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Development and Evaluation of Polyherbal Formulation for the Treatment of Melasma

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Abstract

Liquorice root powder is a BCS class IV antioxidant or anti-inflammatory drug having poor oral bioavailability and is detected at very low levels. Hence, aim of the study was to prepare topical dosage form of liquorice for effective treatment of melasma. Liquorice cream formulations were formulated by fusion method. Box-Wilson Central Composite Design (CCD) was used to optimize concentrations of bees wax, borax, liquid paraffin, citric acid and almond oil as excipients (independent variables) and their effect on three dependent variables (viscosity, phase separation and spreadability respectively). The prepared liquorice topical cream formulations F1 to F43 were assessed for parameters such as physical appearance, phase separation, viscosity, and spreadability. Out of the 43 formulations only formulations F11, F19 and F31 showed satisfactory or passed results for physical appearance, viscosity, phase separation and spreadability as per standard monographs. But formulation F31 exhibited pH of 6.2 which is within the skin pH range as per the monographs. The optimized formulation (F31) was homogeneous and no irritant in nature. Microscopic evaluation of the optimized formulation (F31) confirmed that the prepared cream was water in oil type emulsion. F31 formulation remained stable and exhibited no significant alterations in physical appearance, viscosity, phase separation, spreadability, pH, homogeneity and irritancy test. Hence, it can be concluded that topical dosage form of liquorice root powder represents an innovative therapeutic approach utilizing a drug delivery system for the treatment of melasma.
Major Findings: Liquorice cream formulations F1 to F43 were formulated by fusion method and checked for various parameters to get the optimized preparation having high quality, safety and efficacy to treat melasma skin disorder.

Keywords: Central Composite Design, Liquorice Root Powder, Spreadability, Topical Dosage Form, Viscosity

1. Introduction

Melasma is an acquired skin condition where there are symmetrical brown patches on the face. The term is derived from the Greek "melas", black. A common provoking factor is sun exposure[1]. It is seen most commonly as brown symmetrical patches on the cheeks, upper lip, chin and forehead, although other sun-exposed areas may be involved. Because of its association with pregnancy, this condition is often referred to as "chloasma" from the Greek word meaning "to be green". As the condition is never truly green in color, the term "melasma" is preferred[2]. Whereas anybody may develop melasma, it occurs in a much greater percentage of people having darker skin tones, specifically skin Types IV to VI. This condition, more importantly, affects all ethnic and racial groups[3]. Epidemiological studies indicate that melasma prevails in people having darker skin types: East Asians (Japanese, Koreans, Chinese), Indians, Pakistanis, Middle Easterners, and Mediterranean Africans. It also

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prevails in Hispanic-Americans and Brazilians living in tropical regions, which are subjected to extreme exposure to ultraviolet radiation[4]. However, it has a significantly higher prevalence in women of reproductive age, while it affects around 10% of men. Clinical and histological characteristics of melasma are similar in both sexes[5]. Melasma represents a multifactorial pathogenesis. Key factors include hormonal influence, genetics, and sun exposure. It is not known what precisely provokes this, but it is known to involve the increase of melanin production and deposition in the skin. Hormonal causes, such as pregnancy or birth control, can trigger melasma, and more cases can be noticed in people with darker skin types. Other potential contributors may be certain medications, thyroid diseases, and some cosmetics. Apart from this, sun exposure is responsible for the worsening of melasma; however, avoidance from it may cause fading[6]. Melasma typically consists of brown patches on the face, mainly on the centrofacial pattern on the cheeks, nose, forehead, upper lip, and chin, or in a malar pattern on the cheeks and nose or mandibular pattern along the jawline. While the use of wood's lamp is useful in distinguishing epidermal from dermal and mixed melasma, the differentiation of pigmentation at shallow or deep levels therefore does not appear to be as reliable in darker skin types. Further, the melasma could be classified as transient or persistent[7]. Melasma is a common cosmetic facial skin condition causing severe emotional distress. Though many treatments are available, which include topical creams, chemical peels, and laser therapies, melasma tends to recur. Sun exposure is a primary trigger and prevention is crucial. Hence, effective treatment calls for long-term commitment and education of the patient with respect to including life factors such as hormonal influence, medications, and cosmetics. Topical treatments tend to work better on superficial melasma[8]. Hydroquinone is closely related to melanin precursors; hence, it decreases melanin production by inhibiting the chemical. It affects the function of melanocytes, sometimes leading to cell death. On the other hand, hydroquinone is unstable and decomposes over time, making the product ineffective. While it has been used to treat melasma, the effect is often not sustained, and it holds potential for irritations, allergies, or long-term discoloration of the skin. Safety issues have prompted most countries to act upon heavy restrictions or bans, putting the management of melasma to the use of other regimens[9]. Commonly used treatment for hyperpigmentation includes azelaic acid, kojic acid, and retinoic acid. Azelaic acid is a naturally occurring compound that inhibits the synthesis of melanin. It causes mild irritancy. Kojic acid obtained from fungi is also useful, but it can be irritating. Retinoic acid decreases melanin production but may dry and redden the skin, and it can potentially worsen hyperpigmentation in dark skin types[10]. However, sometimes topical steroids are combined with hydroquinone and retinoic acid in the treatment of melasma but may cause skin thinning, irritation, excessive growth of hair, and blood vessels becoming visible[11].

