems or roots. A study of the antifungal effect of oil of sesame seeds medicinal herb on Candida species was done by Lavaee *et al*. (2019) and demonstrated that no antifungal activity was observed in the oil extract of the sesame seeds. On the contrary, investigation of the antifungal activity of sesame leaves, stems and roots showed that most of the extracts had inhibitory effects on filamentous fungi **[38]**.

On the antimicrobial inhibitory activities of cream formulas, the superiority of F7 over F8 was noticed. This superiority may be due to higher Aloe Vera extract concentration in F7 (dose-dependent phenomenon) since the percentage of Aloe Vera in F7 was 15% while in F8 was only 5% , this attribution corresponds to Main Hayat *et al*., (2016) study **[34]**. They observed that Aloe Vera at different strengths showed remarkable activity against gram-negative as well as gram-positive bacteria and fungi and this activity is varied depending on the concentration. The difference between the antimicrobial activities of Aloe Vera ointments and cream formulations may be due to the solubility of the antibacterial active components of Aloe Vera, which are volatile or saturated compounds that more active in water base formulations **[39]**. Aloe Vera, known as "the healing plant", has been demonstrated to be effective during the healing process in various tissues **[35]**. The plant plays its healing role through fibroblast proliferation, angiogenesis, production of different growth factors, and synthesis of extra-cellular matrix components such as hyaluronic acid, dermatan sulfate and collagen, as well as increasing the number of cross-links between the collagen molecules in skin, bone fractures and gastric lesion. Previous studies have shown that Aloe Vera is efficient in treatment of inflammation, wounds and burns **[40]**. It has also been reported that mannose-6-phosphate, an active constituent in Aloe Vera, has wound healing and anti-inflammation activity. In addition, the antioxidant effect of acemannan, another component of Aloe Vera, has been revealed **[40]**.

CONCLUSION:

In this study, different ointment and cream preparations containing Aloe Vera, sesame oil, shea butter or a mixture thereof were prepared and studied to determine their properties and activities as wound healing remedies. The physical characterization showed that all prepared ointments and creams have good homogeneity and easy spreadability, but, unlike creams, ointments were greasy and difficult to remove after application. Stability testing of the prepared formulations showed that creams and ointments are sensitive to temperature changes, and there is a negative non-linear relationship between temperature and viscosity.

The histological studies performed after testing the prepared formulations showed healed skin structures with faster collagen deposition, reduced inflammation, and control of infection, a result opposite to that when blank preparations were used. Furthermore, when antimicrobial tests were performed, it was found that these plants have antimicrobial and antifungal effects. Both Aloe Vera cream and blend cream showed the highest activity against most of the tested bacteria and fungi. Finally, wound healing activity of the prepared ointment and cream formulations was also studied. The results showed that the blend ointment consisting of equal percentages of Aloe Vera, sesame oil and shea butter (adding up to a total of 15 % herbal mixture) has the most potent wound healing activity in rats compared with other groups. The healing properties of the blend might be due to several mechanisms, including the promotion of faster collagen deposition, the formation of connective tissue, the antioxidant effect, the reduction of inflammation and the control of infection through the antimicrobial effects. In the excision wound model, the results showed the extent of wound healing in the following order: G4 > G 11 > G 3 > G 8 > G 1 > G 2 > G 7 > G 5 > G 12 > G 6 > G 9 > G 10 >G 13.

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