

## FORMULATION AND EVALUATION OF HERBAL WOUND HEALING FORMULATION OF CENTELLA ASIATICA

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### ABSTRACT

Wounds are physical injuries that results in a gap or infringement of the skin. Proper healing of wounds is very essential for the re-establishment of disrupted anatomical continuity and disturbed functional status of the skin. Wound healing is a complex but usually systematic process. Sequential waves of specialized cell types first clear the inciting injury and then progressively build the scaffolding to fill in any resulting defect. Cream is defined as semisolid emulsions which may be oil in-water (o/w) or water- in- oil (w/o) type and these semisolid emulsions are meant for external applications. In this study, we have formulated an herbal cream (O/W type) fulfilling almost all the pharmaceutical parameters which showed better tissue regeneration and healing power. The formulated herbal cream contains a mixture of Centella asiatica extract, corn oil (used to reduce the surface tension),

cetyl alcohol (moisturizer), sodium lauryl sulphate (anionic detergent and surfactant), glyceryl monostearate, propyl paraben (Preservative), Methyl paraben (anti-fungal agent) and purified water (vehicle). The pH of the formulated cream was found to be 7.00 which is good for skin. The creams also showed good Hardness (9.21 g.cm/sec) when measured using a texture After application of the cream the type of smear formed on the skin was found to be non-greasy and easily removed on washing with tap water. The viscosity of the creams was found to be 2150 cps, with 25 rpm, which indicates that the prepared cream was easily spreadable with small amount of shear.

**KEYWORDS:** Cream, O/W emulsion, TLC, Formulation.

## INTRODUCTION

Wound may be defined as a loss or infringement of cellular and anatomic or functional continuity of living tissue. It is produced by physical, chemical, thermal, microbial, or immunological damage to the tissue. Wound healing or wound repair is the body's natural process of regenerating dermal and epidermal tissue. Healing requires the mutual efforts of various tissues and cell lineages. It involves aggregation of platelets, clotting of blood, fibrin formation, and an inflammatory response to wound, alteration in the ground substances, angiogenesis and re-epithelialization. Healing process is not complete until the disrupted surfaces are firmly knit by collagen.<sup>[1,4]</sup> World Health Organization (WHO) is advocating the use of traditional medicines because they are less expensive, easily available and comprehensive, especially in developing countries.<sup>[3,5]</sup> It is also true that eight percent of the world's population relies on medicinal plants for their primary health care. Across the world importance of traditional medicine is recognized has treatment strategies, guidelines and standard for ethno medicine.<sup>[6,9]</sup> Hence after an in depth review regarding pathogenesis as well as different traditional and alternative therapy for wound healing, we have identified *Centella Asiatica* (CA) species which are reported to have good wound healing properties. Leaves extract of *Centella asiatica* (CAE) contain, Asiaticoside, a constituent in CA, has been reported to possess wound healing activity by increasing collagen formation and angiogenesis.<sup>[4,5]</sup>

*Centella* has large amounts of pentacyclic triterpenoids including asiaticoside, brahmoside, Asiatic acid, and brahmie acid (madecassic acid). Other constituents include centellose, centelloside, and madecassoside.<sup>[1,2]</sup> A preclinical study on poly herbal cream containing aqueous CAE applied to open wounds in rats (3 times daily for 24 days) resulted in increased cellular proliferation and collagen synthesis at the wound site, as shown by an increase in collagen content and tensile strength.<sup>[6,7]</sup> The present work is an attempt to develop cream containing aqueous extract of CA using principles of formulation design and design of experiment strategy to desired spreading and drug release properties.

## MATERIALS AND METHODS

The sample of *Centella asiatica* extracts was acquired from Amsar Pvt. Ltd., (Indore, India). Active marker Asiaticoside was obtained from Sigma Pvt. Ltd., (Pune, India). All other ingredients used in the formulation of cream were purchased from local market.

## Development and formulation of cream

### Pre formulation studies

IR studies were carried out on the formulation excipients such as corn oil, cetyl alcohol, SLS and GMS with aqueous extract of *Centella Asiatica* to check for any incompatibilities. These samples were stored at 40 °C for 2 weeks and subjected to FTIR analysis using spectrophotometer between 3000 cm<sup>-1</sup> to 300 cm<sup>-1</sup>.

### Determination of solubility of aqueous extract in oil

The solubility of aqueous extract in oils (corn oil, sesame oil, arachis oil and linseed oil) was determined. The extract (1 g) was taken in 2 mL and kept overnight in orbital shaker (make: CIS-24 BL), the oil was filtered and analyzed using HPTLC method.