This study has focused on licorice, a perennial herb belonging to the genus *Glycyrrhiza glabra*, as a substitute for hydroquinone, azelaic acid, kojic acid, and retinoic acid due to the limitations connected with these agents. Studies have demonstrated that liquorice root extract might be a tyrosinase inhibitor and anti-inflammatory agent. In addition to this, liquorice root powder has been used in clinical trials and proven of value in the treatment of melasma. Increasingly, the cosmetic industry turns to natural ingredients as a result of customer preference for environmental friendliness. This trend concurs with a need for cosmetics that will do more than just beautify a person but also give extra health benefits to the skin. Liquorice root extract, through its bio-actives, manifests by giving both cosmetic value and potential therapeutic advantage in skin disorders such as melasma[12]. Licorice root powder may be useful in skin pigmentation disorders through melanogenesis, the process where melanin-the pigment responsible for the determination of skin color-is produced in melanocytes. Melanin formation is modulated by a great many numbers of factors, such as UV exposure, genetics, and the size of melanocytes. Any kind of imbalance results in various diseases like melasma, hyperpigmentation, vitiligo, and hypopigmentation. Tyrosinases are central enzymes in melanin biosynthesis. Being a copper-dependent enzyme, tyrosinase is involved in the initial step of melanogenesis in which the amino acid L-tyrosine is converted to L-DOPA. In the second step, tyrosinase catalyzes the conversion of L-DOPA to DOPA quinone, which changes to Cysteinyl DOPAs or DOPA-chrome. The latter undergoes an uncontrolled, spontaneous polymerization to give rise to melanin. Note that, although tyrosinase has an ability in the formation of melanin, skin melasma is multifactorial with influences other than the single activity of tyrosinase[13]. Tyrosinase

has emerged as the major molecule for the treatment of melanin hyperpigmentation and attainment of skin-whitening effects. Molecules that can inhibit tyrosinase have become very crucial elements for cosmetics and drugs against several dermatological conditions. In this regard, the study by Ishi *et al*., focused on the *in-vitro* skin depigmenting abilities of *G. glabra* L. belonging to the family *Fabaceae* (synonyms: *mulethi, yastimadhu, atimadhura, atimadhuram etc*.), the root extract to recommend it as a potential medication against melasma[14]. Some studies on formulations containing liquorice root powder have reported safety for external use. In addition, it has been shown that creams formulated with *G. glabra* root extracts exert skin-lightening ability. This is consistent with the study by Kirubakaran *et al*., which confirms the skin-lightening ability of liquorice root powder[15].

A polyherbal formulation using the powder of licorice root may definitely open some promising avenue in topical melasma management. Such delivery may optimize the delivery of active constituents of the licorice root powder to the target site and hence improve the patient compliance and therapeutic efficiency of treatment as compared with conventional modes of treatment.

2. Materials and Methods

2.1 Materials

The materials used in this project were liquorice root powder which was procured from Herbs and Essential Oils Private Limited, Bangalore. The other materials such as beeswax (Herbs and Essential Oils Private Limited, Bangalore), citric acid, borax, liquid paraffin, almond oil, ethanol, ether, sodium hydroxide (SD Fine Chemicals Ltd., Mumbai), potassium dihydrogen-O phosphate and distilled water (Qualigens Fine chemicals, Mumbai). All the materials used in the project were of analytical grades.

2.2 Experimental Methodology

The experimental methodology was divided into three sections such as preformulation studies, formulation development and evaluation.

2.2.1 Pre-formulation Studies

Pre formulation testing is the beginning step in the development process of any dosage form of a drug. In its intention, it is principal to gain vital information regarding the drug substance, information that can then be used to formulate stable, bioavailable dosage forms on a large scale. Pre formulation studies that include physicochemical properties and physical characteristics supply information about the drug substance.

2.2.1.1 Melting Point

The melting point of the drug was determined with a Mel-temp apparatus. A capillary tube was filled with pure drug by sealing one end and placing it in a pile of the drug powder. The drug sample should fill about 1mm of the tube for the measurement to be accurate. The capillary tube was placed in between the observation port and thermometer chimney on the Mel-temp. The sample was heated by turning the voltage control dial to quickly bring the temperature up to about 10° C below the expected melting point. Once the sample had reached this temperature, the dial was turned down and the temperature was allowed to rise at a rate of about one degree per minute through the expected melting point range. The temperature at which the first sign of a liquid droplet was observed was recorded[16].

