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FORMULATION AND EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT POLYHERBAL LOTION

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ABSTRACT

There is increased scientific evidence that plants possess a vast and complex arsenal of active ingredients which have the ability to calm or smooth the skin as well as restore actively, heal and protect the skin. The present work deals with the development and evaluation of the poly herbal lotion containing *Trigonella foenum-graecum*, *Citrus lemon*, *Matricaria chamomilla* and *Cymbopogon citrates*. Different types of formulations oil in water (O/W) herbal lotions namely F1 to F13 were formulated by incorporating different concentrations of stearic acid and Triethanolamine. Formulation of Triethanolamine and stearic acid was optimized as 2.52:9.35. The prepared lotion was evaluated for its antimicrobial, antioxidant and pharmaceutical parameters. The lotion formulation showed no redness, edema, inflammation and irritation during sensitivity test indication that it is safe to use. Stability studies of the lotion showed that the lotion was stable after three months.

Key Words: Herbal formulation, Triethanolamine, Stearic acid, Antioxidant, Antibacterial

INTRODUCTION

Herbal extracts are primarily added to cosmetic preparations due to several associated properties such as antioxidant, antibacterial and anti-inflammatory properties. The lotion as topical suspensions, solutions and emulsions are usually serve as vehicles for topically applied drugs, as emollients, or as protective or occlusive dressings, or they may be applied to the skin and membranes such as the rectal, buccal, nasal, and vaginal mucosa, urethral membrane, external ear lining, or the cornea (Idson & Lazarus 1987, Buhse *et al.* 2005). But the serious problems associated with the formulation and manufacture of topical–mucosal preparations is the establishment of reliable techniques for their characterization, mainly because of the complexity of their physical structure (Tamburic *et al.* 1996).

Herbal remedies enrich the body with nutrients and other useful minerals. There is increased scientific evidence that plants possess a vast and complex arsenal of active ingredients which not only have the ability to calm or smooth the skin but also to restore actively, heal and protect the skin (Gediya *et al.* 2011, Shivanand *et al.* 2010). An herbal lotion that can give effective protection to skin and free from any toxicity or toxic residue or irritation when regularly used should be cosmetically acceptable.

The objective of the present paper was development of formulation of lotion from herbal extracts and their evaluation.

MATERIALS AND METHODS

Plant materials

The medicinal plants *Allium sativum*, *Trigonella foenum-graecum*, *Citrus lemon*, *Matricaria chamomilla* and *Cymbopogon citrates* were collected from the local market of Kathmandu. The bulbs of *Allium sativum*, seeds of *foenum-graecum* were sun dried for seven days. After drying the plants were crushed in powder. The *Citrus lemon* was squeezed to obtain the juice. The plants were identified at Department of Pharmacy, Kathmandu University, Nepal. Herbal specimen was deposited at the Department of Pharmacy, Kathmandu University. The essential oils of *Matricaria chamomilla* and *Cymbopogon citrates* were obtained as a gift sample from Herbs Production and Processing Co. Ltd Nepal.

Preparation of extracts

About 500 g of dried powder of plant was extracted in ethanol for seven days with frequent agitation. The extraction process was carried out three times with the same sample. The filtrates were collected and evaporated in a water bath. Lemon juice obtained by squeezing was further concentrated in water bath.

Phytochemical screening

Phytochemical screening of all extracts was carried out according to standard method (Trease & Evans 1989). The extracts were analyzed for presence of alkaloids, carbohydrates, glycosides, saponin, phytosterol, phenols, tannins and flavonoids.

