



Research Article

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DEVELOPMENT AND EVALUATION OF HERBAL EXTRACT CREAM FOR HYDRATION AND PHOTO-PROTECTION

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ABSTRACT

Despite the increase in the number of cosmetic preparations containing antioxidant, sun protection, moisturizing agents, chiefly, due to their actions in preventing the process of skin aging, there is a need to develop herbal formulation in order to obtain safe, stable and multipurpose high efficient quality products. The present study was to develop and evaluate and compare the hydrating, erythema & sun protection of the herbal o/w cream comprising extract of natural product from Aloe vera, in the appropriate concentration. Different types of formulations oil in water (O/W) were formulated by incorporating different concentrations of different concentrations of the extract, 2.5, 5, 10 and 20 % namely and the base, F1 to F8. The evaluations of all formulations (F1 to F8) were done on different parameters like pH, viscosity, spreadability and stability were examined. Formulations F1 and F4 showed good spreadability, good consistency, homogeneity, appearance, pH, spreadability, no evidence of phase separation and ease of removal. The formulations F1-F4 were selected for the animal studies. All formulations F1-F4 in concentrations of 2.5, 5, 10 and 20 % herbal extract. They showed no redness, edema, inflammation and irritation during irritancy studies. These formulations are safe to use for application to check the other parameters. Formulation F3 in concentration 5% showed the best hydration and photo-protection properties. These studies suggest that composition of the extract and the base of the cream of F3 is more appropriate, stable and safe, it may produce synergistic action.

Key words: Aloe vera, o/w cream, Hydration, erythema, Photo-protection.

INTRODUCTION

The interest of consumers towards natural bioactive compounds as functional ingredients in the cosmetics products has arisen due to their various health beneficial effects. Besides their nutritional and sensorial properties, also, their potential effect in improving skin health has been recognized as acting as protective agents. The skin health promoting benefits of antioxidants from plant is thought to arise from their potential effects on the reactive oxygen/ species. Synthetic commercial antioxidants have been widely used in cosmetics industry to retard the oxidation process. However, synthetic antioxidants such as Butylated Hydroxyl Anisole (BHA) have been reported to cause toxic effect on human and animal studies ¹.

Therefore, restriction on their use is being imposed and the search for natural antioxidants as safe alternatives is becoming important to the food industry.

Aloe vera

The leaves of Aloe vera (*A. barbadensis*) (Fam. Liliaceae) are the source of aloe vera gel. The gel does not include the sap of Aloe vera, which contains anthraquinones. Aloe vera gel is widely used in cosmetics and toiletries for its moisturizing and revitalizing action^{2, 3}. The whole leaf of Aloe varies known to aid cellular repair as well as digestion, assimilation of foods, vitamins, minerals and other vital nutrients to rejuvenate the skin⁴. The fresh gel, juice or formulated products have been used

for medical and cosmetic purposes and to enhance general health⁵.

Several preclinical studies suggest that Aloe vera components may protect skin health by enhancing wound healing activity and reducing UV damage¹³. hydroxylated fatty alcohols derived from Aloe vera suppress inflammatory response and provide sunscreen protection against UV-induced damage in skin cells¹⁴.

The skin care formulation nourishes the health, texture and integrity of skin, moisturizing, maintaining elasticity of skin by reduction of type I collagen and photo protection, etc. These characteristics of cosmetic are due to the presence of synthetic or natural ingredients in skin care formulation, because it helps to diminish the exhibition of free radicals in skin and manage the skin properties for a long time. The cosmetic products are the best choice to reduce skin disorders such as hyper pigmentation, skin aging, skin wrinkling and rough skin texture, etc. The appearance and function of the skin are maintained by an important balance between the water content of the stratum corneum and skin surface lipids¹⁴.

Despite the increase in the number of cosmetic products in the market containing antioxidant, sun protection, moisturizing agents, and due to their toxic actions of their additives, the need of developing a herbal multifunctional cream is necessary. Thus, the objective of this research was to develop and evaluate the selected plant to obtain a multi-purpose high quality product. Different concentrations of the extract were compared with a standard commercial product using animal studies to get the appropriate concentration producing the desired effect. The

evaluated parameters are hydration and photo-protection and erythema behavior.