2.2.1.2 Solubility Study

Qualitative Solubility Study of Pure Drug in Different Solvents

The solubility of pure liquorice root powder was determined by adding 10mg drug to 10 mL of various solvents in Schott-Duran bottles. The different solvents used were distilled water, methanol, ethanol and ether[17,18].

2.2.2 Formulation Development

2.2.2.1 Screening of Excipients

Safety, efficacy, quality and stability of a new drug formulation are dependent on a proper understanding of the components that make up the formulation. Pre-formulation studies characterize active pharmaceutical ingredients and excipients that become important considerations in the manufacture, stability, administration and delivery of the API. Of these excipients, some will enhance processing; others will provide aesthetic appeal,

while others may enhance product performance or enhance patient compliance. In some formulations, they may compose a large part of the formulation. Beeswax, borax, liquid paraffin, citric acid, and almond oil are incorporated into the melasma cream for their special features. All excipients chosen for this purpose were based on physical appearance, homogeneity, viscosity, aroma, and stability. Blank creams of suitable excipients were prepared and individually and in combination evaluated for inclusion in the melasma cream[19].

2.2.2.2 Preparation of Melasma Cream Containing Liquorice by Fusion Method

Take required quantity of bees wax and liquid paraffin in a porcelain dish. Then heat it on a water bath at 75° C. Take borax and distilled water in a beaker, add a measured amount of liquorice in it and heat it on a water bath at 75° C for 10mins. Once all the ingredients melted, both solutions are taken out from the water bath. This borax solution is then added drop wise in a porcelain dish with continuous stirring. After that add citric acid in a porcelain dish and dissolve it. Then add almond oil in it. Add perfume for fragrance. Finally, melasma cream containing liquorice is prepared[20].

2.2.2.3 Optimization of the Melasma Cream Containing Liquorice by Central Composite Design (CCD)

Based on initial screening, final formulation batches were prepared with a CCD (Central Composite Design). It involved five independent variables such as X1 (amount of beeswax), X2 (amount of borax), X3 (amount of liquid paraffin), X4 (amount of citric acid) and X5 (amount of almond oil). For each factor three levels were chosen like -1(low), 0(medium) and +1(high) concentration values respectively. In this research, 43 batches of melasma cream formulations had been prepared and undergone various tests such as viscosity, phase separation and spreadability as shown in the Tables 1 and 2[21].

Table 1. Independent variable factors with different levels

Variable	Low (-1)	Medium (0)	High (+1)
X1: Bees wax (gm)	12	15	17
X2: Borax(gm)	0.20	0.25	0.30
X3:Liquid paraffin(mL)	40	50	60
X4: Citric acid(gm)	0.10	0.11	0.13
X5: Almond oil (mL)	3	5	7

2.2.3 Evaluation of Liquorice Containing Melasma Cream

2.2.3.1 Determination of Physical Appearance

Physical appearance is defined as the observable characteristics of a product which include odor, colour, consistency and state. These properties are quite independent of the real chemical composition of the product and hence give a description of the product that enables it to be distinguished from another. These properties were examined visually against a dark background as well as through smelling sensation[22].

2.2.3.2 Determination of Viscosity

Viscosity is one of the important quality control parameters that signifies the stickiness and thickness of cream. It also impacts flowability, appearance, shelf-life stability and spreadability. The viscosity of the liquorice cream was measured by using a rotational viscometer. Taking 500gm of the formulated cream in a beaker. Calibration of the viscometer was set to auto zero, while the spindle no. 4 was selected according to the viscosity. Dip gently the cream into the spindle, avoiding air bubbles. Take viscosity readings with triplicate experiments[23].

2.2.3.3 Determination of Phase Separation

It is the process whereby a homogenous mixture undergoes separation into two distinct phases upon rapid cooling. This is so particular in the case of immiscible liquids that are oil and water, which is also known as liquid-liquid equilibrium. Oil and water separate quickly because emulsions are generally thermodynamically unstable and differ in density. In the case of phase separation testing which was conducted on the prepared melasma cream by transferring 100gm in a wide mouth container and observed oil and aqueous phases separation for 24 hours[24,25].