### Selection of cream formula

Cream formula was selected from literature.<sup>[9]</sup> The list of ingredients and quantity used to prepare the wound healing cream is depicted in Table 1.

**Table 1: Formula for wound healing cream.**

Ingredients	Formulation %w/w in grams
Centella asiatica extract (mg)	0.2
Corn oil	1.0
Cetyl alcohol	1.4
Sodium lauryl sulphate	0.6
Glyceryl monostearate	0.1
Propyl paraben: Methyl paraben (1:10)	0.33
Purified water	13.2

### Design of experiments

A 3<sup>2</sup> factorial design was chosen to select a formula which will provide optimum viscosity, hardness and drug release. The factor combination chosen with levels is as given in Table. 2.

**Table 2: The factor combination and response chosen for factorial design.**

Variables		Levels		
		+1	0	-1
A	Oil	5	3	1
B	SLS	0.6	0.2	0.3
Responses		Goal	Acceptable Ranges	
Y1	Hardness	Maximize	< 15	
Y2	Particle size	minimize	< 700	
Y4	Drug content	Maximize	99-101 %	

### **Preparation of herbal wound healing cream**

The creams were prepared by fusion method using formulae as per Table 1. The oil phase ingredients (extract, corn oil, GMS and cetyl alcohol) were weighed and mixed with continuous stirring using mechanical stirrer at 100 rpm at the temperature 80°C to form uniform liquid. Similarly the water phase ingredients were weighed mixed with continuous stirring using mechanical stirrer at 100 at 80°C. The phases were mixed with constant stirring using mechanical stirrer at 100 rpm (Remi motor RQT-127 HP1/8) for 30 min till the uniform distribution of the ingredients was achieved. All these batches were allowed to equilibrate for 24 h at room temperature.

### **Evaluation of herbal wound healing cream**

#### **a) Determination of pH**

Accurately weighed quantity of cream was dispersed in water to prepare 1% w/v concentration. Calibrated meter (Make: I, DELUXE-101) was used to determine the pH.

#### **b) Hardness**

Hardness of formulation was determined using a texture analyzer (Brookfield CT-3) cream (20 gm) was filled in conical probe (up to plane surface of top) and Hardness was measured. This apparatus shows Hardness into the comparison of spreadability and adhesive force.

#### **c) Determination of mean globule diameter<sup>[12]</sup>**

The mean globule diameter was determined by using the method of optical microscopy using 1% dispersion of cream in water.

#### **d) Viscosity**

The viscosity of prepared creams was determined using viscometer (Brookfield digital viscometer RVDV Pro) equipped with ULE adapter. The spindle (S06) was rotated at 0.5 rpm. Samples of the cream were allowed to settle over 30 min at the temperature (25±10C) before the measurements were taken. Viscosity was reported in (cP).<sup>[10,11]</sup>

#### **e) In-vitro drug release**

The release of drug from the cream was determined using Franz diffusion cell apparatus for 6h. The receptor medium was phosphate buffer pH 6.8, maintained at 37°C. The membrane filter (cellulose acetate) pore size 0.45µ was soaked in phosphate buffer pH 6.8 for 1h and mounted between the donor and receptor compartment. The gel was placed on receptor

compartment and both the compartments were clamped together. The phosphate buffer pH 6.8 in the receptor compartment (8 ml) was stirred using magnetic stirrer 60 rpm. The samples (1ml) were withdrawn at different time intervals and replaced with an equal volume of buffer. The samples were analyzed spectrophotometrically at 297 nm. The % cumulative drug releases were calculated.<sup>[13]</sup>

### TLC identification

TLC analysis was performed using as a stationary phase precoated silica gel 60 F254 aluminium plates, the mobile phase was ethyl acetate: methanol: water (10: 2.5: 1, v/v/v). Anisaldehyde sulfuric reagent (heated at 100 °C for 5–10 min) was employed as spot detection. The sample solutions were freshly prepared before each analysis. The following solutions were prepared in triplicate:

**Sample solution:** A solution of cream in methanol of 100 mg/ml was prepared employing an ultrasonic bath (45°C, 10 minutes). This solution was cooled at room temperature and filtered.

**Solution of *Centella asiatica* extract:** A solution of *Centella asiatica* dry extract raw material in methanol of 1 mg/ml was prepared using an ultrasonic bath (45 °C, 10 minutes).