MATERIALS & INSTRUMENTS

Instruments

Sartorius balance AG Gottingen, BL210S, CE, Germany. pH meter, Orchidis Laboratories, France and Hanna instruments type, France. Dissolution apparatus type II Dis 6000, Copley scientific, Nottingham, U.K. UV. Visible spectrophotometer Gitra 5, GBC scientific equipment, U.S.A. Oven 50, 400C Memmert 854 Schwabach, W. Germany.

Laboratory Glassware

Graduated cylinder, Volumetric flask, Transfer pipette. Beaker, Funnel, 60 ml Amber glass containers

Materials

The plant was obtained from the garden of Dubai pharmacy college, Dubai, UAE. The plant was authenticated by botanical identification specialist and identification certificate was obtained. All materials are of analytical grade and conform to specifications of pharmacopoeia (BP, USP). Acetyl Alcohol, Glyceryl Monostearate A / S, Liquid Paraffin, Tween 80, Tween 60, Propylene Glycol and Purified Water from DPC store. The animal testing experiments were conducted in the animal facility laboratory. All procedure and protocol get approved from Dubai

Pharmacy College Animal Ethical Committee no (2017/03/209/06).

METHODS

Selection of the herb

The herb used in the multi-purpose formulation is Aloe vera, using the ethanol for the extraction process. Aloe vera, was selected on the basis of a documented literature²⁻⁸.

Collection and Authentication of the Plant

The herb used in formulation was collected in the months of October 2013 from the gardens of Dubai pharmacy college, UAE. Authentication had been done by the head of Pharmaceutical Chemistry and Photochemistry Department, Dubai Pharmacy College, Dubai, and UAE.

Preparation of the Extract

1kg of Avocado was washed and the gel was separated. The plant was homogenized with 70% ethanol (2 liters each) using blender. The alcoholic extract was evaporated under reduced pressure at 50°C using Rota evaporator and the residue was kept for further biological study as well as for the formulation⁹⁻¹².

Preparation of Herbal O/W Cream

The following steps had been followed using the composition in Table 1.

Table 1: Composition of herbal o/w cream

Material Name	Quantity	F1	F2	F3	F4
Herbal Extract		2.5%	5%	10%	20%
Cetyl Alcohol	12.0 g				
Glyceryl Monostearate A / S	16.0 g				
Liquid Paraffin	16.0 g				
Tween 80	6.0 g				
Tween 60	4.0 g				
Propylene Glycol	30.0 g				
Purified Water to	116.0 g				

Step-I (For Oil Phase): The following chemicals and materials were taken in a beaker 120.0 g.

Cetyl Alcohol, 160.0 g Glyceryl Mon stearate A / S, 160.0 g Liquid Paraffin (60.0 g), Tween 80 and Tween 60 (40.0 g). The beaker was heated to 90°C for 60 minutes while stirring, Cooled to 60°C. The temperature was maintained at 60°C - 65°C.

Step-II (for Aqueous Phase): 1160.0 g Purified water were taken in a beaker and heated to 90°C for 30 minutes, then cooled to 60°C. The temperature was maintained to 60°C - 65°C.

Step-III (For Disperse Phase): The oil Phase was maintained to the aqueous phase with a continuous stirring, and temperature 60°C. Homogenized at speed II for 5-10 minutes. Finally, it was cooled to 40°C.

Step-IV (Drug Phase): 25.0 g Propylene Glycol and 20.0 g herbal extract were mixed and warmed. The Propylene Glycol to 40°C in water bath in a beaker. The silver sulfadiazine was dissolved in propylene Glycol (40°C) using homogenizer. The Drug phase was added to the disperse phase while mixing at temperature 40°C.

Step-V (Rinsing): Propylene Glycol: 50.0 g the beaker was rinsed with propylene glycol and add to the disperse phase, mixed and homogenized for 10 minutes at temperature 40°C.

Step-VI: The cream was cooled to 30°C while mixing. Then transferred to the container protecting form direct light.



Formulation of Sunscreen Cream

Methodologies

Preparation of herbal sunscreen emulsions

The nature of commercial sunscreen formulations varies considerably depending on the desired cosmetic and protective properties of the product. The herbal extract was incorporated in a variety of vehicles, including w/o, o/w emulsion and ointment base²⁷. There were variety of preparations for each vehicle involving different concentrations and combinations. They are subjected to primary investigation for the selection of the appropriate vehicle. With this in mind, a model o/w emulsion system was chosen for this study, based on a United States FDA standard sunscreen formulation for SPF testing^[28], the composition as described in Table 2.