2.2.3.4 Determination of Spreadability

Spreadability is the potential of the cream to spread uniformly over the skin which is important for administering uniform medication to achieve therapeutic efficiency. Spreadability was determined by sandwiching 1gm of prepared cream between two glass slides under

Table 2. Formulation parameters for optimization of melasma cream

S. No	Coded Formulation	Ingredients					
		Bees Wax (gm)	Borax (gm)	Liquid Paraffin (mL)	Citric Acid (gm)	Almond Oil (mL)	Water(mL)
1	F1	12	0.3	60	0.1	0.5	60
2	F2	17	0.3	60	0.1	0.2	60
3	F3	14.5	0.215	50	0.115	0.35	60
4	F4	17	0.3	60	0.1	0.5	60
5	F5	12	0.3	40	0.1	0.2	60
6	F6	8.553	0.275	50	0.115	0.35	60
7	F7	12	0.3	60	0.13	0.5	60
8	F8	12	0.25	40	0.13	0.2	60
9	F9	17	0.25	60	0.1	0.2	60
10	F10	12	0.3	60	0.13	0.2	60
11	F11	14.5	0.275	50	0.115	0.35	60
12	F12	14.5	0.275	50	0.115	0.006	60
13	F13	17	0.25	60	0.13	0.5	60
14	F14	17	0.25	40	0.13	0.2	60
15	F15	17	0.3	40	0.13	0.5	60
16	F16	17	0.25	60	0.13	0.2	60
17	F17	12	0.3	60	0.1	0.2	60
18	F18	17	0.25	40	0.1	0.5	60
19	F19	12	0.25	40	0.1	0.2	60
20	F20	14.5	0.275	50	0.150	0.35	60
21	F21	12	0.3	40	O.13	0.2	60
22	F22	17	0.3	40	0.1	0.2	60
23	F23	17	0.3	60	0.13	0.2	60
24	F24	17	0.25	40	0.1	0.2	60
25	F25	17	0.25	60	0.1	0.5	60
26	F26	14.5	0.275	26.215	0.115	0.35	60
27	F27	14.5	0.275	50	0.079	0.35	60
28	F28	12	0.25	40	0.1	0.5	60
29	F29	12	0.25	60	0.1	0.2	60
30	F30	14.5	0.275	73.784	0.115	0.35	60
31	F31	17	0.3	60	0.13	0.5	60
32	F32	12	0.275	60	0.13	0.5	60
33	F33	14.5	0.334	50	0.115	0.35	60
34	F34	12	0.3	40	0.1	0.5	60
35	F35	14.5	0.275	50	0.115	O.706	60
36	F36	12	0.25	60	0.13	0.2	60
37	F37	17	0.25	40	0.13	0.5	60
38	F38	20.446	0.275	50	0.115	0.35	60
39	F39	12	0.25	40	0.13	0.5	60

40	F40	12	0.3	40	0.13	0.5	60
41	F41	17	0.3	40	0.1	0.5	60
42	F42	17	0.3	40	0.13	0.2	60
43	F43	12	0.25	60	0.1	0.5	60

a weight of 10g, whereby a thin layer was formed. The time taken by the cream to travel a length of 3cm was measured. This was repeated three times; the shorter the time, the better the spreadability.

Spreadability = m × l/t

m= Standard weight which is tied to or placed over the upper slide (10g). l= length of a glass slide (3cm), t= time taken in seconds[26].

2.2.3.5 Determination of pH

The pH meter was used to measure the pH of liquorice cold cream. The tool contains a glass electrode that determines the concentration of hydrogen ions in a solution. The reading was calibrated using the standard buffer solutions initially. Then, 50 mL of distilled water was added to about 0.5 grams of the cream and the solution was finally prepared. Then the pH value was noted. The reading had to be done three times for precision[27].

2.2.3.6 Determination of Homogeneity

Homogeneity refers as the even distribution of components, is one of the critical parameters in a cold cream formulation. Homogeneous cream facilitates the delivery of emollients and moisturizers uniformly, thereby providing a smooth texture with an evenly distributed application. Inconsistent distribution will lead to uneven hydration and poor user experience. It is homogeneity that allows for proper skin hydration. The homogeneity of the prepared cold cream was checked through visual and tactile examination. A small amount of cream was squeezed between the thumb and index finger to evaluate instantaneous skin feel-stiffness, grittiness or greasiness[28].

2.2.3.7 Determination of Irritancy Test

Skin irritation testing is conducted as a preclinical safety assessment to determine the product's potential to cause reversible injury to the skin. This requires that the product be applied to the skin surface for a maximum of 4 hours and the resulting reactions checked in the form of redness, itching or pain. The test shall be conducted based on the definition provided by GHS (Globally Harmonized System of Classification and Labeling of Chemicals) of skin irritation, which is the production of reversible damage to the skin following the application of a test substance for up to 4 hours. If skin irritation is a problem, then manufacturers can take measures to prevent its probable adverse reactions. About 30mg of the optimizd cream was applied on the hands and kept unwashed for 4 hours so that we can check for irritation[29,30].

2.2.3.8 Dye Test

The soluble dyes impart colour according to the cream phase nature. The water-soluble dyes show colour in the water phase and oil-soluble dyes do so in the oil phase. A small amount of optimized melasma cream is added to a water-soluble dye like amaranth to find out the type of emulsion. It is mixed properly on the microscope slide, covered with a cover slip and observed under a compound microscope. If colourless droplets appear on a colourless background, cream is water-in-oil; if red droplets are visible on a colourless background, it is oil-in-water[31,32].

