Research Article

Formulation and Evaluation of Herbal Cream containing *Curcuma longa*.

Sujith S Nair[1*], Molly Mathew[2] and Sreena K[3]

[1]Vinayaka Missions University, Salem, Tamil Nadu, India.
[2]Malik Deenar College of Pharmacy, Kasaragod, Kerala, India.
[3]Crescent College of pharmaceutical Sciences, Payangadi, Kannur, Kerala, India.

ABSTRACT

In this study creams were formulated based on the anti-oxidant potential of herbal extracts and its evaluation. Selected plant parts are dried and extracted using 70% alcohol by maceration. The extract was tested for antioxidant activity by superoxide scavenging activity. Quality evaluation of the product was assessed by using different evaluation methods. No change of the physical properties was observed; the pH was in a proper range (approximately pH6). The marker Curcumin was present in the extract, formulation and the peak was comparable with standard Curcumin obtained by HPLC. The formulations showed good spreadability, no evidence of phase separation and good consistency during this study period. It was found that the viscosity of the cream increases when decreasing the rate of shear so the viscosity of creams is inversely proportional to rate of shear (rpm). There is no sign of microbial growth after incubation period of 24hrs at 37^{0C} and it was comparable with the control. The creams were found to be stable during stability study according to ICH guidelines (30 ± 2 °C/ 65 ± 5 % RH and 40 ± 2 °C/ 75 ± 5 % RH) for 2 months. From the present study it can be concluded that it is possible to develop creams containing herbal extracts having antioxidant property and can be used as the provision of a barrier to protect skin.

Keywords: Herbal cream, Superoxide scavenging, Curcumin, HPLC.

INTRODUCTION[1, 2]

Skin aging is the result of continual deterioration process because of damage of cellular DNA and protein. Aging process is classified into two distinct types, i.e. “sequential skin aging” and “photo-aging”. Both types have distinct clinical and historical features. Sequential skin aging is universal and predictable process characterized by physiological alteration in skin function. In the aging process keratinocytes are unable to form a functional stratum corneum and rate of formation from neutral lipids slows down, resulting in dry pale skin with wrinkle. In contrast, photo aging is caused by over exposure to UV rays from sunlight. It is characterized by dry, pale and shallow skin, displaying fine wrinkles as well as deep furrows caused by the disorganization of epidermal and dermal components associated with elastosis and heliodermatitis. Herbs and plants have already proved useful as a tool in complementary medicine. Folkloric use of botanicals was relatively unsophisticated, found usually in the form of infusions, poultices and compresses as well as vinegar, distillates and wines. Only in the last century did it become possible for research chemists to scientifically test, measure and record the effects of plant extracts on the human body, and find new ways of enhancing natural products through the use of sophisticated equipment and testing procedures. Today, holistically balanced and standardized extracts can be reproduced in identical batches, with great emphasis placed on the extract’s final efficacy when incorporated into finished cosmetic products

During the recent times, the whole world has been swept by a green wave with the realization of health hazards and toxicity associated with indiscriminate use of synthetic drugs and antibiotics, that anything in nature is safer than synthetics. More over the expense for developing a synthetic molecule is not affordable even to developed countries. There is a

growing interest in natural antioxidants found in plants. Many antioxidatively acting compounds are isolated from natural herbs and extracts and used as potential antioxidants in cosmetics. The purpose of this study is to develop o/w emulsion (cream) containing the extract and its evaluation.

EXPERIMENTAL

Preparation of Turmeric extract[3]

The Turmeric was collected and dried it for 2 day in sunlight. Then crushed it to fine powder and passed it from sieve # 60. The collected powder was subject to maceration with 70% ethanol in iodine flask for 7 days. The extract was filtered and decolorized with charcoal to get clear liquid. Evaporate this extract to get the semisolid mass.

Preliminary Phytochemicals screening (Qualitative Analysis)[3]

The preliminary phytochemicals studies were performed for testing different chemical groups present in ethanolic extract.

