IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
September 2021 Vol.:22, Issue:2

Development and Evaluation of An Advanced Delivery System Containing Phosphatidylcholine Complex for Skin Lightening

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ISSN 2349-7203
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Submitted: 19 August 2021
Accepted: 25 August 2021
Published: 30 September 2021

Keywords: Anti-oxidant; Liquorice; Potato juice; Phosphatidylcholine complex, Skin lightening

ABSTRACT

Glycyrrhiza glabra and *Solanum tuberosum* have potentially abundant anti-oxidant polyphenols helpful in the prevention of skin darkening. Polyphenolic compounds have lesser skin penetration and high polarity which subsequently results in reduced cutaneous delivery. The present research is tried to develop a new phosphatidylcholine complex cream to enhance cutaneous delivery of polyphenolic compounds for sustained anti-oxidant action. Phytochemical screening and in-vitro anti-oxidant evaluation were done on ethanolic extract of liquorice extract. Total phenolic content, total flavonoid content, and H_2O_2 scavenging activity were done on various ratios of liquorice extract and potato juice. Ratio 1:2 gave the highest total phenolic content (TPC) (226mg gallic acid equivalent (GAE)g−1 extract), total flavonoid content (TFC) (198 mg rutin equivalent (RUE)g−1 extract), H_2O_2 scavenging activity (66.44%), and hence was used for formulation. Solvent evaporation method using dichloromethane with 1:2 extract to phosphatidylcholine ratio was found to have entrapment efficiency (EE) of 54.84%. Evaluation parameters like Fourier transform infrared spectrophotometry (FT-IR) and digital microscopy confirmed the formation of the complex. The phytophospholipid complex was then formulated as water-in-oil cream and evaluated for various parameters. The optimized cream holding 1% complex was found to be stable for 3 months under conditions of stability study. In-vitro diffusion studies show that extract phosphatidylcholine complex cream had good diffusion of polyphenols from the membrane when compared to conventional extract cream giving extended and stronger topical action. Thus, developed polyherbal phosphatidylcholine complex cream safe and stable and it also exhibits a good potential as skin lightening cosmeceutical.

INTRODUCTION: (1)

Skin complexion is a predictable characteristic that is influenced by many factors. Various types of genes, nutrition, and ecological factors regulate the color of the skin. One of the most prominent components of skin that gives that complexion is a pigment known as melanin. Melanin is a pigment that is formed by cells known as melanocytes in the skin of most animals including humans. This pigment has different shades, dependent on the genetic makeup of the individual. Melanin has two basic forms and can have a variety from yellowish-red to dark brown. Eumelanin is the utmost common form of melanin and is brownish. The other basic form is called pheomelanin, which produces reddish-brown color that is frequently associated with freckles and red hair. The production of melanin pigment in an individual is affected by several factors.