Preparation of the herbal sunscreen emulsion

The preparation described above was adapted to incorporate -herbal extract in varying concentrations, by substituting it for the

herbal extract sunscreen. It was decided that the percentage by weight of the other ingredients would remain constant, and varying concentrations of -herbal would be achieved by adding appropriate amounts and varying the water content accordingly. Due to the small nature of the other ingredient quantities, this did not introduce a large variation in the emulsion nature for varying herbal extract concentrations (2.5, 5, 10 and 20 %). It was also decided to exclude the paraben ingredients, as they absorb strongly in the UV region, interfering with spectral analysis. The main purpose of these ingredients is to act as preservatives, to increase the shelf-life of the product, and the small quantity present is unlikely to greatly alter the nature of the emulsion. Thus the final procedure for preparing the emulsions used is described below. Ingredients for parts A and B (see table 2) were weighed into separate containers, and heated using a stirrer hot-plate to approximately 80°C, with constant stirring using magnetic stirrer. Part A was slowly added to part B, with continual stirring of the mixture, and frequent reheating of part A to maintain the temperature. The heat was switched off, and the mixture continually stirred until at room temperature, until a white creamy emulsion was formed.

Table 2: Composition of the herbal sunscreen

	Ingredient	Description	Weight / g	Percent by weight
A (oil phase)	Lanolin	Fatty Substance	1.00	5.00
	Extract mixture	Active ingredient	Y	2.5, 5, 10 & 20
	Mineral oil	Fatty Substance	0.50	2.50
	Steric acid	Fatty substance, Emulsifying agent	0.80	4.00
B (Water phase)	Propyl paraben	Preservative		0.05
	Methyl paraben	Preservative		0.10
	EDTA	Chelate, Preservative, Antioxidant	0.01	0.05
	Propylene glycol	Solvent	1.00	5.00
	Triethanolamine	Emulsifier, Neutralizer	0.20	1.00
	Purified water	Solvent	16.49-x	82.4-y
Total			20	100
The sunscreen was substituted with different concentrations, 2.5, 5, 10 and 20 %				

Composition of herbal sunscreen emulsion of a different concentrations

Concentration of the extract

It was also decided to exclude the paraben ingredients, as they absorb strongly in the UV region, interfering with spectral analysis. The main purpose of these ingredients is to act as preservatives, to increase the shelf-life of the product, and the small quantity present is unlikely to greatly alter the nature of the emulsion. Thus, the final procedure for preparing the emulsions used is described below.

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temperature. The heat was switched off, and the mixture continually stirred until at room temperature, until a white creamy emulsion was formed.

Visual Properties Inspection

The prepared herbal formulations containing different extract concentrations and the commercial preparations were examined for their visual as well as rheological properties.

The prepared formulations were examined for their physical characteristics, namely: color, consistency and homogeneity. Homogeneity of various formulations was tested by visual observation and was ranked as follows:

- +++ = Excellent
- ++ = Very Good
- + = Good
- = Poor

Sensitivity

Small amount of sample was applied on forehead to check irritation effect.

Color Change

The samples were kept for 7 days to note out the color changes.

Water Washability

Small amount of sample was applied in the hand for few minutes and was washed with water to observe the washability.

Consistency

The cone attached to holding rod was dropped from the fixed distance of 10 cm such that it should fall on the center of measuring cylinder filled with SSD cream. The distance travelled by cone was noted down after 10 sec.

Rheological Properties

The prepared formulations were evaluated for the following rheological characteristics (Table 3)

Viscosity Measurements

A Brookfield synchro electric viscometer, Brookfield, MA) was used to measure the viscosity (in cps) of the creams. The spindle was rotated at 2.5 rpm. Samples of the creams were allowed to settle over 30 min at the temperature of test (25±1°C) before the measurements were taken.

Extrudability

Extrudability was determined, using an extrudability apparatus. A closed collapsible tube containing formulation was pressed firmly at the crimped end. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5-cm ribbon of the formulation in 10 seconds was determined. The herbal cream extrusion pressure in grams was reported¹⁶.

Spreadability

One gram of the herbal formulation was placed between the two glass slides and load of 500g was applied. The time required to slip off the slides was measured and Spreadability was calculated using formula

The spreadability was then calculated from the following formula:

$$\text{Spreadability} = m \times l / t$$

m = weight tied to the upper slide (30g)

l = length of glass slide (5cm)

t = time taken in seconds to separate the slides.