Evaluation of Antioxidant Activity[4, 5] by Superoxide Scavenging Activity

The reaction mixture contained 2.650 ml phosphate buffer, 0.1ml NBT, 0.2ml KCN, 0.5ml riboflavin and different concentrations of ethanolic extracts of the plant in a final volume of 3ml. The tubes were illuminated with an incandescent lamp for 15 minutes. Optical density was measured at 532 nm before and after illumination. The percentage of inhibition of super oxide generation was evaluated by comparing the absorbance value of control and test.

$$\text{Percentage inhibition} = \frac{C-T}{C} \times 100$$

C=absorbance of control,
T=absorbance of test

Preparation of Cream[15]

The formulation components used were listed in table 2. The moisturizer conditioner was mixture of propylene gycol: glycerine: sorbitol (2:1:1). All the aqueous soluble ingredients were dissolved in water and all oil soluble ingredients were mixed at 75^0 c± 50c in separate beakers. The aqueous phase was then added to oil phase slowly with constant stirring and the mixture was homogenized for 30 min.

Evaluation of creams[7, 9, 14]

pH : pH was measured by pH meter

Viscosity: Viscosity of cream was determined by Brookefield viscometer.

The viscosity measurements were done using Brookefield DV-II + viscometer using LV-4 spindle. The developed formulation was poured into the adaptor of the viscometer and the angular velocity increased gradually from 0.5 to 20 rpm.

Stability studies[8]

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies were done according to ICH guidelines.

The stability studies were carried out as per ICH guidelines. The cream filled in bottle and kept in humidity chamber maintained at 30 ± 2 °C/ 65 ± 5 % RH and 40 ± 2 °C / 75 ± 5 % RH for two months. At the end of studies, samples were analyzed for the physical properties and viscosity.

Spreadability studies

An important criteria for semisolids is that it posses good spreadability. Spreadability is a term expressed to denote the extent of area to which the cream readily spreads on application to the skin. The therapeutic efficacy of a formulation also depends on its spreading value. A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of the two, better the spreadability.

Two glass slides of standard dimensions were selected. The formulation whose spreadability had to be determined was placed over one of the slides. The other

slide was placed on top of the formulations was sandwiched between the two slides across the length of 5 cm along the slide. 100 g weight was placed up on the upper slide so that the formulation between the two slides was pressed uniformly to form a thin layer.

The weight was removed and the excess of formulation adhering to the slides was scrapped off. One of the slides was fixed on which the formulation was placed. The second movable slide was placed over it, with one end tied to a string to which load could be applied by the help of a simple pulley and a pan.

A 30g weight was put on the pan and the time taken for the upper slide to travel the distance of 5.0cm and separate away from the lower slide under the direction of the weight was noted.

The spreadability was then calculated from the following formula:

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

m = weight tied to the upper slide (30g)
l =length of glass slide (5cm)
t =time taken in seconds.

Determination of marker ingredient by HPLC

The extracts and formulated creams were tested for the presence of marker ingredients by HPLC. Curcumin and the IS emodin were separated on a Diamonsil C(18) analytical column (4.6 x 100 mm, 5 micron) using acetonitrile-5% acetic acid (75:25, v/v) as mobile phase at a flow rate of 1.0 mL/min with detection at 425 nm.

Test for microbial growth in formulated creams[10]

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 cream. The plates were placed in to the incubator and are incubated at 37^0C for 24 hours. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control.

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, flavanoids and phenolic compounds. The IC_{50} of the extract was found to be 293.07± 16.96. The results are summarized in table no. 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 between4.5– 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. 3. Viscosity of creams is different at different revolution per minute. At 0.5 rpm to 20 rpm viscosity was decreased from 6897 to 642 cps. 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 no. 4. The stability studies of the various parameters like visual appearance, nature, p^H 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 no. 5. HPLC chromatogram of extract and formulation confirms the presence of marker ingredient in F1 and the peak was comparable with the standard HPLC chromatogram. 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 37^0C and it was comparable with the control.

CONCLUSION

The prepared formulations showed good spreadability, no evidence of phase separation and good consistency during the study period. Stability parameters like visual appearance, nature, viscosity and fragrance of the formulations showed that there was no significant variation during the study period. The prepared formulations showed proper pH range that

is approximately pH 6; it confirms the compatibility of the formulations with skin secretions. HPLC chromatogram of extract and formulation confirms the presence of marker ingredient and the peak was comparable with the standard HPLC chromatogram. The creams were found to be stable during stability study according to ICH guidelines (30 ± 2 °C/ 65 ± 5 % RH and 40 ± 2 °C/ 75 ± 5 % RH) for 2 months. From the present study it can be concluded that it is possible to develop creams containing herbal extracts having antioxidant property and can be used as the provision of a barrier to protect skin.