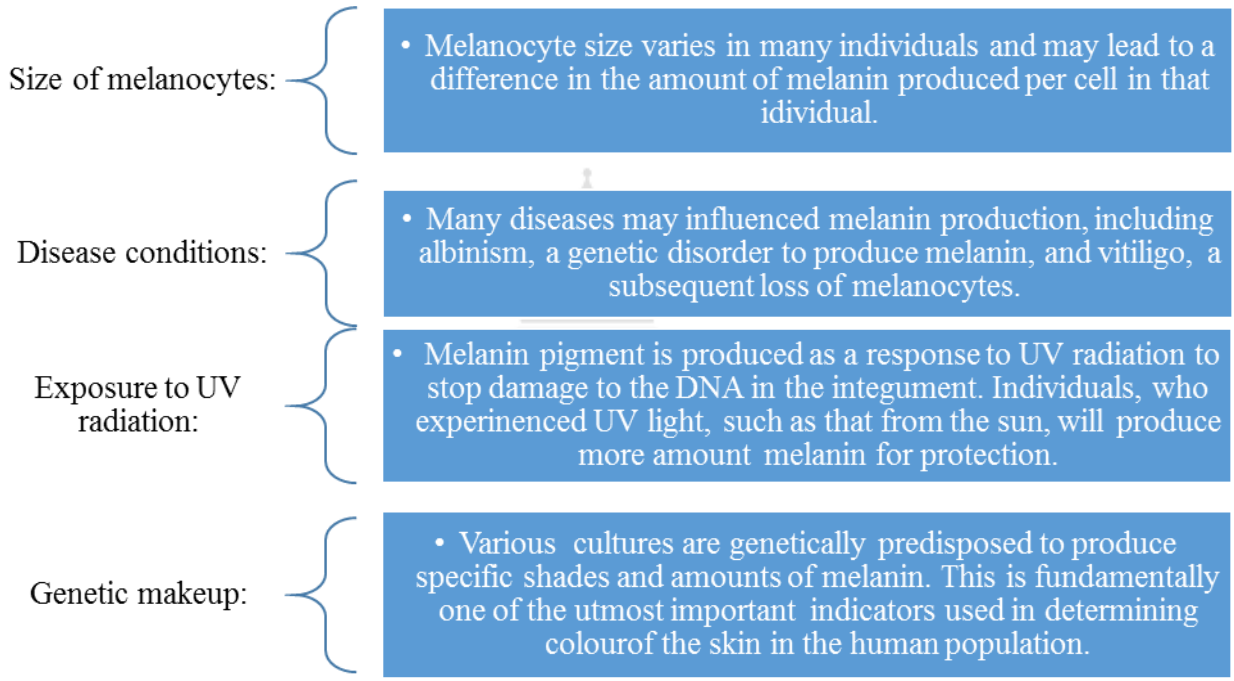

Figure No.1: Factor for production of melanin in the skin

All these aspects play a role in the production and expression of melanin pigment in the skin.

Here the **objective** is to design and develop a skin lightening cream containing phosphatidylcholine complex using herbal extracts of *Glycyrrhiza glabra* (Liquorice) root and *Solanum tuberosum* (potato) juice. The vehicle/ carrier cream dosage form is to be made by emulsion method to deliver skin lightning activity by holding the natural phytoconstituents such as *G. glabra* root extract and *S. tuberosum* juice.

Liquorice (*Glycyrrhiza glabra*, Fabaceae) contains phenolic compounds (flavonoids) (2). The ethanolic extract of liquorice has good anti-oxidant activity because of ROS scavenging, hydrogen-donating, and decreasing abilities of phenolic components present in high amounts (3). It enhances the viscoelastic and hydrating properties of the human skin (4). Potato juice (*Solanum tuberosum*, Solanaceae) is a very effective anti-oxidant, anti-acne, and skin-Lightning agent. Phenolic compounds are secondary metabolites produced in plants that have a common structure that is dependent on an aromatic ring with one or more hydroxyl substituents (5-7). These compounds can be alienated according to their chemical structure into flavonoids, stilbenes, phenolic acids, coumarins, lignans, and tannins. (8,9). Their existence influences the sensory potentials of plant-derived processed foods, including taste, color, and texture (10-12).

Polyherbal formulations give the best therapeutic effect because of the synergistic effect of phytoconstituents (13). Potato juice and liquorice extract have been used separately for their probable cosmetic benefit. So, an effort was made to study the combined effects of extracts on skin darkening.

The stratum corneum of the skin is an outstanding barrier to an external application. To enhance the skin infusion of the active ingredients, it is crucial to use penetration-enhancing agents or appropriate vehicles. Phosphatidylcholine is employed in solubilized form as a penetration enhancer (14,15). The significant advantage of phosphatidylcholine is a decreased skin irritation potency when compared to a typical penetration enhancing agent (16,17).

Phytosomes® is a patented technology established and commercialized by Indena to integrate standardized plant extracts or water-soluble compounds into phospholipids such as phosphatidylcholine (PC) resulting from soybean to make lipid-compatible molecular complexes, called Phytosomes®. Phosphatidylcholine complexes (complexes of phytoconstituents and phospholipids) are beneficial in improving skin absorption and the bioavailability of active constituents. Phosphatidylcholine complexes give superior biological activity when linked to an equal amount of the active constituent or extract, orally and topically (18).

Thus, this work is focused on the incorporation of phosphatidylcholine complex into an appropriate topical dosage form for delivery to the skin, ensuring enhanced cutaneous absorption and retention of polyphenolic compounds in the skin for greater and stronger topical action against skin darkening.

MATERIALS AND METHODS

- **MATERIALS:**

Glycyrrhiza glabra extract was a free sample procured from Amsar private limited, Indore. *Solanum tuberosum* used in this work was procured from the local market in Pune in November 2020 and was authenticated by the Botanical Survey of India, Pune, India. Gallic acid was acquired from SD Fine-Chem Ltd., India. Rutin and ascorbic acid were obtained from Loba Chemie, India. All the reagents and chemicals used in the study were of A.R. grade.