Antibacterial property

Antibacterial property was evaluated by well diffusion method (Collins et al 1995). The microorganisms used were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*. Total 20 μ l of test solutions of the sample and standard prepared (2%, 4%, and 6%) were then pipetted with the help of the micropipette and poured in the prepared bore in each nutrient agar plate swabbed with different bacteria with great care. Similarly, 20 μ l of DMSO (10%) solution was also poured in another bore as control. The nutrient agar plates were allowed to stand for about 1 hour till the test solution completely diffuses in the media. The plates were then incubated at the temperature of 37°C for 48 hours. After 48 hours, zone of inhibition was measured (Junaid et al. 2006). Minimum Inhibitory Concentration (MIC) test was carried out by serial dilution method (Mhatre et al. 2014). The extracts and EO were serially diluted to obtain the concentration from 200 mg/ml to 6.25 mg/ml. A loop full of microbial suspension which was already prepared was added to each test tube. The test tubes were then incubated at 37°C for 24 hours. The MIC is the concentration of the highest dilution tube in which the bacterial growth was absent, hence no turbidity observed.

Determination of DPPH free-radical scavenging activity

The antioxidant activity of the extracts and the essential oil based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Mensor et al. (2001) with a slight modification. In the DPPH radical scavenging assay, antioxidants react with DPPH, and convert it to the yellow colored a, a-diphenyl- β -picryl hydrazine. Ascorbic acid was used as standard.

The DPPH radical scavenging activity percentage was calculated by using the formula:

$$\text{DPPH radical scavenging activity (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

Where, A_{control} is the absorbance of a DPPH solution without sample, and A_{sample} is the absorbance of the sample.

Formulation of lotion

Topical lotion formulation was designed using central composite design (CCD) with two factor and 3 levels. Stearic acid and Triethanolamine were chosen as independent variables while spreadability, pH and viscosity as dependent factors. The lotion was prepared by adding non-polar phase to the polar phase with rapid stirring to avoid separation of water and oil phase. Non-polar phase was first melted together and slowly added to the preheated mixture of polar phase. Triethanolamine and stearic acid when mixed, undergo an acid-base reaction to yield an ionic compound (a salt). This salt has a nonpolar section and a charged section, so it is a good emulsifying agent. Cetosteryl alcohol in combination with other ingredients in the formulation such as Triethanolamine and stearic acid forms an emulsion. This stops oil and water from separating.

Statistical analysis

Mathematical relationships for the measured dependent variable (response) and the independent variables were developed using statistical software Minitab. Thirteen tests were conducted as per the software. The three output variables (responses), such as viscosity (Y1), pH (Y2), spreadability (Y3), were evaluated. The experimental results were fitted to a polynomial quadratic model by applying multiple regression analysis for pH, viscosity and spreadability. The effect of formulation variables on different dependent or response variables was assessed by the generated regression coefficients and r^2 values. The fitted quadratic equations relating the responses such as viscosity (Y1), pH (Y2), spreadability (Y3), to the transformed factor are given in equations respectively below:

$$Y_1 = +5656.6 - 101.1 X_1 + 1755.8 X_2 - 365.7 X_1 X_2 + 374.8 X_1^2 + 872.8 X_2^2 \dots\dots\dots (1)$$

$$Y_2 = +5.77400 + 0.64897 X_1 - 0.21598 X_2 - 0.17750 X_1 X_2 - 0.10613 X_1^2 - 0.02388 X_2^2 \dots\dots\dots (2)$$

$$Y_3 = +9.64 + 0.00214 X_1 - 0.78373 X_2 + 0.32000 X_1^2 - 0.14500 X_2^2 - 0.02500 X_1 X_2 \dots\dots\dots (3)$$

Where; X_1 is concentration of Triethanolamine, X_2 is the concentration of stearic acid.

Optimization of Formulation

The formulation was optimized using response optimizer in Minitab Software. The excipients used as variables were Triethanolamine and Stearic acid. Desirability function was calculated for pH (Y1), viscosity (Y2), and spreadability (Y3) using the software. On the basis of the ratio obtained from

the software final optimized formulation was developed.

Evaluation of Pharmaceutical parameters of lotion

Preliminary Pharmaceutical evaluation of lotion formulations was carried out as follows:

PH: Lotion pH was measured with a digital pH meter. 10% solution of lotion was prepared and the solution was immersed in the pH meter and the measured pH was recorded (Namita & Nimisha 2013).