2.2.3.9 Short Term Stability Studies

The principle of stability testing is to assess the effects of a variety of environmental factors such as temperature, humidity and light on the quality of a drug substance or drug product over a period of time. This type of testing assures the re-test period for drug substances and the shelf life of drug products. The stability study of an optimized melasma cold cream was carried out by storing and sealing cream in plastic container at room temperature for 30 days. The cream, after a period of one month was evaluated for different parameters[33].

3. Results and Discussions

3.1 Pre-Formulation Studies

The results of pre formulation studies carried out in this study are presented below. Physical properties of liquorice root powder were seen as appearance and odour. Appearance: Light yellow to brown powder. Odour: Intensely sweet.

Pre formulation studies of liquorice root powder was carried out by performing various parameters *viz*., determination of melting point of drug and determining the solubility profile.

3.1.1 Melting Point Determination

Liquorice root powder did not show any melting temperature.

3.1.2 Qualitative Solubility Study of Liquorice Root Powder in Different Solvents

Solubility profile image of liquorice root powder in different solvents was shown in Figure 1.

The solubility profile of liquorice root powder in various media were checked out and the results were presented in Table 3.

The solubility profile of liquorice root powder was determined using various solvents such as normal distilled water, hot distilled water, ethanol and ether. Liquorice root powder was found to be insoluble in normal distilled water, soluble freely in hot distilled water, soluble sparingly in ethanol and ether insoluble which was displayed in Figure 1 and tabulated in Table 3.

3.2 Formulation Development

3.2.1 Excipients Screening

Blank cold cream image is shown in Figure 2.

The formulation of melasma cream containing liquorice was achieved by fusion method. Different excipients (bees wax, borax, liquid paraffin, citric acid and almond oil) in different concentrations were screened based on their effects they impart to the formulation physical appearance, homogeneity, viscosity, aroma and stability. By observing the above formulation parameters, it was found that the combination of all excipients is suitable for preparing melasma cream containing liquorice of desirable.

3.2.2 Preparation of Melasma Cream Containing Liquorice by Fusion Method

Preparation of melasma cream containing liquorice by fusion method is shown in Figure 3.

Melasma cream containing liquorice was fabricated by fusion method. This is a simple, economic, less time-consuming technique.

3.2.3 Optimization of the Melasma Cream Containing Liquorice by CCD (Central Composite Design) is Presented in Table 4.

Optimization of the melasma cream containing liquorice by CCD (Central Composite Design) is presented in Table 4.

Based on the preliminary work which resulted in identifying crucial formulation variables, optimization by CCD was employed to investigate the factors. In the central composite design, five factors were investigated, three levels each and experiments were performed on all forty-three possible combinations among these three formulations were considered as control formulation. Viscosity, phase separation and spreadability were selected as dependent variables. Formulation code of all

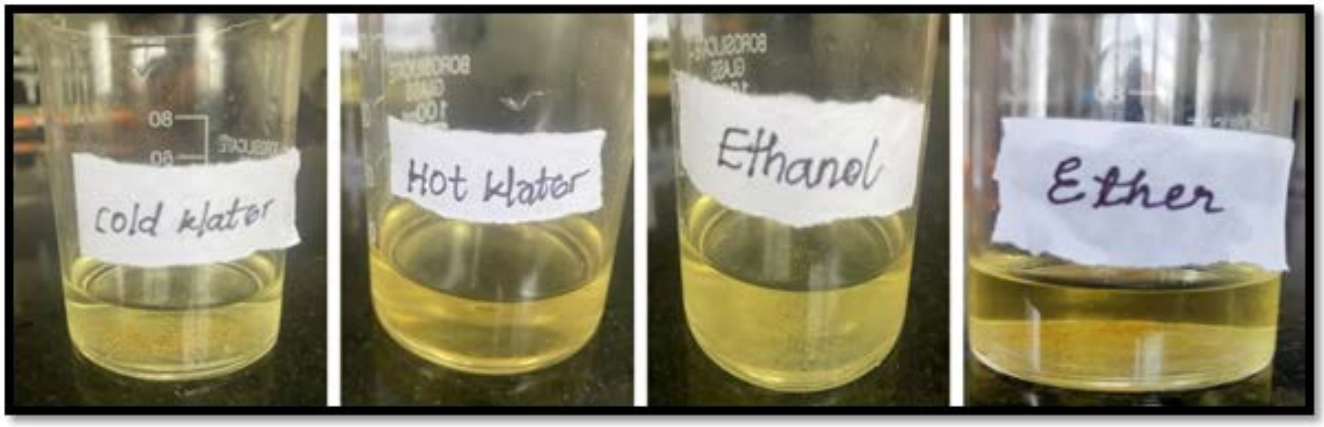


Figure 1. Image of liquorice root powder solubility profile in different solvents.