Table 1: Antioxidant activity

Extract		Ascorbic acid	
Cocentration (µg/ml)	**% Scavenging**	**Concentration (µg/ml)**	**% Scavenging**
100	27.17±1.61	10	28.39±2.43
150	31.91 ±1.97	20	39.62±2.19
200	35.42 ±2.02	30	46.74± 2.36
250	40.21 ±2.84	40	55.63±2.77
300	51.32 ±1.92	50	63.57± 3.48
IC_{50}	**293.07±16.96**	----	**34.79±0.64**

Values are mean ± SEM, n=3.

Table 2: Formulation of cream

Ingredients	F1(%w/w)	F2(%w/w)
Extract in Isopropyl alcohol	2	2
Stearic acid	10	12
Triethanolamine	1.3	1.6
Mineral oil	3.5	3
Moisturizer conditioner	10	12
Cetyl alcohol	2	1.5
Propyl paraben	0.02	0.02
Sodium metabisulphite	0.1	0.1
EDTA	0.1	0.1
Water qs to100ml	q.s	q.s

Table 3: Spreadability and pH

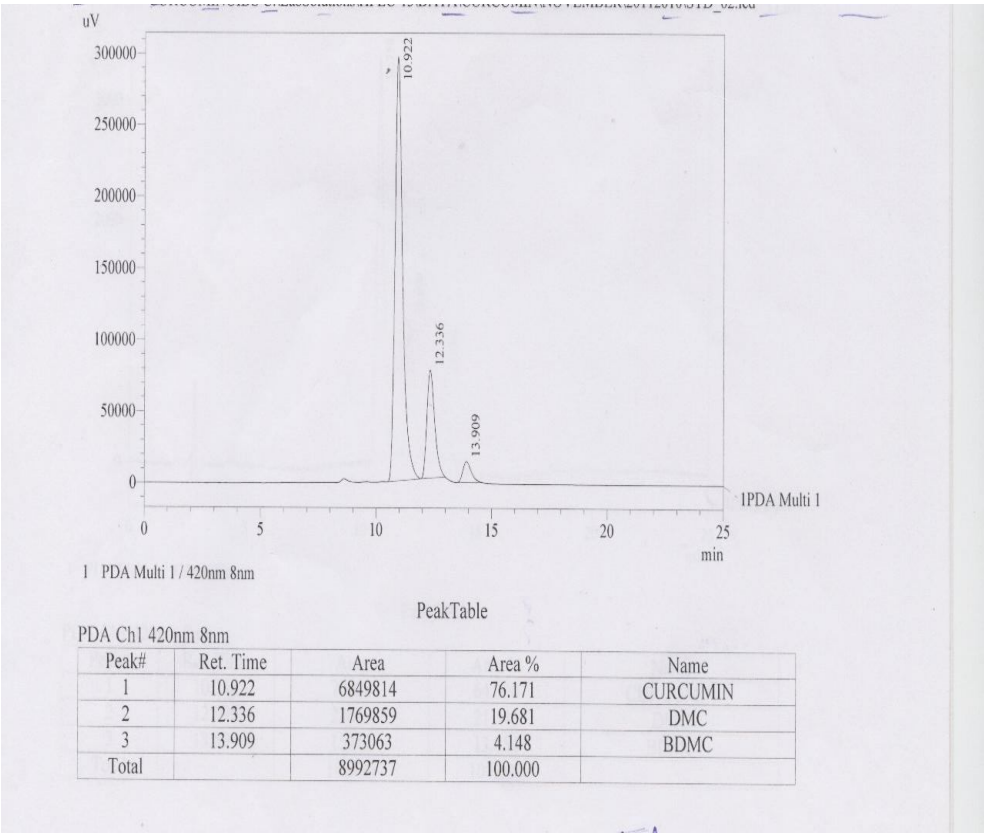
Formulations	Time in seconds	Spreadability (g cm/sec)	pH
Formulation-I	11	12.78	6.4
Formulation-II	12	10.67	6.2
Marketed cream	10	15	6.8

Table 4: Viscosity

rpm At	Viscosity of cream (cps)		
	F1	F2	Marketed cream
20	642	629	658
10	964	939	986
5	1621	1597	1618
1	3271	3217	3343
0.5	6825	6783	6896

Table 5: Stability studies (Observation after 2 months)

Formulations	pH	Colour & Appearance	Viscosity at 20 rpm
F 1	5.8	Yellowish brown & non glossy	595
F 2	6.2	Yellowish brown & non glossy	601
Marketed cream	6.4	Pinkish white & non glossy	625