A range of concentrations from 0-20 % by weight of herbal formulations were made using this method. The concentrations quoted are guidelines, and emulsion natures varied slightly from sample to sample. The emulsions used had the consistency of a smooth cream that did not pour. However, overnight, samples tended to become milky and less viscous, indicating that the emulsions were not stable for extended periods of time. Samples were therefore tested as soon as possible after preparation. This problem largely prevented extract the stability studies done following ICH guidelines⁷, due to the changing nature of the emulsion *vita* time.

Phytochemical Screening of the Plant

Preliminary Phytochemicals screening (Qualitative Analysis)¹⁷

The preliminary phytochemicals studies were performed for testing different chemical groups present in ethanolic extract of samples from Cucumber, Avocado and *Aloe vera* were tested for some constituents, like CHO, glycoside, tannins, anthraquinone, cardiac glycosides and flavonoids. The results are shown in table 1^{4,5}.

Quality Control Tests for Creams containing the Herbal Extract

Test for Non-Irritancy

Non-irritancy of the preparation is evaluated by patch test. In this test 24 rats were selected. Definite quantity of cream was applied under hair removed skin for 21 days. Daily the type of pharmacological action observed.

Skin Irritation Test

The herbal creams containing different concentrations of the extract, were applied on the skin. The test cream and cotton swab covering it were secured firmly on the applied surface with the help of adhesive tapes. Then observations were made for any sign of erythema and ranked as follows as per the state of the applied site¹⁸

+++ = Severe erythema

++ = Moderate erythema

+ = Slight erythema

- = No irritation^{11, 12}.

Test of Rate of Penetration

Weighed quantity of the preparation was applied over selected area of the skin for a definite period of time. Then the preparation left over is collected and weighed. The difference between the initial and final weights of the preparation give the amount of penetrated through the skin^{13, 14}.

Stability Study

The formulation was kept at different temperatures (10°C, 30°C and 45°C). The Samples were observed for pH, viscosity and their appearance for 4 weeks^{15, 16}.

Toxicity Test

The toxicological studies have been performed for longer period of 28 days.

SPF Determination Transmission Spectrum Transpore Tape Method

Samples were applied on transpore tape, spread uniformly with help of capillary to form a thin film. The strip was placed inside UV-Vis cuvette in such that the formulation touches transparent side of cuvette. It was allowed to equilibrate for 15 mins to ensure levelling of the formulation between transpore tape and wall of the cuvette¹⁸⁻²².

The cuvette was placed inside UV Spector-photometer (Shimadzu) and a transmission spectrum was recorded from 290-400 nm. The data was appropriately processed to calculate UVA and UVB protection factors using the following formulas²¹⁻²⁵.

Spontaneous Erythema and Edema in Hairless Rats

Male Wistar rats weighing around 300g were used. Hair on the dorsal skin is clipped and carefully shaved. An area (1.5 × 2.5cm) biphasic erythema is observed. One side of the flank was irradiated for 15min (1.5cm²) at a vertical distance of 20cm with UV-B lamps. Immediately after irradiation, initial faint erythema appears, disappearing within 30 min. The second phase of erythema starts 6hrs after the irradiation and gradually increases, peaking between 24 and 48hrs. Pieces of the scale are relatively thick. The irradiated rats were sacrificed after various time intervals by decapitation under diethyl ether anesthesia. Skin biopsies were taken immediately, fixed in 10% formalin and embedded in paraffin. Tissue sections (4µm thick) were stained with hematoxylin and eosin. The numbers of the keratinocyte layers, including the basal layer, are counted by direct microscopy²⁶.

Rehydration Effect in Hairless Rat

Male Wistar rats weighing around 300g are used. Hair on the dorsal skin was clipped and carefully shaved. Applied the cottons seed oil to increase the epidermal skin size for the period of 7 days on the hairless rat skin and treated by the standard and test cream for the period of 14 days. Skin biopsies were taken immediately, fixed in 10% formalin and embedded in paraffin.

Tissue sections (4 µm thick) are stained with hematoxylin and eosin. The numbers of the keratinocyte layers, including the basal layer, are counted by direct microscopy^{6,11,12,13,14,15,16,17}.

Microbiological Studies for the herbal creams

The formulated creams were inoculated on the plates of Muller Hinton agar media by streak plate method and a control was

prepared by omitting the cream¹⁸. The plates were placed in to the incubator and are incubated at 37 °C for 24 hours. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control^{26,27}.