Viscosity: viscosity was evaluated in Brookfield viscometer using LV-64 spindle. The rotation rate was adjusted to 25 RPM. The formulated lotion was directly immersed into the spindle and the viscosity was measured (Kumar *et al.* 2011, Neuwald 1966).

Spreadability: The spreadability of lotion was determined by the parallel plate method. Two glass slides of 20/20 cm were selected. About 1 g of the lotion formulation was placed over one of the slides. The other slide was placed upon the top of the lotion such that the lotion was sandwiched between the slides and 125 g weight was placed upon the upper slide so that lotion between the two slides was pressed uniformly to form a thin layer. The weight was removed and the spread diameter was measured (Garg *et al.* 2002).

Stability Test: The formulated lotion was stored at different temperatures and humidity conditions of 25±2 °C / 60±5% RH (at room temperature), 40±2 °C / 75±5% RH (accelerated temperature) for a period of three months and studied for pH, viscosity and spreadability (Negi *et al.* 2012).

Sensitivity Test: A portion of lotion was applied on the forearms of 6 volunteers and left for 20

minutes. After 20 minutes any kind of irritation if occurred was noted (Draize *et al.* 1944).

Washability Test: A portion of lotion was applied over the skin of hand and allowed to flow under the force of flowing tap water for 10 minutes. The time when the lotion completely removed was noted.

Appearance: The color, odor and homogeneity of the lotion were visually determined.

Type of emulsion test: Dye solubility and dilution test was conducted to determine the type of emulsion formed (Tharwat 2013).

RESULTS AND DISCUSSION

Phytochemical screening

The plants were undertaken for phytochemical screening of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids. It was investigated that tannin, phenols and phytosterols were commonly found. Alkaloid was absent in *Allium sativum* and *Citrus lemon*. Carbohydrate and glycosides were absent in all samples. Flavonoids, exhibits the large number of biological activities like anti-inflammatory, antioxidant and antimicrobial properties (Ojala, 2000).

Antibacterial activity study

Ethanol extract of the plants were undertaken for antibacterial activity. All of the extracts showed significant antibacterial activity at 6 percent concentration. Among five extracts *Allium sativum* showed less activity against bacteria while *Citrus lemon* showed highest activity. Afterwards, five different formulations were prepared using four of the five plant extract excluding *Allium sativum* since it possesses minimum antibacterial activity.

Table 1. Zone of inhibition of plant extracts against different bacteria

Extract	Zone of inhibition (mm)				
	Concentration (%)	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
<i>Allium sativum</i>	2	5.66±1.52	6.66±0.58	5.66±0.58	4.33±0.58
	4	8.66±1.52	9.33±1.15	8±1	7.33±1.52
	6	9.66±1.52	11±1	10.66±0.58	10±1
<i>Trigonella foenum graceum</i>	2	15±1	14.66±0.58	14.33±1.52	12±1
	4	17.33±0.58	17.33±1.15	16±1	15.33±1.52
	6	20.66±0.58	19±1	19±1	18.66±0.58
	2	14.66±1.52	17±2	17.33±1.15	18.33±1.52

<i>Citrus lemon</i>	4	20±1	20±1	20±1	19.33±1.52
	6	21±1	22±1	21±1	21.33±1.52
<i>Matricaria chamomilla</i>	2	13.66±1.52	13±1	12.66±1.15	12±1
	4	17.33±1.15	19.33±1.52	14.66±1.52	14.66±0.58
	6	21.33±1.52	22±1	18±1	19.33±1.52
<i>Cymbopogon citrates</i>	2	15.33±2.08	16±1	13.66±1.15	13.66±1.52
	4	18.66±0.58	17.33±0.58	17±1	17.33±0.58
	6	22.33±2.08	22.33±1.52	20±1	19.66±1.15
Standard cipro	6	24.33±1.15	25.66±1.52	25±1	24.33±0.58
Standard Amox	6	24.33±0.58	24±1	24.66±1.15	25±1

Among the five different combination of extracts, ratio 2 (R2) showed good antibacterial property as compared to remaining others. Since R2 combination shows highest antibacterial activity, same was used for lotion preparation.