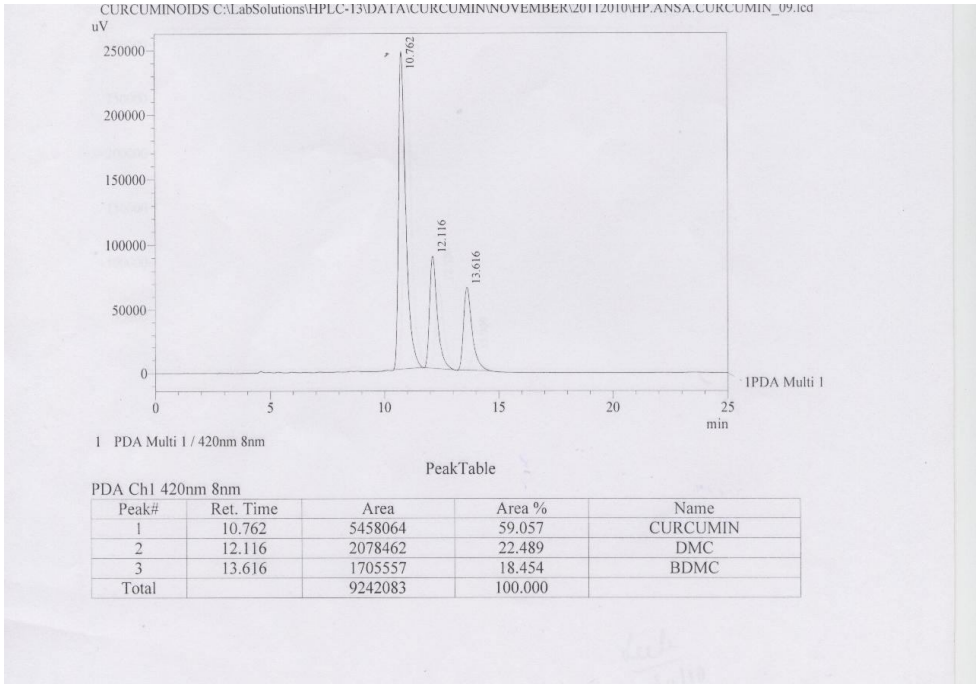


PeakTable

PDA Ch1 420nm 8nm

Peak#	Ret. Time	Area	Area %	Name
1	10.922	6849814	76.171	CURCUMIN
2	12.336	1769859	19.681	DMC
3	13.909	373063	4.148	BDMC
Total		8992737	100.000	

Fig. 1: HPLC of Standard Curcumin

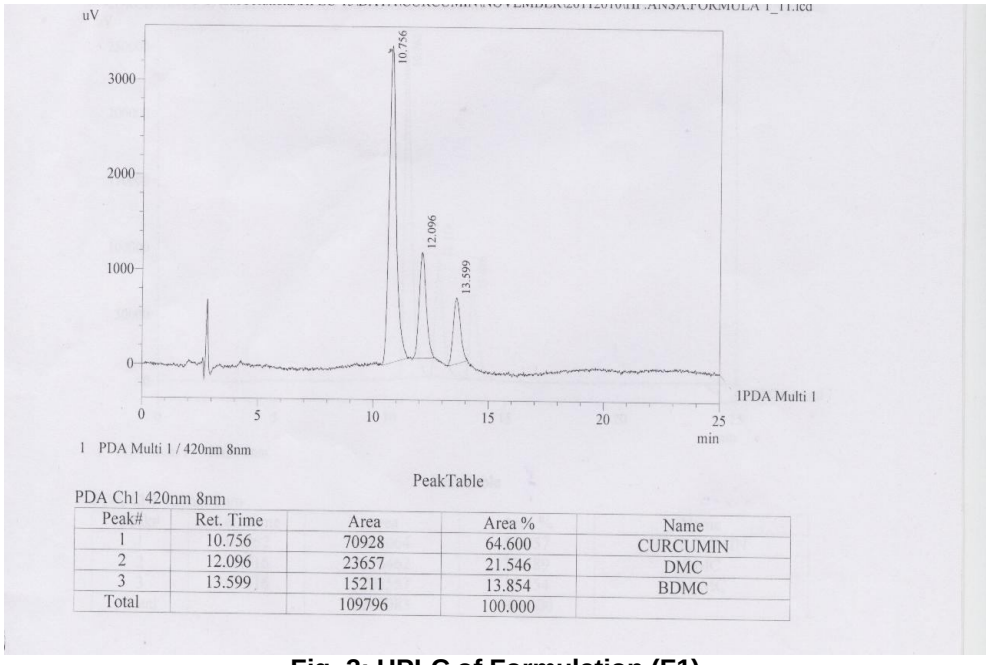


PeakTable

PDA Ch1 420nm 8nm

Peak#	Ret. Time	Area	Area %	Name
1	10.762	5458064	59.057	CURCUMIN
2	12.116	2078462	22.489	DMC
3	13.616	1705557	18.454	BDMC
Total		9242083	100.000	

