



FORMULATION AND EVALUATION OF CREAM INCORPORATING *ALOVERA* AND *TOMATO* EXTRACTS

Dr Jairam Patel¹, Dr Neeraj Upmanyu², Mrs Manisha Tandon³, Ms Rajshree Mishra*

¹Principal, ²Associate Professor, ³Assistant Professor & *M.Pharm Student

RKDF College of Pharmacy, Bhopal, M.P., INDIA

Abstract

The present study would involve the formulation of w/o emulsion based cream incorporating Aloe vera and tomato extracts. The water phase would be incorporating Aloe vera extract as a moisturizer and would be used for reducing skin irritation. The Aloe vera extract would be consisting of aloin and saponins which would be responsible for moisturizer activity. The tomato extract consists of lycopene which consists of conjugated double bonds responsible for its red colour and antioxidant capacity. As it is water insoluble I can be incorporated in to the organic solvent phase. The oil phase would be mixed into the water phase at same temperature and mixed property till an even consistency is achieved. The cream formulated would be evaluated for its determination of pH, drug content uniformity, viscosity (in cps) and stability studies. The cream would be incorporating the extracts and will be acting as free radical scavenger and retains moisture on skin surface maintaining the emolliency.

Introduction

Aloe vera

Aloe vera is a succulent plant species that is found only in cultivation, having no naturally occurring populations, although closely related aloes do occur in northern Africa. The species is frequently cited as being used in herbal medicine since the beginning of the first century AD. Extracts from *A. vera* are widely used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing, or soothing properties. There is, however, little scientific evidence of the effectiveness or safety of *Aloe vera* extracts for either cosmetic or medicinal purposes, and what positive evidence is available is frequently contradicted by other studies.

Aloe vera is a stemless or very short-stemmed succulent plant growing to 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm (35 in) tall, each flower being pendulous, with a yellow tubular corolla 2–3 cm (0.8–1.2 in) long. Like other *Aloe* species, *Aloe vera* forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil. *Aloe vera* leaves contain phytochemicals under study for possible bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, anthraquinones, such as emodin, and various lectins.

Tomato

The tomato is the edible, often red fruit of the plant *Solanum lycopersicum*, commonly known as a tomato plant. The species originated in the South American Andes and its use as a food originated in Mexico, and spread throughout the world following the Spanish colonization of the Americas. Its many varieties are now widely grown, sometimes in greenhouses in cooler climates. There are around 7500 tomato varieties grown for various purposes. Heirloom tomatoes are becoming increasingly popular, particularly among home gardeners and organic producers, since they tend to produce more interesting and flavorful crops at the cost of disease resistance and productivity. The tomato's medicinal properties had already been endorsed in Continental Europe in the 16th C. and their consumption was believed to benefit the heart among other things, as it contains lycopene, one of the most powerful natural antioxidants which, especially when cooked, has been found to help prevent prostate, lung, stomach, pancreatic, colorectal, esophageal, oral, breast and cervical cancers. Lycopenes, bioflavonoid closely related to beta carotene, are potent antioxidants present in tomatoes and seem to be responsible for these natural cancer-fighting properties.

Material and Method

Formulation of Cream

Water in oil (w/o) emulsion-based cream (semisolid formulation) was formulated. The Span 80 (emulsifier) and other oil soluble components (white bees wax, liquid paraffin) and extract of tomato were dissolved in the oil phase (Part A) and heated to 75° C. The preservatives and other water soluble components (Propyl paraben) extract of *Aloe vera barbadensis* were dissolved in the aqueous phase (Part B) and heated to 75°C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until cooling of emulsifier took place. Perfume was added after cooling.

Preparation of aqueous extract

Aloe vera leaf was subjected to aqueous extraction.