Table 2. Zone of inhibition of ratios of extracts against different bacteria

Microorganisms	R1	R2	R3	R4	R5	Std. Cipro	Std. Amox
<i>Staphylococcus aureus</i>	18±1	20.66±1.15	17.33±1.15	15.33±0.58	15.66±0.58	25±1	26±1
<i>Pseudomonas aeruginosa</i>	16.33±0.58	20.66±0.58	15.66±0.58	16.33±0.58	12.66±0.58	25±1	26±1
<i>Escherichia coli</i>	14.33±0.58	18.66±1.53	16±1	13±1	15±1	25.66±1.15	25.66±2.08
<i>Klebsiella pneumoniae</i>	14±1	16.33±1.15	14.66±1.53	12±1	12.33±0.58	24.66±1.15	26.66±1.15

R1= 1:1:1:1; R2= 1:1:1:2; R3=2:1:1:1; R4=1:2:1:1; R5=1:1:2:1 (*Trigonella graecum foenum: Citrus lemon: Matricaria chamomilla: Cymbopogon citratus*).

Table 3. Antibacterial activity of final formulation compared to the marketed product.

Zone of Inhibition (mm) of final formulation (F2)		
Microorganisms	Formulation	Std.
<i>Staphylococcus aureus</i>	20±1	23.33±0.57
<i>Pseudomonas aeruginosa</i>	19.33±1.53	22.33±2.08
<i>Escherichia coli</i>	17.33±0.58	22.66±1.15
<i>Klebsiella pneumoniae</i>	16.33±1.52	22.66±0.58

Antioxidant activity test

The antioxidant activity of the extracts, oils and final product was evaluated. The antioxidant property of the formulation was significant as compared to the standard Ascorbic acid. The IC₅₀ value of Ascorbic acid was 4.8225 µg/ml, while R2 formulation was

10.8921 µg/ml. Among extracts and essential oils *Cymbopogon citrates* has maximum antioxidant property with IC₅₀ value of 5.6788µg/ml. Similar result was reported on lemongrass and therefore our finding is in agreement with Cheel *et al.* 2005 and Lu *et al.* 2014.

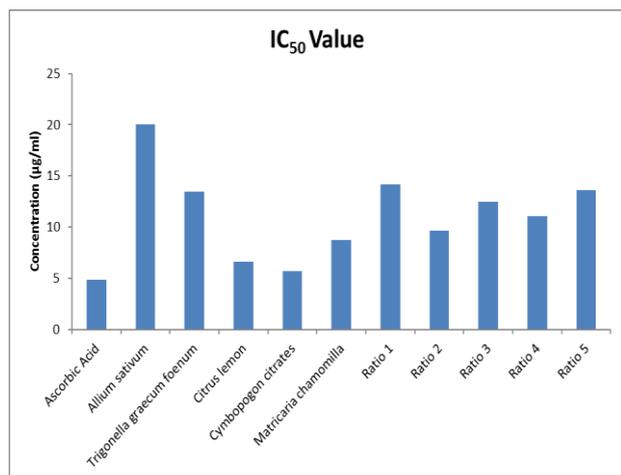


Fig 1: Comparison of IC₅₀ value of antioxidant property of different plant extracts, oils and ratios.