**Asiaticoside reference solution:** A solution of 1 mg/ml in methanol of asiaticoside was prepared.

**Excipients blank solution:** It was prepared in the same manner as the sample solution.

The sample solution, the *C. asiatica* extract solution, the asiaticoside solution and the excipient blank solution were applied as a 10 mm band on TLC plate using a capillary tube. The development distance was 9.0 cm.

## RESULTS AND DISCUSSION

### Development and formulation of cream

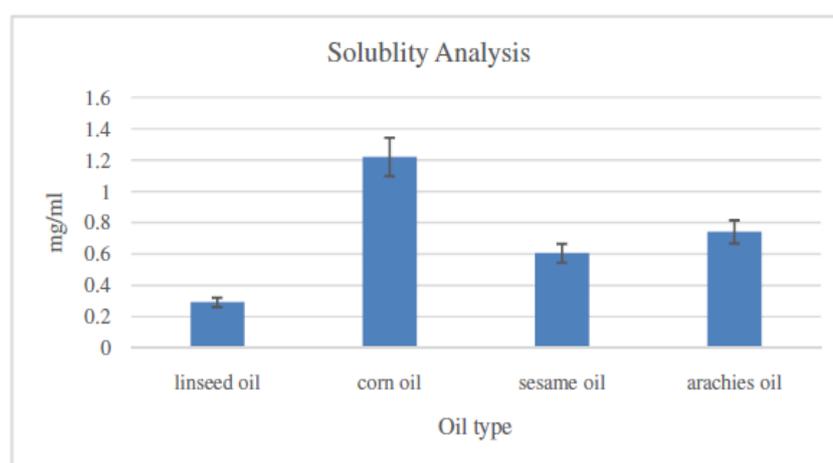
#### Pre formulation studies

Asiaticoside promotes fibroblast proliferation and extracellular matrix formation in wound healing. The use of synthetic and semi synthetic excipients in cosmetics causes long term side effects besides causing pollution. The CAE has been traditionally used and well documented natural ingredient having surface active activities. The use of surfactants like sodium lauryl

sulphate (SLS) is fraught with the disadvantage that apart from sebum and dirt they can also readily dissolve the skin's natural oil. A solution of 2% SLS can increase skin thickness, cause irritation, inflammation and increase other forms of immune activity in the skin. SLS can also cause an increase in enzyme levels in the skin, leading to redness and swelling. The objective of present work is to determine the utility of the dried aqueous CAE as emulsifier for skin creams which will not only provide biocompatible skin friendly cosmetic preparations but also reduce the environmental pollution because of its biodegradability.

### Determination of solubility of aqueous extract in oil

Highest solubility of extract was seen in corn oil, the solubility's are depicted in Fig. 1.



**Fig. 1: Solubility of Centella Asiatica extract in different oils.**

### Design of Experiments

Optimization of the formula was done using 2 factor 3 level factorial design using Design Expert 10.4 trial version. The responses such as spreadability, particle size and viscosity were chosen for optimization which depended mainly on quantity of oil phase and the surfactant hence these were selected as factors. The three levels give good idea about interaction between factors if any.

### Evaluation of herbal wound healing cream

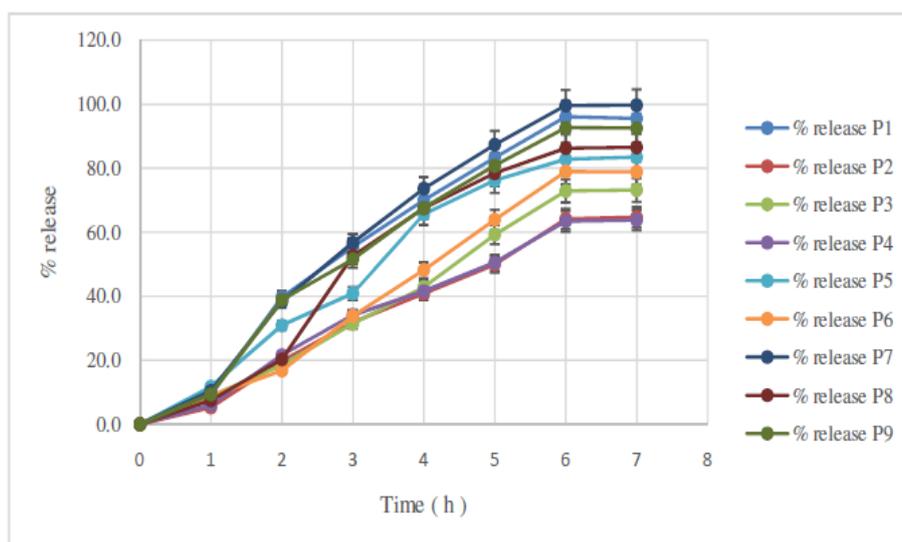
All the developed formulations were found to be within the limits. The drug content of P8 formulation was found to be highest. P8 formulation complies with requirements of pharmaceutical parameters. Cetyl alcohol and glyceryl monosterate was melted in liquid paraffin at 60°C to form oil phase. Propyl paraben was added in the same phase. Aqueous CAE was dissolved in corn oil and methyl paraben was added to this phase. It was heated at