- **Equipment:**

Fourier Transform Infrared Spectrophotometry (FTIR), UV-Visible spectrophotometer (Shimadzu UV-1800), Brookfield viscometer, mechanical stirrer.

METHODS:

a. Phytochemical and pharmacognostic study of Glycyrrhiza glabra extract:(19)

Initial phytochemical screening of the extract of *Glycyrrhiza glabra* root was carried out by established methods. The extract was screened for different chemical as well as physical tests.

Table No. 1: Test for identification of phytoconstituents

Sr. No.	Phytochemical analysis
1. Test for alkaloids: The extract was dissolved in water. It was shaken well and filtered. The filtrate was used to perform the following tests.	a. Dragendroff's test: Few drops of Dragendroff's reagent were added to 2-3 ml filtrate. The presence of alkaloids is specified by the formation of an orange-brown precipitate. b. Mayer's test: 1-2 drops of Mayer's reagent were added to 2-3 ml filtrate. The presence of alkaloids is specified by the formation of a cream-colored precipitate. c. Hager's test: 1-2 drops of Hager's reagent were added to 2-3 ml filtrate. The presence of alkaloids is designated by the formation of yellow precipitate. d. Wagner's test: 1-2 drops of Wagner's reagent were added to 2-3 ml filtrate presence of alkaloids is indicated by formation reddish

	brown ppt.
2. Test for glycoside:	Bontrager's test: To 3 ml extract, add diluted sulfuric acid which is then boiled and filter, to cold filtrate was then added with an equal volume of chloroform or benzene. Shake well. Separate the organic solvent. Then add ammonia solvent, ammoniacal layer changes to pink or red.
3. Test for saponins:	Foam test: Herbal drug extract or dry powder which is vigorously shaken with water. The persistent foam was then observed.
4. Test for carbohydrates:	Molisch's test: 3 ml of the aqueous herbal extract was added to 2ml of Molisch reagent and then the resulting mixture was shaken appropriately, then 2ml of concentrated sulfuric acid was poured cautiously down the side of the test tube. A violet ring at the interface shows the occurrence of carbohydrates.
5. Test for tannins:	About 2ml of aqueous extract was stirred with 2ml of distilled water and few drops of $FeCl_3$ solution were added then the formation of green precipitate was an indication of the presence of tannins.

b. Physicochemical Evaluation of Crude Drug

Table No. 2: Physicochemical Evaluation of Crude Drug (For Liquorice)

Sr.No.		**Procedure**
	• **Determination of ash values (20)**	
1	**Total ash**	Weigh and ignite flat, thin, porcelain dish. Weigh 1 gm of the powdered drug into the dish. Support the dish on a pipe clay triangle on the ring of the retort stand. Heat with the help of a burner, with a flame about 2cm high and supporting about 7cm, above the flame, heat till vapors almost cease to be evolved, then lower the dish and apply heat more strongly until all the carbon burnt off. Cool in a desiccator. Weigh the formed ash and calculated for the % of total ash with regards to the air-dried sample of the crude drug.
2	**Acid insoluble ash**	Using 25ml of dil. HCL washing of the ash from the dish used for total ash into a 100ml beaker. Put wire gauze over a Bunsen burner and boil for five min. Filter through a filter paper, wash the remainder twice

		with hot water. Ignite a porcelain dish with the help of flame, cool, and weigh. Put a filter paper and residue together into the porcelain dish, heat gently until vapors cease to be evolved, then more strongly until all carbon has been removed. Cool in a desiccator. The residue was then weighed and calculated for acid-insoluble ash of the crude drug concerning the air-dried sample of the crude drug.
3	**Sulfated ash value:**	This is determined by a method in a way for acid insoluble ash, 25ml of dilute sulfuric acid, in place of dil. Hydrochloric acid.
4	**Water-soluble ash**	This is determined by a method in a way for acid insoluble ash, 25ml of water in place of dil. Hydrochloric acid.
	• **Determination of Extractive Values (21)**	
1	**Alcohol soluble extractive value**	Fill a 100ml graduated volumetric flask to the delivery mark with the solvent (90% alcohol) wash out the weighing flask and then pour the washings collected with the remaining solvent into a conical flask. Cork the flask and set weigh about 5gm of the powdered drug in a considering bottle and transfer it to a dry 250ml conical flask. Aside from the 24 hours, shaking frequently. Filter into a 50ml cylinder. When satisfactorily filter has collected, transfer 25ml of the filtrate to weighed porcelain dish. Evaporate it to dryness on a water bath and complete drying it in the oven. Cool in a desiccator, weigh. Calculate the % w/w of extractive about the air-dried drug.
2	**Water-soluble extractive value**	Weigh 5gm of the drug in a weighing bottle and transmission it to dry 250ml conical flak. Fill 100ml graduated flask to the delivery mark with the help of chloroform water wash out the weighing bottle and pour the washings composed with the remain of solvent. Cork the flask and keep it aside for 24 hours with shaking. Filter it into a 50ml cylinder. Addition of filtrate to porcelain dish. Evaporate it on a water bath and then oven. Cool in a desiccator. Calculate the % w/w of extractive with regards to air-dried drugs.