Table 3. Solubility profile of liquorice root powder

S. No.	Solvent	Solubility
1	Normal distilled Water	Insoluble
2	Hot distilled water	Freely soluble
3	Ethanol	Sparingly soluble
5	Ether	Insoluble

the 43 batches along with viscosity, phase separation and spreadability is shown in the Table 4. From the data, it is evident that formulations F11, F19 and F31 showed good viscosity and spreadability without phase separation.

3.3 Evaluation of Liquorice Containing Melasma Cream

3.3.1 Determination of Physical Appearance

Optimized formulations F11, F19 and F31 physical appearance image is shown in Figure 4.

Physical appearance studies are carried out for all the formulations F1 to F43. All formulations are cream-white in colour, aromatic and semisolid in nature. In terms of consistency formulations F11, F19 and F31 passed the physical appearance test as per the monographs, shown in the Figure 4.



Figure 2. Image of blank poly herbal cream.

3.3.2 Determination of Viscosity

Viscosity of formulations F11, F19 and F31 is shown in Table 5.

Viscosity studies are carried out for all the formulations F1 to F43 as shown in the table 3.3.But formulations F11, F19 and F31 exhibits viscosity test as per the monographs.

3.3.3 Determination of Phase Separation

Phase separation of formulations F11, F19 and F31 is shown in Table 6.

Phase separation investigation for all the formulations F1 to F43 are carried as shown in the Table 6. Majority of the formulations failed the test due to phase separation. But formulations F11, F19 and F31 did not show any

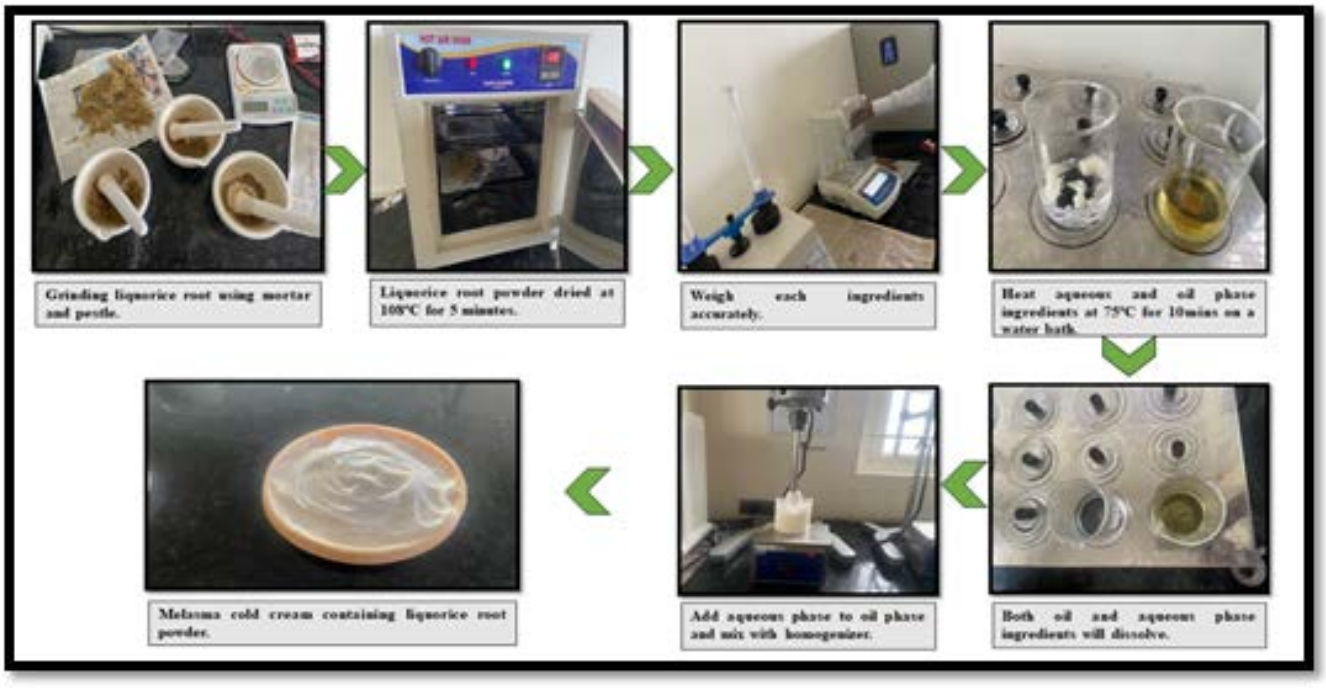


Figure 3. Preparation image of melasma cream containing liquorice by fusion method.