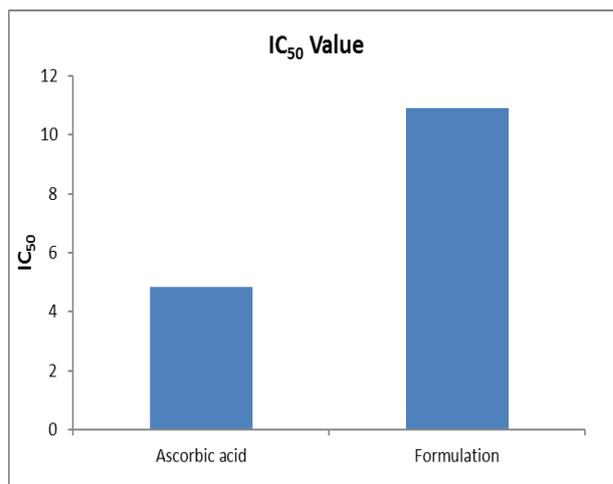


Fig. 2. Comparison of IC₅₀ value of Ascorbic acid and final formulation.

Minimum Inhibitory Concentration

MIC of 25 mg/ml was shown by *Trigonella foenum-graecum*, *Cymbopogon citratus* and *Matricaria chamomilla* against the *Staphylococcus aureus*. MIC of 25 mg/ml was shown by *Citrus lemon* against *Pseudomonas aeruginosa*. Against *Escherichia coli* the best MIC (50 mg/ml) was shown by *Cymbopogon citratus* as compared to

other extracts and essential oil. Against *Klebsiella pneumonia* all of the extracts and essential oils showed MIC of 100 mg/ml which is not very effective. Since lower MIC is indication of better antibacterial property, it was concluded that *Citrus lemon* is best antibacterial agent as compared to other extracts and oils which was followed by *Cymbopogon citratus*.

Table 4. MIC of plant extracts against different microorganism

Plant extract	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>
<i>Trigonella foenum graecum</i>	25	25	100	100
<i>Citrus lemon</i>	12.5	25	100	100
<i>Matricaria chamomilla</i>	25	25	100	100
<i>Cymbopogon citratus</i>	25	25	50	100

Formulation of lotion

Thirteen tests were conducted as per the software. The three output variables (responses), such as viscosity (Y1), pH (Y2), spreadability (Y3), were evaluated. The effect of formulation variables on different dependent or response variables was

assessed by the generated regression coefficients and r^2 values. The fitted quadratic equations relating the responses such as viscosity (Y1), pH (Y2), spreadability (Y3), to the transformed factor are given in equation 1 to 3 respectively.

Table 5. Chemical composition of formulation in percentage

Formulation no.	Stearic Acid	Cetostearyl alcohol	Lanolin	Mineral oil	TEA	Propylene glycol	Water
F1	10	1.21	9.75	12.19	3	1.21	Qs to 100 ml
F2	10	1.21	9.75	12.19	3	1.21	Qs to 100 ml
F3	5	1.21	9.75	12.19	4	1.21	Qs to 100 ml
F4	17.07	1.21	9.75	12.19	3	1.21	Qs to 100 ml

F5	15	1.21	9.75	12.19	2	1.21	Qs to 100 ml
F6	15	1.21	9.75	12.19	4	1.21	Qs to 100 ml
F7	10	1.21	9.75	12.19	4.41	1.21	Qs to 100 ml
F8	10	1.21	9.75	12.19	3	1.21	Qs to 100 ml
F9	2.92	1.21	9.75	12.19	3	1.21	Qs to 100 ml
F10	10	1.21	9.75	12.19	3	1.21	Qs to 100 ml
F11	10	1.21	9.75	12.19	1.58	1.21	Qs to 100 ml
F12	10	1.21	9.75	12.19	3	1.21	Qs to 100 ml
F13	5	1.21	9.75	12.19	2	1.21	Qs to 100 ml

Note: Active plant extracts and essential oils combination was 5% in the formulation composition.

Effect of independent variables on lotions

Effect on viscosity of lotion

It was observed that Triethanolamine have negative effect on the viscosity whereas, the stearic acid

have positive effect on the viscosity. The increase in the concentration of Triethanolamine decreases viscosity and the increase in concentration of stearic acid increase the viscosity.