c. Standard calibration curve of Liquorice:

The standard calibration curve of licorice was taken using a UV spectrophotometer. The UV absorbance of liquorice standard solution in the range of 10-60µg/ ml of drug in ethanol was prepared and the absorbance is checked.

d. Total Phenolic Content (22)

It is calculated by Folin– Ciocalteu reagent procedure as determined by Singleton and Rossi with slight changes. Gallic acid was used as a solvent for reference whose concentration ranges from 20 to 100ppm. The other solvent used was water. Take 0.50ml of extract and reference solvent reacting with 2.5ml of FC reagent (with 1:10 by diluting it with water), after 2ml of saturated $NaHCo_3$ solution was mixed in the reacting mixture. Absorbance is taken with the help of a UV–vis spectrophotometer at 765 nm. The results were documented as milligram of gallic acid equivalent per gram extract weight (mg GAE g^{-1} extract weight).

e. Total Flavonoid Content (23)

It is calculated by the method described by Marinova et al. 1ml of licorice extract and reference solvent is taken respectively. and then it was reacted with 3.4 ml of CH_3OH, 0.15ml of $NaNO_2$, 0.15ml of $AlCl_3$, and 1ml of NaOH. Rutin is used as a reference solvent whose concentration ranging from 100 to 500 ppm. The experiment is carried out in a triplicate manner. Absorbance is taken with the help of a UV–vis spectrophotometer at 506 nm. The results were documented as milligram of rutin equivalent per gram extract weight (mg $RUEg^{-1}$ extract weight).

f. Hydrogen Peroxide Scavenging Activity (24)

The potential of the herbal extracts to hunt hydrogen peroxide (H_2O_2) was determined according to the method given by Ruch et al. with some modifications. A solution of H_2O_2 (43mM) was prepared in phosphate buffer pH 7.4. mixed were 0.1ml (10– 60 ppm) extract solution, 3.4 ml 0.1 M phosphate buffer (pH 7.4), and 0.6 ml 43 mM H_2O_2, and therefore the absorbance (Abs) of the mixture was then measured at 230nm concerning a blank solution with phosphate buffer without the hydrogen peroxide. Control solutions were prepared for each sample concentration by replacing the sample/standard with phosphate buffer. Ascorbic acid was used as the standard. The experiment was performed in triplicate. H_2O_2 scavenging activity was mentioned as the inhibition percentage calculated using the following formula,

$$\% \text{ Inhibition} = \frac{\text{CONTROL Abs. - SAMPLE Abs.}}{\text{CONTROL Abs:}} \times 100$$

g. Correlation Study:

Total phenolic content, total flavonoid content, and antioxidant assays were done on both the extracts of liquorice extract and potato peel juice.

h. Combination Study:

Different ratios of liquorice extract and potato juice (1:1, 1:2, 2:3, 3:2, 2:1, 1:0, 0:1) were used for determination of TPC, TFC, and anti-oxidant assays. The ratio that gave the maximum polyphenolic content and the anti-oxidant effect was selected for the formulation.

i. Phosphatidylcholine Complex Preparation

Various preparing methods were tried in this study for complex formation, namely,

1. Solvent evaporation method.
2. Salting-out, and
3. Film formation,

1. Refluxing with Methanol (Solvent Evaporation Method) (25)

Specific amounts of extracts (1.00 g liquorice extract and 1.00 ml of Potato juice) and phosphatidylcholine (1 g) were refluxed at 50°C for 3 h in 100ml round-bottom flask and with 50 ml of dichloromethane as reaction medium. The resulting clear mixture evaporated with the help of a vacuum and then dried residue was then placed in desiccators overnight and stored at room temperature in an amber-colored glass bottle.