About 200 gm of *Aloe vera* leaves were taken. The spines were removed. The leaves were then cut longitudinally and placed in a beaker in upright position for the gel to ooze out from the leaves and collected in a 500 ml beaker. It was cold extracted for 7 days with occasional shaking and warming. At the end of the seventh day, the clear filtrate was obtained by filtering through a funnel. The filtrate was concentrated by vacuum distillation, cooled and transferred into a china dish and dried in an oven at 60°C for a period of five minutes. Finally, the aqueous extract was kept in desiccator for 5 days to remove the excessive moisture and was used for further studies.

Preparation of tomato extract

250 gm of Tomato was taken, it was chopped into fine dices. The dices were filtered through a filter paper and the pulp was subjected to ethyl acetate extraction. Ethyl acetate was added to the pulp and subjected to extraction. The extracted material (Lycopene) was concentrated in vacuum. Tomato extract was ready for use.

Method Description ;It measures viscosity by the force required to rotate a spindle in a fluid. On the DV-II, the viscosity can be read directly.

Apparatus / Reagents ;Brookfield Digital Viscometer Model DV-II ,Wide mouth 8 oz. sample jar (O. Berk cat# 1AC08F8) ,Spindle ,Calibrated thermometer, Viscosity Standards, National Bureau of Standards or Brookfield Engineering Laboratories.

Procedure

The sample container and quantity should be approximately the same as for the Calibration Standard. Check to confirm that the viscometer has been calibrated. Equilibrate the temperature of the sample to the temperature designated in the specification ($\pm 1^\circ\text{C}$). Confirm that the viscometer is level using the bubble level on the back of the instrument. For the Brookfield DV-II, AUTO ZERO the instrument with no spindle attached and the speed set as designated in the product specification. The main display will flash 00.0 after 10 seconds. For the DV-II, choose the units by pressing the desired unit key (CPS for centipoises). Set the speed as designated in the product specification, start the viscometer and read at constant reading. For manual models, use the conversion chart to convert the dial readings to centipoises. When done, turn motor and power off. Clean spindle and place in spindle holder. The DV-II, If EEE is displayed, the reading is overage. Correct as in 9. For other troubleshooting, see manual "The Brookfield Viscometer Model DV-II Operating Instructions" No. M/85-160-F or the manual for the model being used.

Evaluation Parameters for Cream

Dye test:-The Sudan red III dye was added with the cream, the dispersed globules appear colourless in the ground.

Homogeneity:- The appearance of the cream was judged by its color, pearlsense and roughness and graded.

After feel:- Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

Type of smear:-After application of cream, the film or smear formed on the skin was checked.

Removal:-The ease of removal of the cream applied was examined by washing the applied part with tap water.

Irritancy test: Marked an area (1 sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs and reported.

Dilution test:- The oil was distributed uniformly in the emulsion, so the emulsion is of w/o type.

Physical Stability of Cream:- The ability of cream to maintain its consistency was determined by keeping it at 25 °C for 30days.

Rubout and Emolliency:- It included spread ability and emolliency. A fixed amount of cream was applied on dorsal skin surface of human volunteer and the properties were observed.

Primary Skin Irritation Test in Rabbits

Primary skin irritation is the production of reversible inflammatory changes in the skin following the application of a test substance as it involves the interaction of chemicals with the sensory receptors in the skin at the site of application.

Test and Control of Formulation(Husbandry):- All rabbits were kept in metal cages fitted with perforated floors. Water and standard rabbit feed were given .The room temperature was maintained at 22 ± 3 °C with 30-70 % Relative Humidity. The light conditions were controlled to give 12 hours artificial light (8 a.m. -8 p.m.) each day. A minimum of 7 days acclimatization was allowed before the commencement of the study.

Experimental Procedure

Skin Preparation: The skin of the rabbit was prepared before the test (dose application), hair on the back and flanks of the rabbit were clipped exposing (approximately 6 cm² area) of skin. Dose: 0.5 gm / animal.

Application Procedure:- 0.5 gm sample of cream was applied evenly to a small area (approximately 6 cm square) of the closely clipped skin of each of the three rabbits. The site of application was covered with a cotton gauze patch. At the end of 4 hours period, the bandages were removed and treated sites were wiped with wet gauze to remove any test substance remaining.