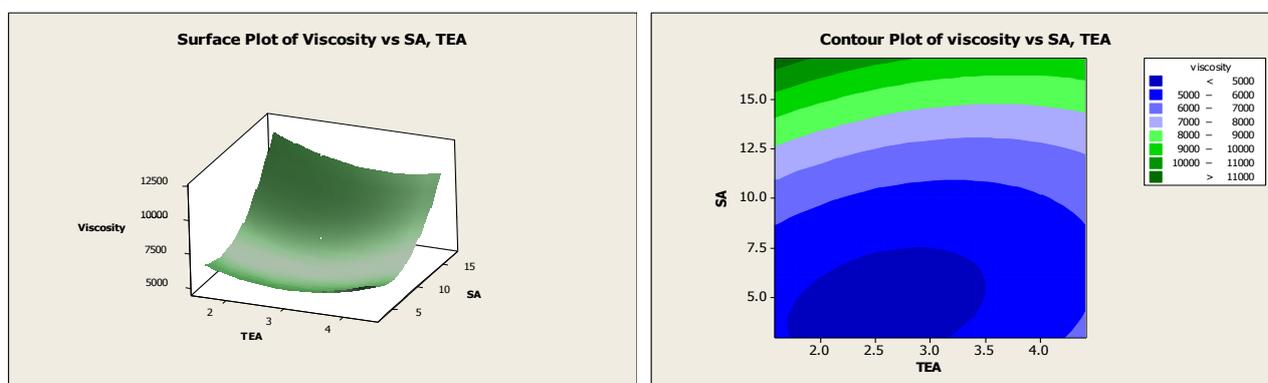


Fig. 3. Estimated contour plot and response surface plot elucidating the relationship between the viscosity and the independent variables (Triethanolamine and stearic acid).

Effect on pH of lotion

The positive magnitude of the coefficient of Triethanolamine represents the positive effect between the Triethanolamine and the pH. The increase in concentration of Triethanolamine leads to the increase in the pH of the formulation.

Similarly, the negative magnitude of the coefficient of stearic acid denoted the inverse relation between the pH of the formulation and the stearic acid. The increase in concentration of stearic acid causes the decrease of the pH of the formulation.

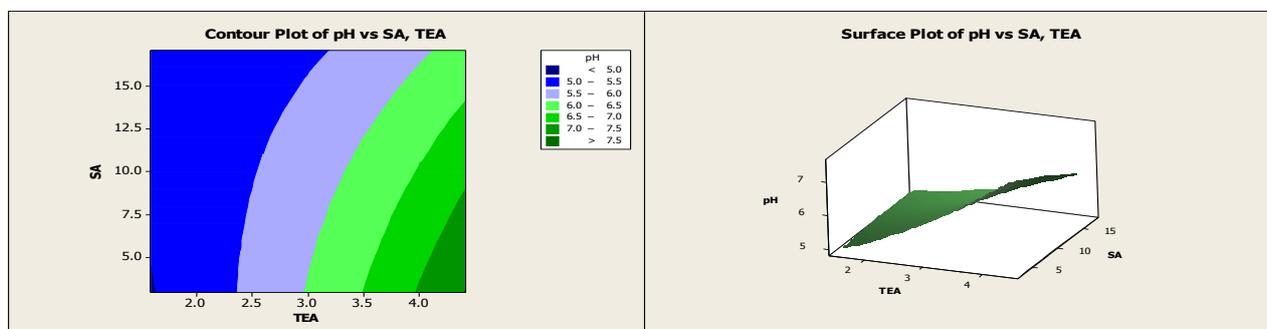


Fig. 4. Estimated contour plot and response surface plot elucidating the relationship between the pH and the independent variables (Triethanolamine and stearic acid).

Effect on spreadability of lotion

Triethanolamine have positive effect so the increase in concentration of Triethanolamine increases the spreadability of lotion. In contrast, as the concentration of stearic acid increases the

spreadability of the lotion decreases. The positive coefficient value of Triethanolamine indicated the increase in spreadability as the concentration of Triethanolamine increases.