2. Refluxing with Dichloromethane (Salting-Out Method) (26)

Specific amounts of extracts (1.00 g liquorice extract and 1.00 ml of Potato juice) and phosphatidylcholine (1 g) were refluxed at 50°C for 3 h in 100ml round-bottom flask and with 50 ml of dichloromethane as reaction medium. The resulting clear mixture was evaporated, and 20 ml of n-hexane was added to it with stirring. The precipitate was filtered and it is dried under and dried residue placed in desiccators overnight and stored at room temperature in an amber-colored glass bottle.

3. Film Formation Method (27)

Specific amounts of extracts (1.00 g liquorice extract and 1.00 ml of Potato juice) and phosphatidylcholine (1 g) were taken into a 100ml round-bottom flask and dispersed in 30 ml methanol. The dispersion was then gently stirred in a warm water bath (45–55°C), and the formed solution was then frequently heated (45–55°C) using a rotary evaporator to evaporate methanol. The obtained Phyto phosphatidylcholine complex film was then dried overnight in an oven at room temperature and which is then stored in a desiccator till next use.

The method which gives good results for % yield was selected for further evaluations.

- **Determination of % yield**

The subsequent formula calculated for determination of % yield of phosphatidylcholine complex:

$$(\%)\ \text{Yield} = \frac{(\text{Practical yield})}{(\text{Theoretical yield})} \times 100$$

j. Optimization of Extract: phosphatidylcholine Ratio

The complexes were ready with extract (Liquorice extract and potato juice) and soy lecithin (phosphatidylcholine) in the ratio of 1:1, 1:2, 2:3, 3:2, and 2:1 respectively. The complexes were then equated based on entrapment efficiency (EE).

Entrapment Efficiency (28):

Entrapment efficiency (EE) was measured with the help of an 1800 UV–visible spectrophotometer (Shimadzu). A known quantity of the prepared phytophospholipid complex was ultracentrifuged at 5000 (Rotation per minute) rpm for 15 min in methanol. The number of phenolic compounds within the supernatant was then analyzed by UV–visible spectrophotometer at λmax 255 nm; the concentration of phenolic compounds was measured. All measurements were performed in triplicate.

The EE was calculated using the subsequent equation:

$$EE\ (\%) = (T\text{-}S)/T^* 100$$

Where,

T= Total amount of phenolic compounds present in a quantity of phosphatidylcholine complex taken

S= Amount of phenolic compound within the supernatant

T-S= Amount of phenolic compounds entrapped

k. Evaluation of Phosphatidylcholine Complex

The optimized phosphatidylcholine complex was therefore evaluated for the following evaluation parameters.

1. **Visual technique:** Phosphatidylcholine complex was taken and shaken with distilled water and then it was viewed under a digital microscope at an objective lens of 400X.

2. **Particle size distribution and zeta potential:** The vesicle size and potential zeta were examined by dynamic light scattering system spectroscopy through a Malvern Zeta sizer Nano Z (UK) computerized system.

3. **Fourier Transform Infrared Spectrophotometry:** Fourier transform infrared spectrophotometry (FT-IR Spectrometer, SHIMADZU, IR A 1S WL ENG230V) was employed to study the interaction between extracts and phospholipids and to acknowledged the structure and chemical stability of phosphatidylcholine complex, phospholipid, and extracts. The Infra-Red Spectra of herbal extracts, phospholipids which are soy lecithin, phytophospholipid complex, and physical mixture were obtained by the potassium bromide (KBr) method. KBr pellets were prepared by moderately mixing a 1-mg sample with 100 mg KBr. Spectral scanning was completed in the range between 4000 and 400 cm^{-1}.

l. Development of Cream Containing Liquorice extract & Potato juice–Phosphatidylcholine Complex:

With the help of **Design-Expert** software version, 13 various batches are determined depending upon the concentration of olive oil and stearic acid.

Table No. 3: Table showing parameters for Design expert software®

File Version	13.0.3.0
Study Type	Response Surface
Subtype	Randomized
Design Type	Central Composite
Design Model	Quadratic
Runs	9

Table No. 4: Composition of Creams containing liquorice & potato juice phosphatidylcholine