Observations:- Skin reaction at the site of application was observed and scored once daily at 1, 24, 48, 72 hours, 7 and 14 days after cream removal .The reaction at the site of application was assessed and scored according to the following system.

Skin Reaction	Score
(A) Erythema And Eschar Formation	
No Erythema	√
Very Slight Erythema (Barely Perceptible)
Moderate To Severe Erythema
(B) Edema Formation	
No Edema	√
Very Slight (Barely Perceptible)

In-vitro Antioxidant activity:**Requirements****Reagents and Chemical** - DPPH ,Ethyl Acetate**Glassware**-Graduated pipette,Volumetric flask (5 ml/10 ml) or, test tubes

Beaker (25/50 ml)

Instrument used -Digital weighing balance,Spectrophotometer,Incubator**Methodology**

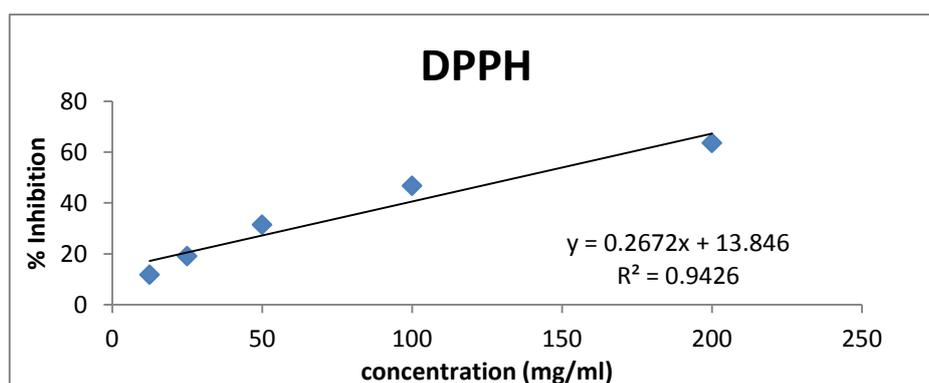
Prepared 0.1mM DPPH solution (4mg/100ml) in methanol.Prepared different concentrations (200, 100, 50, 25 & 12.5 mg/ml) of test sample with ethyl acetate.Added 2 ml of test sample and 1ml of DPPH solution. Incubated at room temperature for 10 min. The absorbance was taken at 515 nm against blank (methanol).Calculated % Inhibition = $[(AC\ 515\ \text{nm} - AS\ 515\ \text{nm}) / AC\ 515\ \text{nm}] \times 100$.Plotted a curve for % inhibition and concentration and using line of regression estimated IC_{50} .

Results & Discussion

Physiochemical Evaluation of cream:- After the formulation the herbal cream was evaluated using the following parameters Titratable acidity is 7.0 ,Water content is 6.5%, Standard plate count is fair,coil form count is satisfactory, Specific gravity 1.06068 g/ml, Refractive index 1.33, pH 7 (neutral).

Free radical scavenging assay (DPPH assay)% Inhibition of DPPH by Plecebo

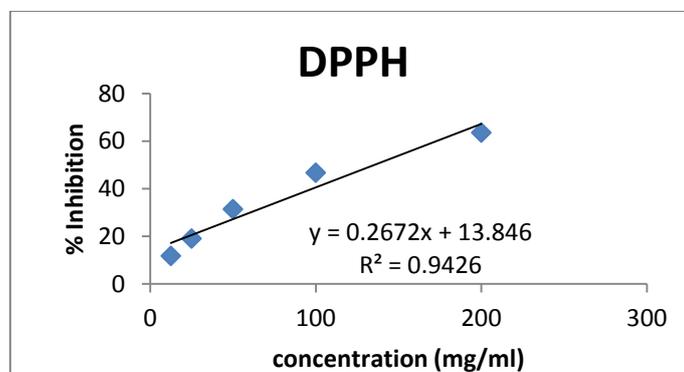
S. No.	1	2	3	4	5
Conc. (mg/ml)	12.5	25	50	100	200
% Inhibition	11.80	19.10	31.46	46.77	63.62