600C to form the aqueous phase. The oil phase was added to the aqueous phase with constant stirring until an emulsion was formed.

**Table 3: Design and evaluation of herbal cream.**

Formulation code	Corn Oil	SLS	pH	Viscosity (cP)	Hardness (g.cm/sec)	Particle Size	Drug content %
P1	5	0.4	6.67	3050	44.31	732.9	96.1
P2	1	0.4	6.65	1110	44.31	475	64.1
P3	1	0.6	6.45	1420	6.41	466.5	72.9
P4	1	0.2	6.97	1300	6.38	481	63.4
P5	3	0.4	6.00	2510	10.77	518.5	82.8
P6	3	0.2	6.25	1970	16.68	618.9	78.9
P7	5	0.6	6.95	3170	38.46	702	99.5
P8	3	0.6	7.00	2150	9.21	492.3	86.2
P9	5	0.2	6.87	3450	45.34	759.2	92.6

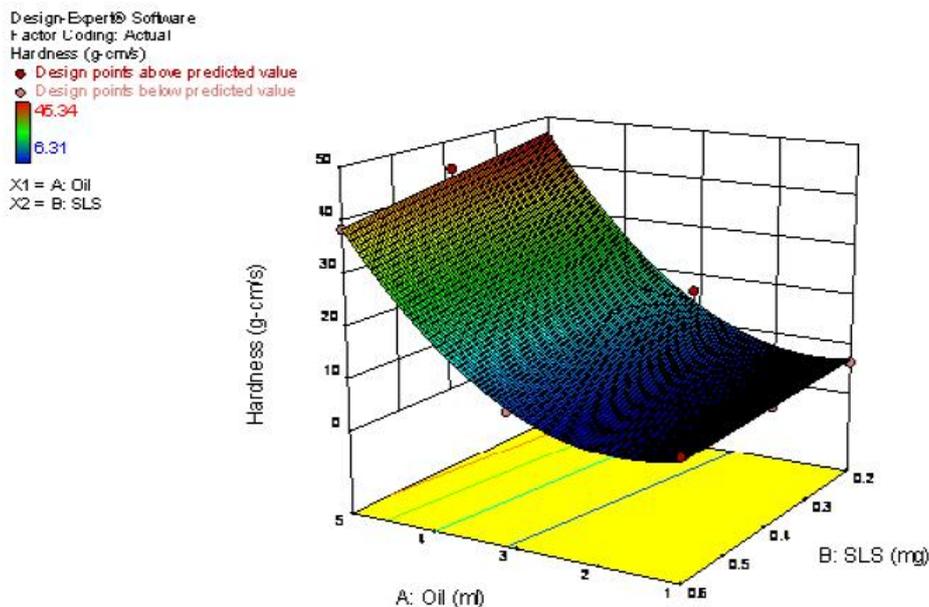
The pH of the formulation 7 which is acceptable for topical formulation. Since topical systems are directly applied on the skin, their pH should be compatible with the skin pH.



**Fig 2: % in vitro drug release from optimization batches.**

Fig. 3 gives the response surface curve for the hardness of the cream. It indicates that as the concentration of oil goes on increasing the hardness also goes on increasing and the SLS has no effect on the hardness of the cream. Eq. 1.