Sr. No.	Ingredients (In %)	B1	B2	B3	B4	B5	B6	B7	B8	B9
1	Phosphatidylcholine complex	1	1	1	1	1	1	1	1	1
2	Steric acid	1.5	20	10	11	7.92	20	15	22.07	10
3	Cetyl alcohol	5	5	5	5	5	5	5	5	5
4	Olive oil	3.96	5	10	7.5	7.5	10	11.03	7.5	5
5	Jojoba oil	10	10	20	10	10	10	10	10	10
6	Vitamin E	1	1	1	1	1	1	1	1	1
7	Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
8	Propyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
9	Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Ingredients enumerated in Table No.4 were accurately weighed and placed in separate beakers (for the oil phase and water phase). The beakers were heated at 70°C with help of a water bath. On the whole melting of the ingredients, the ingredients of the water phase were added to the oil phase with continuous stirring. The resulting emulsion was stirred until it becomes cool and congealed. 1% phosphatidylcholine complex was combined into the base by shear. Conventional (1%) cream was prepared by combining the known quantity of the extract in the water phase and then adding it to the oil phase with stirring using the similar method as above. The cream base in which phosphatidylcholine complex was stable was further evaluated and kept for stability studies.

m. Evaluation of Cream

9 formulation batches as provided by design expert software were further evaluated for the following parameters.

1. **Organoleptic Properties:** Appearance and emollience of cream were checked.

2. **Presence of Foreign Particles/Grittiness:** A small amount of cream was taken and spread on a glass slide free from grease and was detected against diffused light to check for the presence of foreign particles.

3. **pH:** The pH meter was calibrated with help of a standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50 ml of (DW) distilled water, and its pH was measured.

Rheological Properties:

I. **Viscocity (29):** Viscocity of the cream formulation was analyzed by Brookfield viscometer using spindle no 18.

II. **Spreadability (30):** Spreadability is a term mentioned to denote the extent of the area to which the topical formulation spreads on application to the skin. The spreadability of the cream was measured using the glass slide apparatus.

$$S=W^* L / t$$

Where,

S =Spreadablity (g cm s^{-1})

W= Weight on upper slide (g)

L= Length of slide (cm)

T= Time (s)

4. ***In-vitro* Diffusion Studies:**

Diffusion of the topical formulation was accomplished using cellophane membrane and modified Franz's diffusion cells. The membrane was stored in a refrigerator at −20°C in phosphate buffer. The receptor section was filled with 11 ml of phosphate buffer (saline) solution, and the donor section contained 1 g of each formulation. The receptor solution was uninterruptedly stirred at 200 rpm. The temperature was kept at 37°C by circulating water

through an external water jacket and protected from light with aluminum foil. All determinations were performed in triplicate. 1mL of aliquot solution within the receptor chambers was withdrawn through the sampling port at 0.5, 1, 2, 3, 6, 9, 12, and 24 h, and cells were refilled with fresh phosphate buffer solution so on keep the quantity of receptor solution constant during the experiment. The solution in the receptor compartment was analyzed spectrophotometrically using an 1800 UV–visible spectrophotometer (Shimadzu) at λmax 255 nm; the concentration of phenolic compounds diffused through cellophane membrane was measured. A comparison between diffusion profiles of phosphatidylcholine cream and extract cream was done.

RESULTS AND DISCUSSION

a. Test for identification of phytoconstituents

Table No. 5: Test for identification of phytoconstituents (For Liquorice)

Sr. No.	Phytoconstituents	Test performed	Result
1.	Test for alkaloids	Dragendroff's test Mayer's test Hager's test: Wagner's test	+ - + -
2.	Test for glycoside	Bontrager's test	-
3.	Test for saponins	Foam test	+
4.	Test for carbohydrate	Molisch's test	+
5.	Test for tannins	$FeCl_3$ test	+

b. Physicochemical Evaluation of Crude Drug

Table No. 6: Physicochemical Evaluation of Crude Drug (For Liquorice)

- **Determination of ash value**

1.	**Ash value**	**found to be 13%.**
2.	Acid insoluble ash value	was found to be 4%
3.	Sulfated ash value	was found to be 6%.
4.	Water-soluble ash value	was found to be 3%.

- **Determination of extractives value**

1.	**Alcohol soluble extractive value**	**was found to be 8%**
2.	Water-soluble extractive value	was found to be 22.4%.

c. Standard calibration curve of Liquorice:

The standard solution of liquorice in methanol shows UV absorbance at 255nm. The linearity was plotted for absorbance against concentration with R^2 value of 0.9947 and with a slope of 0.0169.