RESULTS AND DISCUSSION

The phytochemical screening of the extract revealed the presence of alkaloids, tannins and phenolic compound. The antioxidant activity of the extract showed that it was a potent free radical scavenger and antioxidant due to the presence of tannins, flavonoids and saccharide compounds. The IC₅₀ of the extract was found to be 293.07± 16.96. The results are summarized in table 1. The pH of the prepared cream with the extract was found to be around 6 which is suitable for topical application because the pH of the skin is between 4.5–6. The spreadability study showed that formulation I have better spreadability when compared with the marketed cream. The results of pH and spreadability are summarized in table no.4. Viscosity of creams is different at different revolution per minute. At 0.5rpm to 20rpm viscosity was decreased from 6897 to 642cps. So, if we decrease the rate of shear it increases the viscosity of cream. Viscosity of creams is inversely proportional to rate of shear (rpm) and the results are showed in table 5. The stability studies of the various parameters like visual appearance, nature, pH and viscosity of the formulations showed that there was no significant variation after two months of the study period and the results are summarized in table 6.

The formulated creams were tested for the presence of pathogenic microorganisms by culturing it with Muller Hinton agar medium. There were no signs of microbial growth after incubation period of 24 hours at 37°C and it was comparable with the control.

Table 3: Evaluation of physical properties

Sl. No	Evaluation Parameters	F 1	F 2	F 3	F 4
1.	Sensitivity	Not Sensitive	Not Sensitive	Not Sensitive	Not Sensitive
2.	Color Change	Observed	Not Observed	Not Observed	Not Observed
3.	Water Wash ability	Washable	Washable	Washable	Washable
4.	pH	7	8	7	7
5.	Spreadability				

Physical Evaluation

Table 4: Spreadability and pH

Formulations of o/w	Time in seconds	Spreadability (g cm/sec)	pH
20%	11	12.78	5.9
10%	10	11.12	5.7
5%	10	10.84	6.1
2.5%	9	10.12	5.9
Marketed	13	11.91	5.7

Table 5: Viscosity

Rpm at	Viscosity of cream (cps)				
	20%	10%	5%	2.5%	Marketed
20	642	642	642	642	642
10	964	957	988	911	994
5	1121	1129	1094	1618	1590
1	3271	3217	3211	3226	3234
0.5	6825	6783	6896	6920	6532

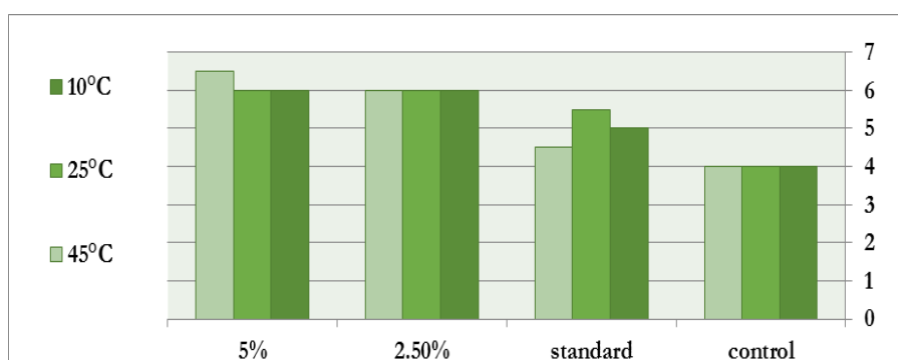
Table 6: Stability studies (Observation after 2 months)

Formulations	pH	Color & Appearance	Viscosity at 20 rpm
20%	6.2	Yellowish brown & non-glossy	601
10%	6.3	Yellowish brown & non-glossy	612
5%	5.9	Yellowish brown & non-glossy	597
2.5%	5.7	Yellowish brown & non-glossy	579
Marketed	6.1	Yellowish brown & non-glossy	599