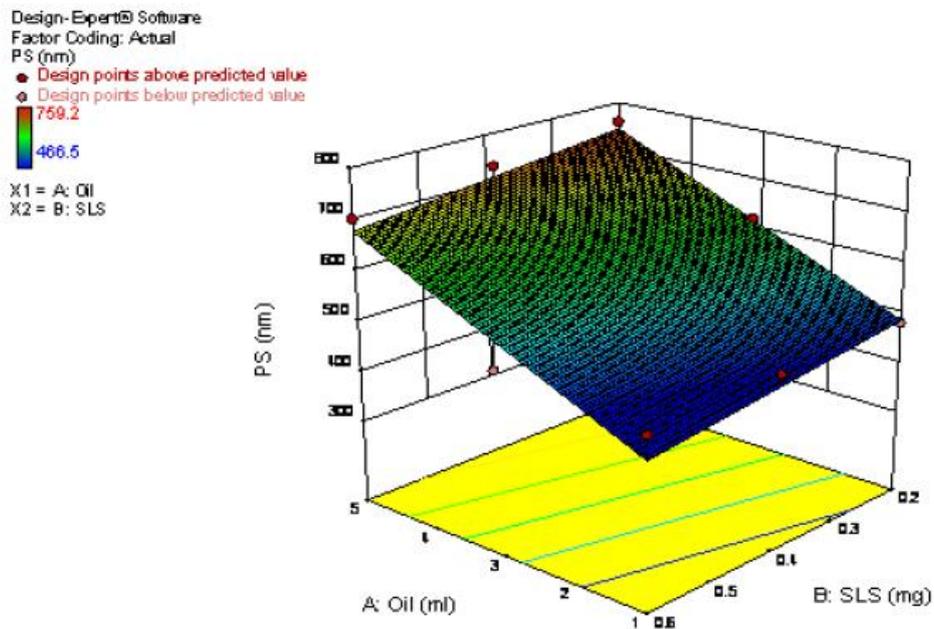
$$\text{Hardness} = +12.25 + 18.16 * A - 2.38 * B - 1.72 * AB + 12.31 * A^2 - 0.05 * B^2 \text{ --- (1)}$$



**Fig. 3: Response surface depicting effect of oil and SLS on spreadability.**

Fig. 4 shows the response surface curve for the particle size for the cream. Concentration of oil has a significant positive impact on the particle size and because concentration of oil direct affects the globule size. Also the concentration of SLS has a slight negative effect on the particle size of the cream and reduces it. Eq 2

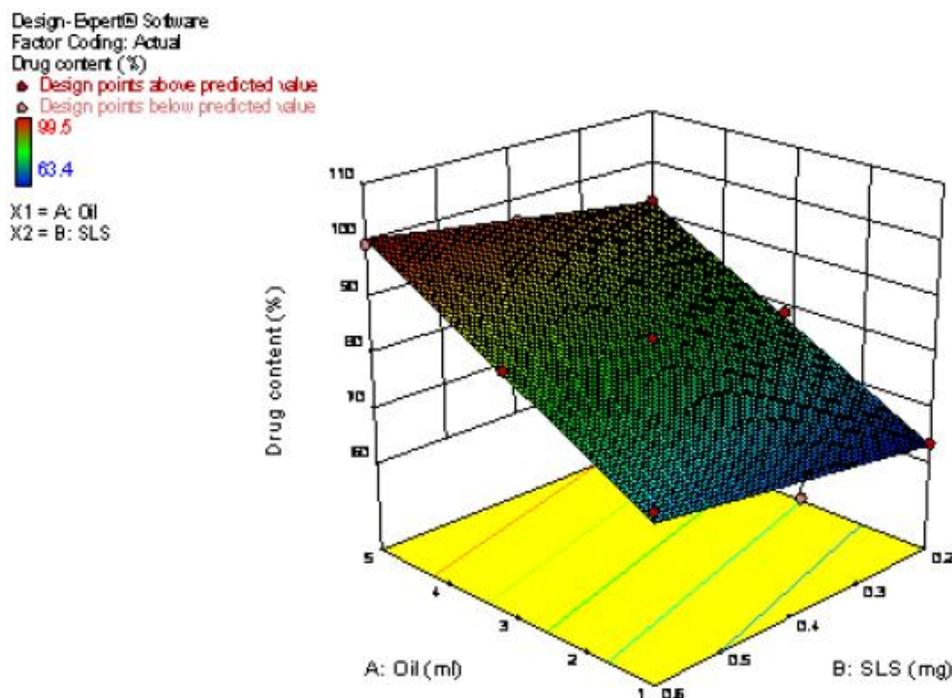
$$PS = +582.92 + 128.60 * A - 33.04 * B \text{ ---- (2)}$$



**Fig. 4: Response surface depicting effect of oil and SLS on particle size.**

Fig. 5 represents the response surface curve for the drug content in the formulation. As per the response surface curve, Oil has significant positive impact on the drug content and as the concentration of oil goes on increasing there is increase in the drug content of the formulation and thus proves that drug is readily soluble in oil. Concentration of SLS has no impact on drug content. Eq. 3