Scavenging assay (DPPH assay) % Inhibition of DPPH by Plecebo

Graph No.1: Standard curve of Placebo (Graph represent regression curve of placebo by DPPH assay method)

 IC_{50} of Placebo = 135.43 mg/ml table No. 10: % Inhibition of DPPH by Cream

S. No.	1	2	3	4	5
Conc. (mg/ml)	12.5	25	50	100	200
% Inhibition	18.12	27.25	43.40	60.39	75.84



Graph No.2: Standard curve of Cream

(Graph represent regression curve of cream by DPPH assay method)

IC₅₀ of Cream = 94.65 mg/ml

Conclusion

The formulated herbal cream was found to be of w/o type as confirmed by dye test and dilution test. The PH of herbal cream was found to be 7.00 as measured by the pH meter. The stability and homogeneity, spread ability of the cream was found to be good. The irritancy test for sensitivity testing result had shown the formulation to be safe with respect to irritation and allergic sensitization. The formulated herbal cream is mainly for cosmetic use. The herbal cream incorporates lycopene which is an antioxidant, bactericide, and anti-inflammatory agent. *Aloe vera* was also used as skin moisturiser, in ulcerative skin condition, wound, burns and relieves burning sensation. The formulated herbal cream should be beneficial to normal human keratinocytes. The *in vitro* antioxidant activity of the cream was performed by DPPH method and it was found to have antioxidant potential.

References

1. Akinyele BO, Odiyi AC (2007). Journal of Plant Sciences 2 (5): 558–563.10.3923, 2007.558.563.
2. Ernst E. (2000). "Adverse effects of herbal drugs in dermatology". Br J Dermatol 143 (5): 923–929.
3. Boudreau MD, Beland FA (2006). "An Evaluation of the Biological and Toxicological Properties of *Aloe barbadensis* (Miller), *Aloe vera*".Journal of Environmental Science and Health Part C 24: 103–154.
4. Gong M, Wang F, Chen Y (January 2002). "Study on application of arbuscular-mycorrhizas in growing seedings of *Aloe vera* Zhongyaocai, Journal of Chinese medicinal materials 5 (1): 1–3. PMID 125-231.
5. Eshun, K.; He, Q. (2004). "Aloe Vera: A Valuable Ingredient for the Food, Pharmaceutical and Cosmetic Industries—A Review". Critical Reviews in Food Science and Nutrition 44 (2): 91–96.
6. Dabelstein, Werner; Reglitzky, Arno; Schütze, Andrea; Reders, Klaus (2007). "Automotive Fuels". *Ullmann's Encyclopedia of Industrial Chemistry*.
7. Gulçin I, Elias R, Gepdiremen A, Boyer L. Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus* L.). Eur Food Res Technol 2006; 223: 759-767.
8. Lycopene: Its role in human health and disease, Rao 'et al.', AGRO Food industry hi-tech, July/August 2003 .
9. USDA study on Carotenoid content of gac fruit.
10. Journal of the American College of Nutrition: Role of Antioxidant Lycopene in Cancer and Heart Disease.
11. Meschino Health. "Comprehensive Guide to Lycopene". Retrieved 10 May 2012.
12. Helmut (1997). "Oxidative stress: Oxidants and antioxidants". *Experimental Physiology*82 (2): 291–5.
13. McKinlay A, Diffey BL. A reference action spectrum for ultraviolet induced erythema in human skin. In human exposure to Ultraviolet Radiation. Risks and Regulations, Elsevier, Amsterdam, Netherlands, 1987, p 83.
14. INDIAN PHARMACOPOEIA (2007); Volume 2 page no. 17
15. Khandelwal K.R. Practical Pharmacognosy, Nirali Prakashan, 19th edition, 2008, page no. 149 -153.
16. Ernst E. (2000). "Adverse effects of herbal drugs in dermatology". Br J Dermatol 143 (5): 923–929