Table 4. Optimization of melasma cream formulation by CCD

Formulation	Independent Variables					Response Variables		
	Bees wax (gm)	Borax (gm)	Liquid Paraffin (mL)	Citric acid (gm)	Almond oil (mL)	Viscosity (cP)	Phase Separation (Yes/No)	Spreadability g×cm/sec
F1	12	0.3	60	0.1	0.5	1738.2	Yes	2.4
F2	17	0.3	60	0.1	0.2	2167.5	Yes	1.7
F3	14.5	0.21	50	0.115	0.35	1945.7	Yes	2.6
F4	17	0.3	60	0.1	0.5	2367.9	Yes	1.7
F5	12	0.3	40	0.1	0.2	1745.8	Yes	2.3
F6	8.55	0.27	50	0.115	0.35	1569.4	Yes	3.1
F7	12	0.3	60	0.13	0.5	1893.7	Yes	2.5
F8	12	0.25	40	0.13	0.2	1678.9	Yes	2.2
F9	17	0.25	60	0.1	0.2	2243.9	Yes	1.6
F10	12	0.3	60	0.13	0.2	1787.2	Yes	2.3
F11	14.5	0.27	50	0.115	0.35	5432.4	No	6.25
F12	14.5	0.27	50	0.115	0.006	2106.4	Yes	1.7
F13	17	0.25	60	0.13	0.5	2289.5	Yes	1.8
F14	17	0.25	40	0.13	0.2	2277.1	Yes	1.8
F15	17	0.3	40	0.13	0.5	2299.4	Yes	1.7
F16	17	0.25	60	0.13	0.2	2231.6	Yes	1.7
F17	12	0.3	60	0.1	0.2	1765.9	Yes	2.3
F18	17	0.25	40	0.1	0.5	2189.6	Yes	1.1
F19	12	0.25	40	0.1	0.2	5031.2	No	5.96
F20	14.5	0.27	50	0.150	0.35	1896.4	Yes	2.5
F21	12	0.3	40	0.13	0.2	1725.9	Yes	2.3
F22	17	0.3	40	0.1	0.2	2195.6	Yes	1.6
F23	17	0.3	60	0.13	0.2	2276.1	Yes	1.8
F24	17	0.25	40	0.1	0.2	2268.9	Yes	1.9
F25	17	0.25	60	0.1	0.5	2184.2	Yes	1.5
F26	14.5	0.27	26.21	0.115	0.35	1982.5	Yes	2.6
F27	14.5	0.27	50	0.07	0.35	1890.7	Yes	2.5
F28	12	0.25	40	0.1	0.5	1735.2	Yes	2.4
F29	12	0.25	60	0.1	0.2	1823.3	Yes	2.5
F30	14.5	0.27	73.78	0.115	0.35	1967.4	Yes	2.6
F31	17	0.3	60	0.13	0.5	4137.6	No	7.2
F32	12	0.27	60	0.13	0.5	1736.6	Yes	2.4
F33	14.5	0.33	50	0.115	0.35	2012.3	Yes	1.3
F34	12	0.3	40	0.1	0.5	1729.2	Yes	2.9
F35	14.5	0.27	50	0.115	O.70	2038.22	Yes	1.2
F36	12	0.25	60	0.13	0.2	1262.8	Yes	2.5
F37	17	0.25	40	0.13	0.5	2259.8	Yes	1.6
F38	20.44	0.27	50	0.115	0.35	2508.9	Yes	1.8

F39	12	0.25	40	0.13	0.5	1792.7	Yes	2.4
F40	12	0.3	40	0.13	0.5	1783.6	Yes	2.4
F41	17	0.3	40	0.1	0.5	2284.95	Yes	1.6
F42	17	0.3	40	0.13	0.2	2265.8	Yes	1.5
F43	12	0.25	60	0.1	0.5	1739.9	Yes	2.4



Figure 4. Physical appearance image of optimized formulations F11, F19 and F31.

Table 5. Viscosity of F11, F19 and F31 optimized melasma cold cream

S. No	Formulation	Viscosity (cP)
1	F11	5432.4±0.213
2	F19	5O31.2±0.324
3	F31	4137.6±0.211

N=3

Table 6. Phase separation of F11, F19 and F31 optimized melasma cold cream

S. No	Formulation	Phase Separation (Yes/No)
1	F11	No
2	F19	No
3	F31	No

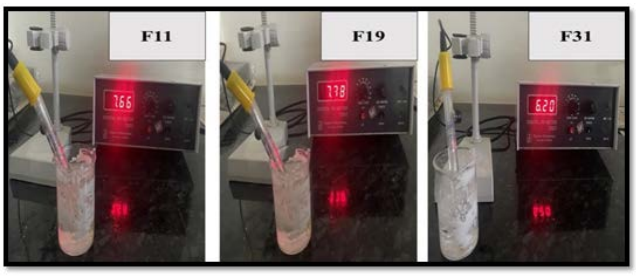


Figure 5. pH image of formulations F11, F19 and F31.