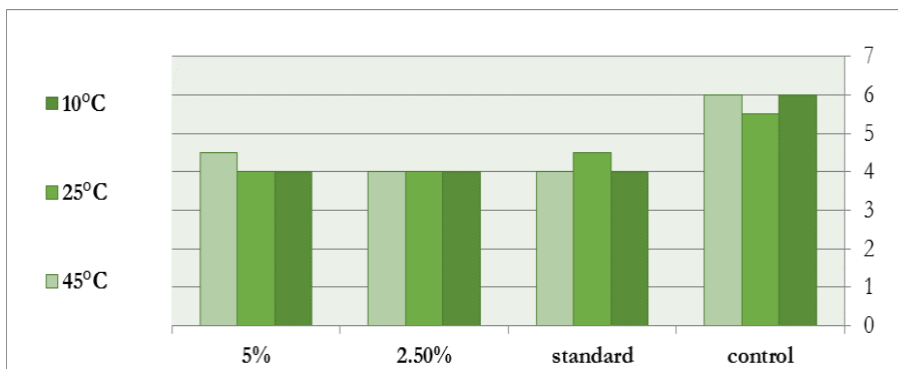
Table 7: Phytochemical screening of the plant

Chemical class	Result
Antraquinone aglycon	-ve
Antraquinone glycoside	-ve
Tannin	+ve
Saccharide	+ve
Flavonoid	+ve
Alkaloid	-ve

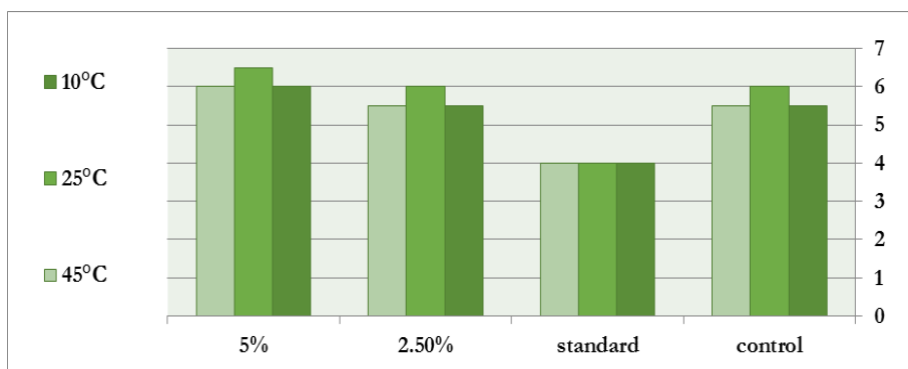
Quality control tests for creams containing herbal extract



Anti-inflammatory Cream



Sun Protective Cream



Rehydration Cream

Figure 1: pH values of different formulations at various temperature after 2week.

1. Toxicity test: No toxicity has been found in the herbal extract.
2. Determination of SPF for cream containing herbal extract:

In vitro sunscreen activity of all developed cream containing herbal extract were checked by transmission spectroscopy. Range 290nm-400nm

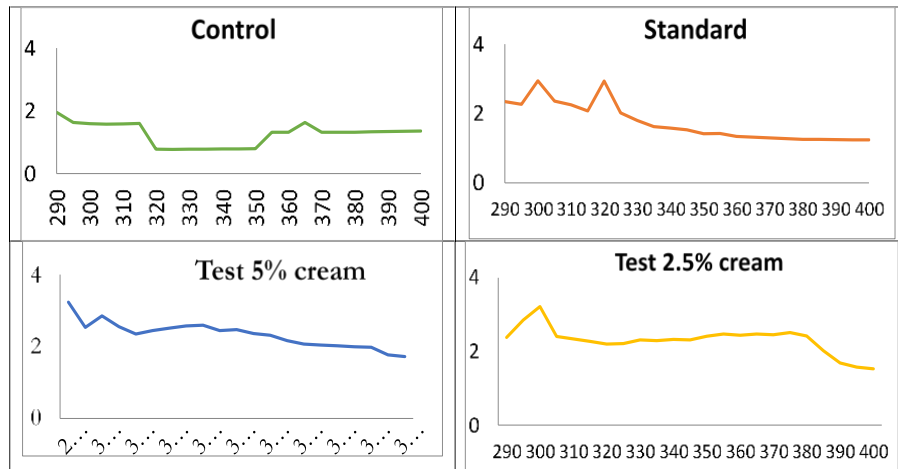


Figure 2: Absorbance spectrum of the herbal cream for determination of SPF.

Spontaneous erythema and edema in hairless rats

- Physical observation: Soften in the damage rats skin under investigation was observed. Complete healing in the rate skin was also observed.

- The erythema and edema had cured in the test 5% cream compare to standard cream during the period of the 14 days.