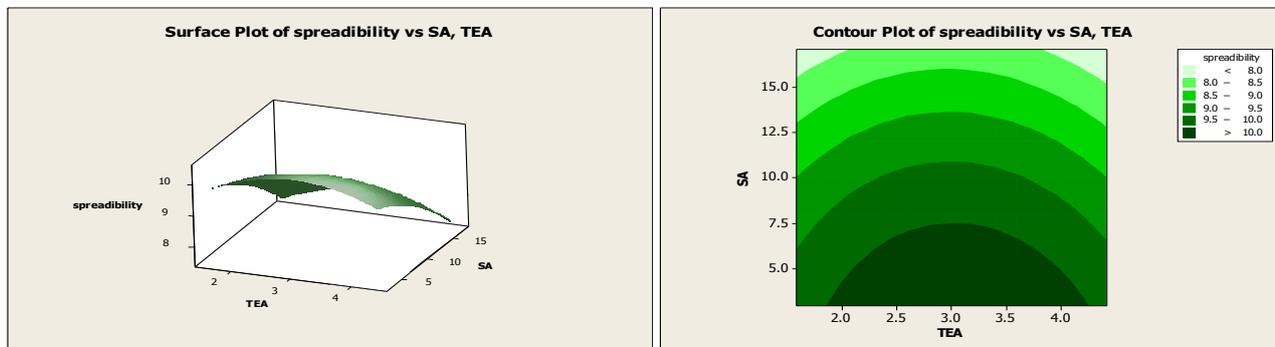


Fig. 5. Estimated contour plot and response surface plot elucidating the relationship between the spreadability and the independent variables (Triethanolamine and stearic acid).

Evaluation of pharmaceutical parameters of lotions

The pH of the lotion was within the limit which indicated that the lotion is safe to use for skin and is stable. The pH of lotion influences the stability. The stability of lotion increases with an increase in

viscosity of the medium. Among thirteen different formulations the value of viscosity differed from 4908 cps to 10187 cps. The range of spreadability for lotion was from 7 to 13 cm. The measured minimum spread diameter of the lotion was 8.2 cm and the maximum spread diameter was 10.3 cm.

Table 6. Value of Viscosity, pH and spreadability obtained for 13 different formulations

Formulation	pH (4-7)	Viscosity (<10000) cps	Spreadability (7-13) cm
F1	5.57	5962	9.3
F2	5.74	5681	9.7
F3	7.02	5013	10.2
F4	5.41	10187	8.2
F5	5.13	9236	8.4
F6	6.19	7572	8.5
F7	6.78	6925	8.8
F8	5.5	5829	9.5
F9	5.96	4908	10.3
F10	6.01	5439	9.8
F11	5.11	6178	9
F12	6.05	5372	9.9
F13	5.25	5214	10

The lotion was not irritating to the skin and it was water removable. The final appearance of lotion was found to be yellowish white in color. Emulsion formed during the formulation of lotion was

evaluated for the type of emulsion. Oil miscible dye Sudan III was mixed with the sample and observed under microscope. Small red droplets of oil can be observed surrounded by water phase. This helps to

identify that the lotion was oil-in water emulsion. The formed lotion was oil-in water type.

Optimization of formulation

The range of pH suitable for hand lotion was 4 to 7 and for spreadability the desired range was 7 to 13 cm. The viscosity should be such that the lotion can

be easily spread over the skin surface. The optimized formulation was evaluated for the response parameters and the value pH of optimized formulation was 5.6, value of viscosity was 5561 cps and the value of spreadability was 9.8. All the three parameters were within the limit and suitable for application over the skin.