Table 7. Spreadability of F11, F19 and F31 optimized melasma cold cream

Si. No	Formulation	Spreadability (g×cm/sec)
1	F11	7.97±0.220
2	F19	8.51±0.323
3	F31	9.81±0.213

N=3

Table 8. pH of F11, F19 and F31 optimized melasma cold cream

S. No	Formulation	pH±SD
1	F11	7.66±0.112
2	F19	7.79±0.221
3	F31	6.20±0.011

N=3

separation in phase and passed the test as per the monographs.

3.3.4 Determination of Spreadability

Spreadability of formulations F11, F19 and F31 is shown in Table 7.

Spreadability studies are carried out for all the formulations F1 to F43 as shown in the Table 7. But



Figure 6. Homogeneity image of optimized formulation F31.

Table 9. Irritancy test of F11, F19 and F31 optimized melasma cold cream

Si. No	Formulation	Irritancy Test
1	F11	Slightly irritant
2	F19	Slightly irritant
3	F31	Non-irritant

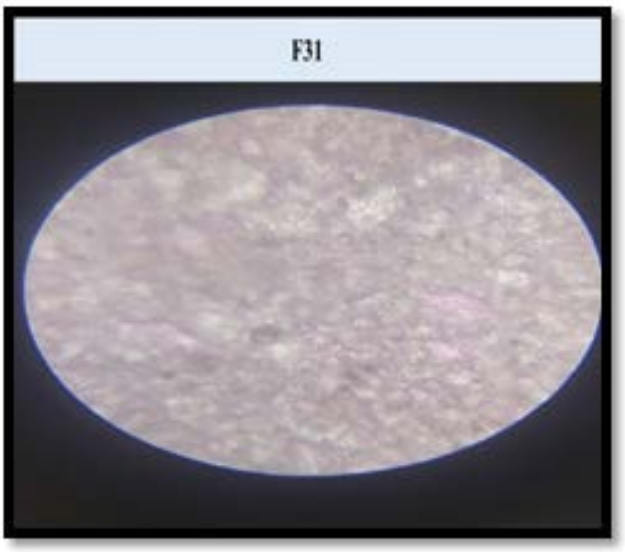


Figure 7. Compound microscope image of F31 formulation at 10x magnification.

formulations F11, F19 and F31 exhibits good spreadability as per the monographs.

3.3.5 Determination of pH

pH image of optimized formulations F11, F19 and F31 is shown in Figure 5.

Table 10. Optimized formulation (F31) short term stability parameters

F31 Formulation	0 Month ± SD	1 Month ± SD
Physical appearance	Normal	Normal
Viscosity	4137.6±0.211	4002.6±0.395
Phase separation	No	No
Spreadability	9.81±0.213	9.54±0.103
pH	6.20±0.011	6.29±0.201
Homogeneity	Homogeneous	Homogeneous
Irritancy test	Non-irritant	Non-irritant

pH studies are carried out for the optimized formulations F11, F19 and F31 as shown in the Figure 5 and Table 8. The pH of formulation F31 among other only lies with the range as per the monographs[34].

3.3.6 Determination of Homogeneity

Homogeneity image of optimized formulation F31 is shown in Figure 6.

Homogeneity study is carried out for the optimized formulation F31 as shown in the Figure 6. The optimized cold cream (F31) is found to be homogeneous in nature.

3.3.7 Determination of Irritancy Test

Irritancy test of formulations F11, F19 and F31 is shown in Table 9.

Irritancy test is carried out for the formulations F11, F19 and F31 as shown in the Table 9. Slight irritation is reported for the formulations F11 and F19. But for optimized formulation F31 no irritation case is reported.

3.3.8 Dye Test

Compound microscope image is shown in Figure 7.

Dye test for the formulation F31is carried out as shown in the Figure 7. The microscopic image of the optimized formulation (F31) confirmed that the prepared cream is water in oil type.

3.3.9 Short Term Stability Studies

Stability studies for the F31 formulation are carried out for one month under the storage conditions of room temperature.

Optimized formulation (F31) short term stability parameters are shown in Table 10.

As shown in the Table 10, F31 formulation remained stable and exhibited no significant alterations in physical appearance, viscosity, phase separation, spreadability, pH, homogeneity and irritancy test.

4. Conclusion

The objective of the research was to formulate a topical cream utilizing liquorice in the management of melasma. F31 was found suitable after screening and optimization processes. It possessed desirable characteristics like homogeneity, good spreadability and was non-irritant. However, formulations F11 and F19 were mildly irritant. The optimized cream, F31 was found to be a stable water-in-oil emulsion. Hence, these findings indicate that the topical liquorice cream may be a potential drug for the treatment of melasma.

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