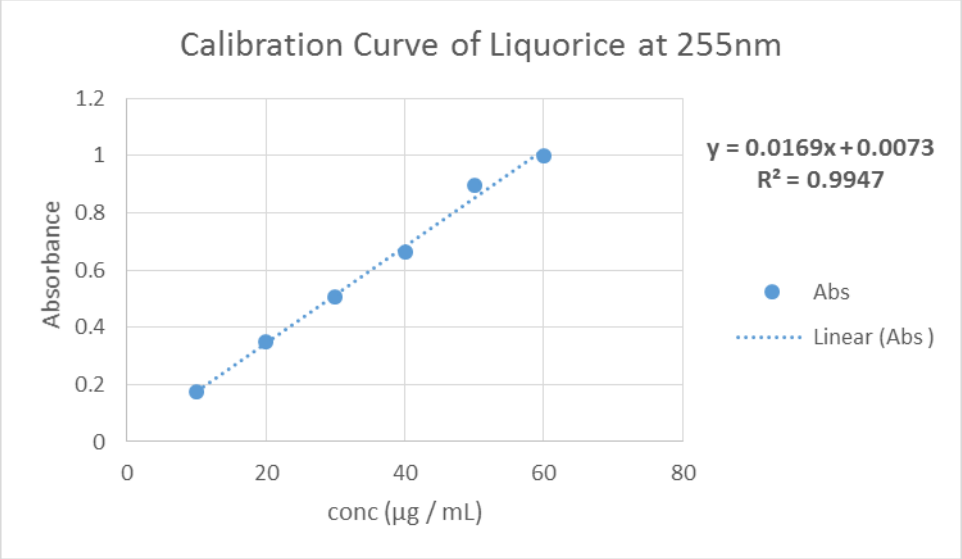

Figure No.2: Calibration curve of liquorice

d. Total Phenolic Content:

The TPC potato juice and liquorice extract determined by the Folin–Ciocalteu method is mentioned in terms of gallic acid equivalent (the standard curve equation: y = 0.0013x - 0.0148, R^2 =0.9972). The values gained for the concentration of total phenols are expressed as milligrams of GAE per gram of extract.

e. Total Flavonoid Content:

The TFC of potato juice and liquorice determined by the $AlCl_3$ method is expressed in terms of rutin equivalent (the standard curve equation: y = 0.0002x + 0.0056 R^2 = 0.9983). The values gained for the concentration of total flavonoids are expressed as milligrams of RUE per gram of extract.

f. Hydrogen Peroxide Scavenging Activity:

The anti-oxidant activity of the liquorice extract was expressed as (IC50), which was defined as the concentration (µg ml^{-1}) of extract required to scavenge 50% of radicals. The IC50 of liquorice extract in H_2O_2 of scavenging assay was 7.34 µg ml^{-1} in comparison to standard ascorbic acid 47.62 µg ml^{-1}.

g. Correlational study:

Results of correlation studies were tabulated in table no. 7. It shows a high phenolic content (350.15mg GAE g^{-1} extract) in potato while high flavonoid content in liquorice (448.97 mg RUE g^{-1} extract). Both liquorice extract and potato juice have good anti-oxidant values. So, the combination of these two herbal constituents plays a vital role in a skin lightening cream.

Table No. 7: Consolidated data of TPC, TFC and H_2O_2 assay for individual herbal drug

Sample	TPC	TFC	H_2O_2
Unit	GAE/g extract	RUE/g extract	IC 50
Liquorice Extract	153.23	448.97	71.34
Potato juice	350.15	602.04	48.39
Ascorbic acid	NA	NA	47.62

h. Combination Studies:

Results of TPC, TFC, and H_2O_2 scavenging activity carried out on different ratios of liquorice extract and potato juice are consolidated in Table no.8. As evident from the results, ratio 1:2 (Liquorice and potato juice) gave maximum TPC, TFC, H_2O_2 scavenging activity. Therefore, a 1:2 ratio was used for the next procedure.

Table No. 8: Consolidated data of TPC, TFC, and H_2O_2 assay of ratios of liquorice extract & potato juice phospholipid

Ratio (Liquorice -potato juice Phosphatidylcholine complex)	TPC GAE/g extract	TFC RUE/g extract	% Inhibition activity (in %)
1:1	180.2	73	50.75
1:2	**226**	**198**	**66.44**
2:3	175.3	144	54.09
3:2	136.5	103.5	47.41
2:1	123.33	89.5	38.39
1:0	88.75	80	47.91
0:1	93.44	52	51.75

i. Phosphatidylcholine complex preparation

Liquorice extract and potato juice phosphatidylcholine complex were prepared by using 3 methods as stated earlier. The solvent evaporation method gives 55% yield which highest amongst all. On the other side the remaining 2 methods give 43 and 33.5 % which was less. Thus, the solvent evaporation method was found to be suitable for the formation of the phosphatidylcholine complex of Liquorice- Potato juice.