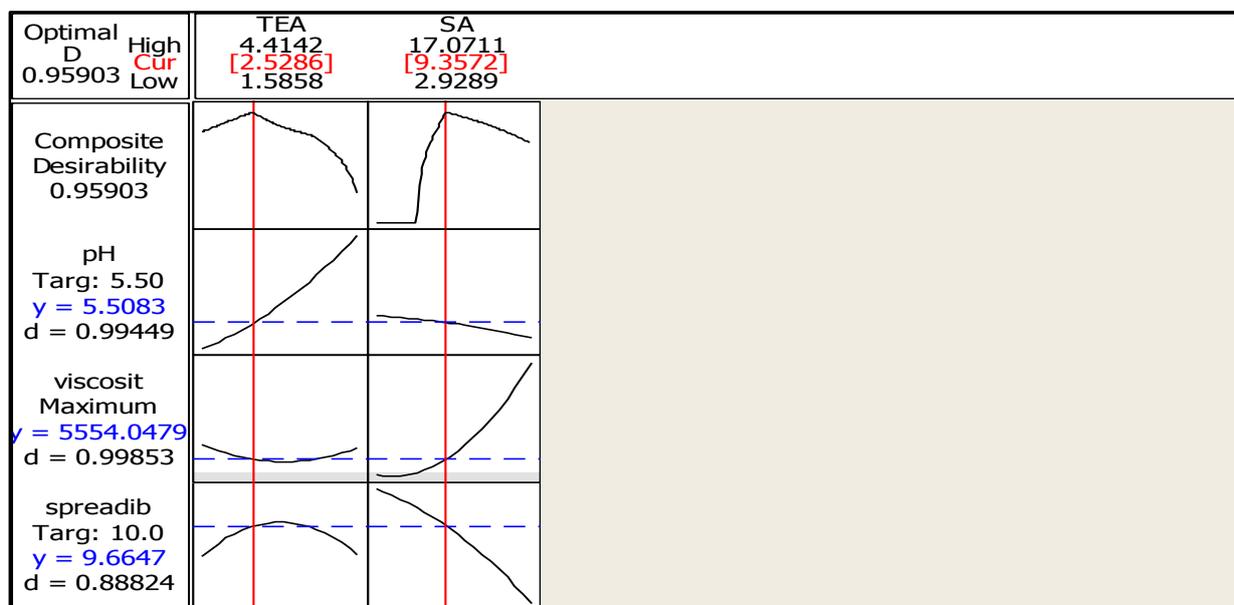


Fig. 6. Response Optimization plot.

The desirability results showed (2.52:9.35) ratio of Triethanolamine and Stearic acid as optimum. So, on the basis of the ratio obtained from the software final optimized formulation was developed.

Stability test

The stability test of final optimized lotion was

carried out and it was found that the lotion was stable in room temperature and accelerated temperature for at least three months. The value of pH, viscosity and spreadability all lay within the required range. There was no major difference in values of pH, viscosity and spreadability as compared to the initial value.

Table 7. Stability test at room temperature and accelerated temperature (of optimized formulation)

Parameters	Initial value	Room temperature (25±2)°C			Accelerated temperature (40±2)°C/75% RH		
		1 month	2 month	3 month	1 month	2 month	3 month
Viscosity	5561 cps	5554 cps	5565 cps	5559 cps	5550	5564	5571
pH	5.6	5.7	5.9	5.8	5.8	5.9	6.1
Spreadability	9.8 cm	9.6 cm	9.9 cm	9.7 cm	9.6 cm	9.9 cm	10.0 cm

CONCLUSION

Oil in water type lotion was formulated using formulation of *Trigonella foenum-graecum*, *Citrus lemon*, *Matricaria chamomilla* and *Cymbopogon citratus* at the ratio of 1:1:1:2. This optimum

formulation was designed with 2.52:9.35 ratios of triethanolamine and stearic acid. The lotion formulation showed no redness, edema, inflammation and irritation during sensitivity test indicating it is safe to use. By combining the

different plant extracts it is possible to improve as well as synergize the cosmetic properties of prepared products compare to individual extracts. From the above study it has been revealed that that the lotion was stable in room temperature and accelerated temperature for at least three months.

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