

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 2.529.35. 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 one months.*

Keywords Herbal formulation, Triethanolamine, Stearic acid, Antioxidant, Antibacterial

I. 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.

1.1 Advantages of Lotion

Lotions are highly beneficial for a person. These are necessary for the healthy maintenance of the skin and its glow. Here are the few advantages of using lotion

A. Hydration and Moisturizing

The primary function of lotion is to hydrate the skin. All lotions are mostly low-viscosity and contain hydrating elements. The lotions form a barrier around the skin and prevent excessive loss of moisture. These have a formulation that keeps the skin hydrated for long times. Thus, lotions are a great way for skin management due to their moisture-retaining ability.

B. Smooth and Softens

As the skin remains hydrated, it will naturally become smooth. This will make the skin soft. There are substances in lotions that soften and moisturize the skin and thus prevent scaling and flaking. Also, some lotions might contain

substances known as emollients. These chemicals enable the creams to sink deeper into the skin. Thus, this property makes the skin soft and feel smooth.

C. Anti-Aging Effect

Many advanced lotions are known to contain anti-aging effects. These lotions reduce the appearance of wrinkles by plumping the skin. So lotions will brighten the skin by reducing the presence of wrinkles. These contain the ingredients that improve skin tone and texture. Thus, moisturizers can help improve the skin and prevent anti-aging temporarily. However, do not rely on these lotions completely for anti-aging.

D. Fragrance to Skin

Most lotions have a pleasant scent that makes the skin aromatic. These products add fragrance to the skin. The traditional creams and lotions had added scent to them. However, advanced lotions have an organic origin and contain scents due to numerous essential oils in their formulation. Thus, a person wearing these lotions does not necessarily have to use perfumes or cologne for fragrance.

E. SPF Protection

Lotions protect the skin from damaging, cracking, and drying. Some advanced lotions also contain SPF substances that keep the skin safe from harmful UV radiation. These are called sunscreen as function as a screen against sun rays. With the increasing effect of these rays, it is advised to everyone to wear sunscreens when going out in the open. Also, several skin disease concerns are rising, so these will protect the skin from all these.

F. Healing and Soothing

The lotions will boost the natural ability of the skin to heal. These provide the skin will everything it needs to repair itself. Also, moisturizers help to fight wrinkles and make them less apparent. Thus, lotion can heal the skin and soothe it.

G. Nutrition and Essential Oils

The moisturizers, lotions, and creams are made up of healthy ingredients. Also, with the increasing awareness of organic material, more and more lotions with natural ingredients have been introduced. These contain many beneficial vitamins, essential oil, and organic compounds. These most common are vitamin D, aloe vera, vitamin E, and keratin.

1.2 Disadvantages of Lotion

Apart from all the mentioned advantages, lotions hold some drawbacks. These can have a few negative impacts on the users. Here are some disadvantages of lotions that everyone needs to know

A. Allergies

Lotions and moisturizers are likely to contain some synthetic chemicals. These synthetic substances can be harmful to the skin. People with sensitive skin are especially susceptible to these. Also, a person can have an allergic reaction to a few chemicals. Some people have complained about itching, redness, and irritation due to few products. Scratching or rubbing can also cause blisters and pain. So people with sensitive skin need to be alarmed about redness, swelling, itching, and hives.

B. Clogging Pores

Clogging of pores is quite harmful to people in the long term. If a person over moisturizes their skin, it can cause clogged pores. These will increase acne and face marks. Also, the clogged pores increase the blackheads and whiteheads.

C. Expensive

Some of the lotions, moisturizers, acne, and dark circle creams are extremely expensive. These become luxury products and are not affordable for the general public. Thus, these items need to be cost-effective so that more audiences can benefit from them.

D. Breathing Trouble

The breathing issue is the adverse effect of some lotion and creams. This is especially the case with the lotions that have added synthetic fragrances. These can cause allergies to the skin and also respiratory issues. Some people start sneezing and coughing and have issues breathing due to the scents.

1.3 Properties

1. **It retains moisture and helps your skin glow** When you use our Kukui Body Lotion daily, you'll quickly notice a difference in your skin's hydration levels. This lotion is amazing for adding moisture and hydration to your skin so that you can spend your days looking your best after this super easy step. When your skin is nice and hydrated, it gets this amazing glow, which is bound to have your friends asking what you've been using to help your skin look so amazing.
2. **It helps with anti-aging and wrinkles** Using a moisturizer like our Kukui Body Lotion daily will help you reap benefits in the long run as well. This body lotion helps with anti-aging and prevents wrinkles, so the more you use it, the more likely you are to look younger, longer.
3. **It lengthens the time of your self-tanner** We're in the middle of a hot summer, so you might be using a self-tanner to help you feel like your most confident self. Self-tanners are a great and safe way to get golden sun-kissed skin. Using a natural lotion like our Kukui Body Lotion that contains natural Hawaiian Botanicals is a fantastic way to help your faux tan last longer, for a golden sun-kissed look that lasts all summer long.
4. **It softens your skin and soothes rough patches** Summer also means dry skin, which is another thing our Kukui Body Lotion specializes in. When you use this superpowered body lotion, you'll be able to achieve softer skin overall, and soothe any rough patches your skin has. This means you'll be able to both feel and look like your most confident self with soft, hydrated skin (all year long).
5. **It can be used as a nice light scent** If you want to smell nice, but don't want to use an overpowering perfume, our body lotion can provide a nice and light scent. This means you'll be able to smell nice, while letting our powerful Hawaiian botanicals treat your skin right.
6. **Massaged into the skin it can be quite relaxing** When you use body lotion daily, you're taking time to treat yourself. Use our Kukui Body Lotion to relax by massaging it lightly into the skin, so that you can chill out at the end of the day, while allowing kukui nut oil and aloe vera to soothe your skin, helping it to feel its best at the end of a long day.