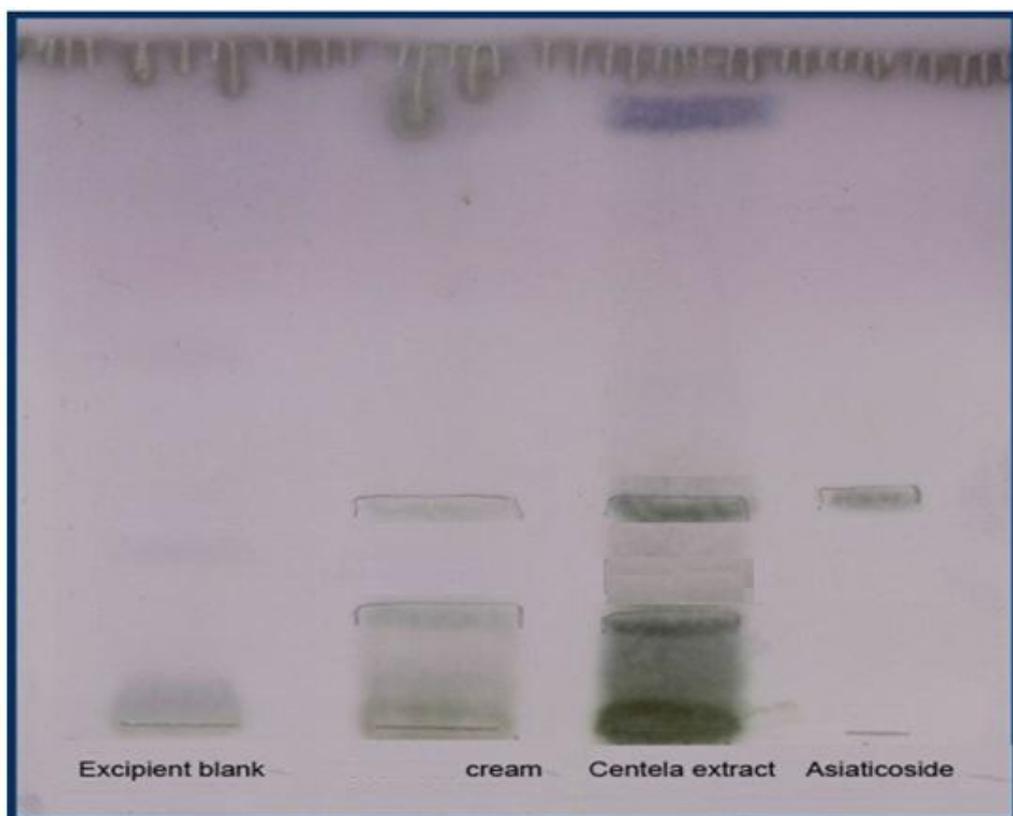
$$\text{Drug content} = +81.83 + 14.63 * A + 3.95 * B \text{---- (3)}$$



**Fig. 5: Response surface depicting effect of oil and SLS on drug content.**

### TLC Identification

After treatment with anisaldehyde sulfuric reagent, similar spots at  $R_f$  and colour with the profile of *C. asiatica* extract solution were observed in the chromatographic profile of the cosmetic cream. In the lower third of the chromatogram, a dark green spot ( $R_f$ : 0.48) corresponding to asiaticoside (Figure 1) was observed. The extract of *C. asiatica* raw material was analyzed by TLC using asiaticoside as a marker because of its greater commercial availability and lower cost. In that way, asiaticoside, the major active principle in the raw material extract employed, was identified. (Fig. 6).



**Fig. 6: TLC analysis. Excipients blank; cream; Centella extract; Asiaticoside.**

## CONCLUSIONS

P8 formulation or cream was found to be best and satisfactory compared to all other formulations. It had light brown appearance, gave a cool and smooth feel on application. The pH of the formulation was found to be 7.00 which is good for skin. The creams also showed good Hardness (9.21 g.cm/sec) when measured using a texture analyzer, the apparatus consist of two glass cone shaped probe. Fill one probe with 20 gm. of cream (up to plane surface of top) and measure the Hardness. This apparatus shows Hardness into the comparison of hardness and adhesive force was calculated. After application of the cream the type of smear formed on the skin was found to be non-greasy and easily removed on washing with tap water. The viscosity of the creams was found to be 2150 cps, with 25 rpm, which indicates that the prepared cream was easily spreadable with small amount of shear.

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