Fig. 2: HPLC of Extract



PeakTable

PDA Ch1 420nm 8nm

Peak#	Ret. Time	Area	Area %	Name
1	10.756	70928	64.600	CURCUMIN
2	12.096	23657	21.546	DMC
3	13.599	15211	13.854	BDMC
Total		109796	100.000	

Fig. 3: HPLC of Formulation (F1)

ACKNOWLEDGEMENT

The Authors are thankful to the management authoritories of CCOPS for providing necessary facilities to carry out this study.

REFERENCES

1. Kaur IP, Kapila M, Agrawal R. Role of novel delivery systems in developing topical antioxidants as therapeutics to combat photo ageing. Ageing Res Reviews 2007;6:271-288.
2. Watson, Ogden S, Cotterell LF, Bowden JJ, Bastrilles JY, Long SP, Griffiths CE.A cosmetic 'anti-ageing' product improves

photo aged skin: a double-blind, randomized controlled trial British. J Dermatol 2009;161;419–426.
3. Ochani PC, D'Mello P. Antioxidant and antihyperlipidemic activity of Hibiscus sabdariffa Linn. Leaves and calyces extracts in rats. Indian J Exp Biol 2009; 47(4):276-82.
4. Arumugam P, Ramamurthy P, Thyagarajan S, Santhiya and Ramesh A Antioxidant activity measured in different solventfractions obtained from Mentha spicata Linn.: An analysis by ABTS + decolorization assay. Asia Pac J Clin Nutr 2006;119-124.
5. Ravishankara, M.N. Shrivastava, Neeta; Padh, Haris; Rajani M.(2002) Antioxidant activity of methanolic extract of Hemidesmus indicus root bark in several in vitroand ex vivo models. Phytomedicine: International Journal of Phytotherapy & Phytopharmacology March 1, 2002.
6. Siddhuraju P. and Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata (L.)* Walp.) Seed extracts, Food Chemistry. 2007,101: 10–9.
7. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharm Exp Thera, 1944, 82, 377-90.
8. Note For Guidance on Stability Testing. Stability Testing of New Drug Substances and Products.
9. Puvabanditsin P, Vongtongsri R. Efficacy of aloe vera cream in prevention and treatment of sunburn and suntan. J Med Assoc Thai 2005 Sep;88(4):173-6.
10. MarieLode N, Buraczewska I, Halvarsson K. Facial anti-wrinkle cream: influence of product presentation on effectiveness: a randomized and controlled study. Skin Res Technol 2007;13:189–194.
11. Ravichandran G, Bharadwaj S, Kolhapure SA. Evaluation of the efficacy and safety of “Anti-Wrinkle cream” in the treatment of facial skin wrinkles: A prospective, open, phase III clinical trial. The Antiseptic 2005;102(2):65-70.
12. Srinivas KVNS, Rao YK, Mahender I, et al. Flavonoids from Caesalpinia pulcherrima. Phytochemistry; 2003, 63: 789–93.
13. Kamlesh J. Wadher, C.L Lakhotia and M J Umekar Formulation and evaluation of cream of Azadirachta indica leaves extracts on skin renewal rate, International Journal of Chem Tech Research, 2009, 1, 88-95.
14. Kawada A, Konishi N, Momma T, Oiso N, Kawara S Evaluation of anti-wrinkle effects of a novel cosmetic containing retinol using the guideline of the Japan Cosmetic Industry Association. J Dermatol 2009; 36:583–6.
15. Kawada A, Konishi N, Momma T, Oiso N, Kawara S. Evaluation of anti-wrinkle effects of a novel cosmetic containing Niacinamide. J Dermatol 2008;35:637–642.
16. Kamkaen N, Phuntuwate W, Samee W, Boonrod A, Treesak C. The investigation of the rabbit and human skin irritation of herbal Anti-wrinkle cream. Thai Pharm health Sci J 2007;2(1):20-5.