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II. MATERIALS AND METHODS

2.1 Plant Materials

The medicinal plants *Allium sativum*, *Trigonella foenum-graecum*, *Citrus lemon*, *Matricaria chamomilla* and *Cymbopogon citrates* were collected. 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. The essential oils are *Matricaria chamomilla* and *Cymbopogon citrates*.

2.2 Plant Profile

Garlic (*Allium sativum*)

Family Amaryllidaceae

Synonyms wordhippo Thesaurus

Uses

People commonly use garlic for high blood pressure, high levels of cholesterol or other fats in the blood, and hardening of the arteries. It is also used for the common cold, osteoarthritis, and many other conditions, but there is no good scientific evidence to support these uses.



Fig - 1 Garlic

Fenugreek (*Trigonella foenum-graecum*)

Family - Legumes

Synonyms - Genus *Trigonella*

Uses

Fenugreek is used as an ingredient in spice blends and a flavoring agent in foods, beverages, and tobacco. Fenugreek extracts are also used in soaps and cosmetics. In North Africa, Asia, and southern Europe, fenugreek was traditionally used for diabetes and to increase milk supply in women who were breast feeding.



Fig -2 Fenugreek

Lemon (*Citrus lemon*)

Family - Rutaceae

Synonyms - Pomelo

Uses

Lemons are high in vitamin C, fiber, and various beneficial plant compounds. These nutrients are responsible for several health benefits.



Fig -3 Lemon

Matricaria chamomilla (German chamomile)

Family Asteraceae.

Synonym Matricaria recutita

Uses

German chamomile reduces inflammation, speeds wound healing, reduces muscle spasms, and serves as a mild sedative to help with sleep.



Fig -4 Matricaria chamomilla

Lemongrass (Cymbopogon citrates)

Family Grasses

Synonym Malabar grass

Uses

Lemongrass might help prevent the growth of some bacteria and yeast. Lemongrass also contains substances that are thought to relieve pain and swelling, reduce fever, improve levels of sugar and cholesterol in the blood, stimulate the uterus and menstrual flow, and have antioxidant properties.



Fig -5 Lemongrass

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.

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.

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.
- **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.
- **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.

- **Stability Test:** The formulated lotion was stored at different temperatures and humidity conditions of 25 ± 2 oC / $60\pm 5\%$ RH (at room temperature), 40 ± 2 oC / $75\pm 5\%$ RH (accelerated temperature) for a period of three months and studied for pH, viscosity and spreadability.
- **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.
- **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.

III. RESULTS AND DISCUSSION

3.1 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.

3.2 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 four extracts *Allium sativum* showed less activity against bacteria while Citrus lemon showed highest activity. Afterwards, four different formulations were prepared using four of the four 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 (%)	Staphylococcus aureus	Pseudomonas aeruginosa	Escherichia coli
Allium sativum	2	5.66±1.52	6.66±0.58	5.66±0.58
	4	8.66±1.52	9.33±1.15	8±1
	6	9.66±1.52	11±1	10.66±0.58
Trigonella foenum graceum	2	15±1	14.66±0.58	14.33±1.52
	4	17.33±0.58	17.33±1.15	16±1
	6	20.66±0.58	19±1	19±1
Citrus lemon	2	14.66±1.52	17±2	17.33±1.15
	4	20±1	20±1	20±1
Matricaria chamomilla	6	21±1	22±1	21±1
	2	13.66±1.52	13±1	12.66±1.15
	4	17.33±1.15	19.33±1.52	14.66±1.52
Cymbopogon citrates	6	21.33±1.52	22±1	18±1
	2	15.33±2.08	16±1	13.66±1.15
	4	18.66±0.58	17.33±0.58	17±1
Standard cipro	6	22.33±2.08	22.33±1.52	20±1
	6	24.33±1.15	25.66±1.52	25±1
Standard Amox	6	24.33±0.58	24±1	24.66±1.15

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

Microorganisms	R1	R2	R3	R4	R5	Std. Cipro	Std. Amox
Staphylococcus aureus	18±1	20.66±1.15	17.33±1.15	15.33±0.58	15.66±0.58	25±1	26±1
Pseudomonas aeruginosa	16.33±0.58	20.66± 0.58	15.66±0.58	16.33±0.58	12.66±0.58	25±1	26±1
Escherichia coli	14.33±0.58	18.66±1.53	16±1	13±1	15±1	25.66±1.15	25.66±2.08

R1= 1111; R2= 1112; R3=2111; R4=1211; R5=1121 (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.
Staphylococcus aureus	20±1	23.33±0.57
Pseudomonas aeruginosa	19.33±1.53	22.33±2.08
Escherichia coli	17.33±0.58	22.66±1.15

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.

Minimum Inhibitory Concentration

MIC of 25 mg/ml was shown by Trigonella foenum-graecum, Cymbopogon citratus and Matric ariachamomilla 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 citrates 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	Staphylococcus aureus	Pseudomonas aeruginosa	Escherichia coli
Trigonella foenum graceum	25	25	100
Citrus lemon	12.5	25	100
Matricaria chamomilla	25	25	100
Cymbopogon citratus	25	25	50

Formulation of Lotion

One 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 r2 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

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.

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.

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.

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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 one 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

Formulation	pH (4-7)	Viscosity (<10000) cps	Spreadability (7-13) cm
F1	5.57	5962	9.3

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.

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 one 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	1 month
Viscosity	5561 cps	5554 cps	5550
pH	5.6	5.7	5.8
Spreadability	9.8 cm	9.6 cm	9.6 cm

IV. 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 2.529.35 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 the lotion was stable in room temperature and accelerated temperature for at least one months.

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