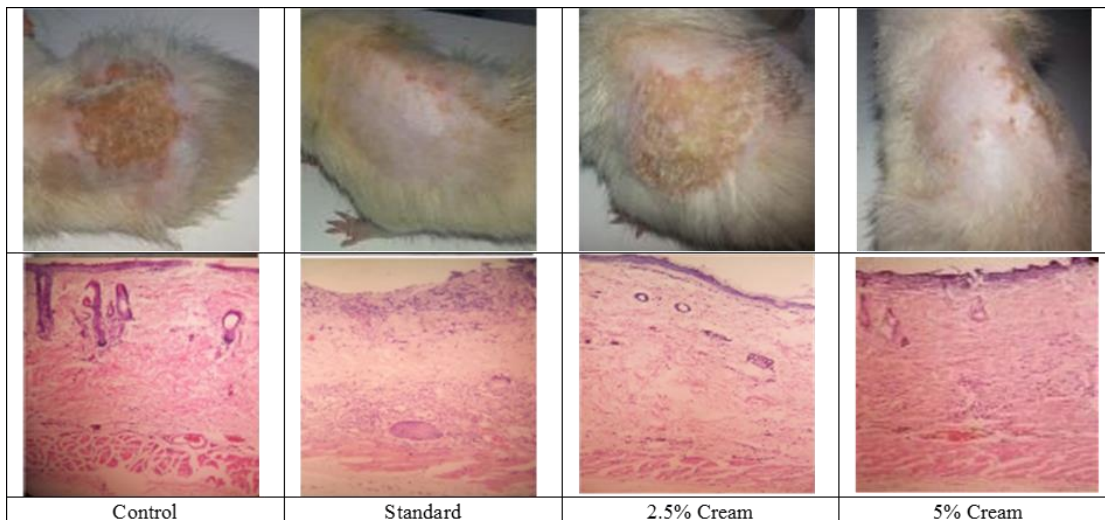


Figure 3: Spontaneous erythema, edema has cured in the test 5% cream

Rehydration effect in hairless rat

The rehydration creams showed significant effect in reducing the epidermal skin layer of rat skin and make smooth of the skin in the test 5% cream compared to the standard cream.

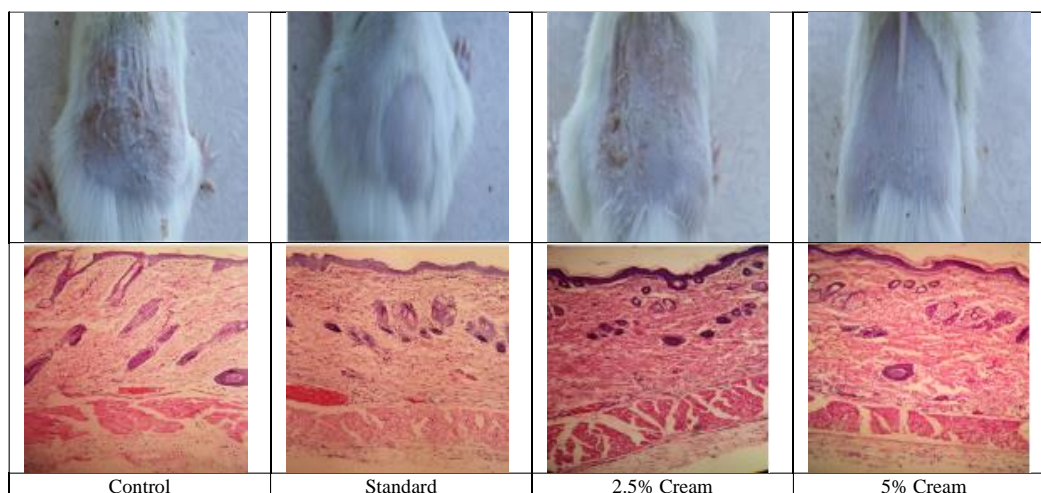


Figure 4: The epidermal (E) of skin size is significantly reduce in the test 5% cream sample compare to the standard rehydration cream

CONCLUSION

The extent of efficiency of medicinal and cosmetic property of single plant extract, can possibly be increased by the selection of the appropriate plant and the appropriate concentration incorporated in the suitable type of the cream base. The suitable cream base was found to improve as well synergize the cosmetic properties of the prepared product.

This study suggests that composition of *aloe vera* extract in 5% concentration in o/w emulsion of F3 was more stable and safe; it may produce synergistic action, in hydration, photo-protection and anti-aging properties.

Further research will be needed to check scientifically the synergistic action of selected formulation on human subjects.

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