Table No. 9: Consolidated data for % practical yield of phosphatidylcholine complex preparation by various method

Sr. No	Method	TY	PY	P/T=Y	Y*100 (in %)
1	**Solvent evaporation method**	**2**	**1.1**	**0.55**	**55**
2	**Salting out method**	2	0.86	0.43	43
3	**Film formation method**	2	0.67	0.335	33.5

j. Optimization of Extract: Phosphatidylcholine Ratio:

Phosphatidylcholine complex of 1:1, 1:2, 2:3, 2:1, and 3:2 was prepared and evaluated for EE.

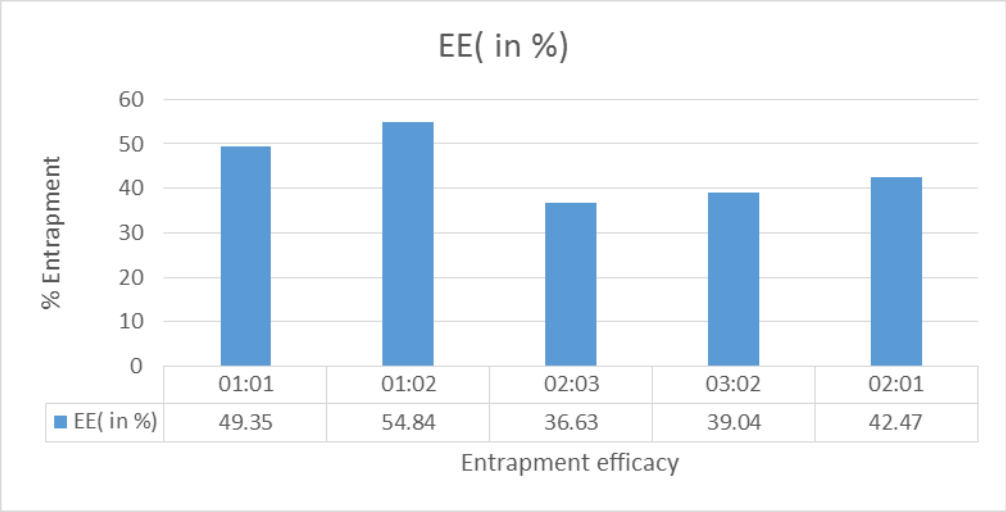

Figure No. 3: Entrapment Efficacy for phosphatidylcholine complex

As depicted in Fig No.3, results displayed that complex with ratio 1:2 gives the highest EE. **Therefore, 1:2 was selected as the optimum ratio for further characterization of complex and formulation of cream.**

l. Evaluation of phosphatidylcholine Complex:

1. Visual technique: The morphology of phosphatidylcholine complex was observed using digital microscopy, using a digital microscope, and viewed at 400X lens. Liquorice-potato juice phosphatidylcholine complex shows spherical morphology.

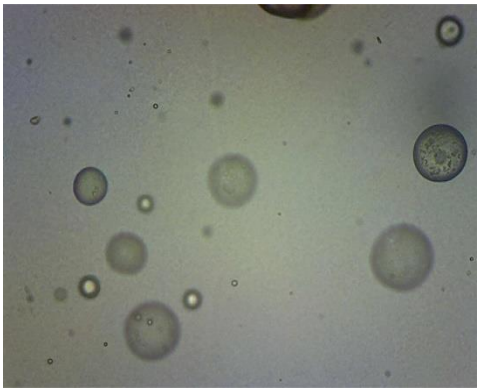

Figure No. 4: Digital Microscopic image of Liquorice-potato juice phosphatidylcholine complex

2. Particle size distribution and zeta potential:

The results for particle size distribution and zeta potential are demonstrated in Table No.10. B1 had a normal diameter volume of 123.08 nm. The results also showed that B1 has a zeta potential value of -32.1 mV.

Table No.10: Characteristic of phosphatidylcholine complex for particle size and zeta potential

Formula	Morphology	Dmean volume (nm)	Zeta Potential (mV)
1:1	Spherical	123.08	-32.1
1:2	Spherical	250.23	-25.2
2:3	Spherical	326.55	-23.4
3:2	Spherical	398.23	-14.2
2:1	Spherical	476.78	-10.9

3. Fourier Transform Infrared Spectrophotometry:

FT-IR was used to confirm the interaction between extracts and phospholipids in the phosphatidylcholine complex.

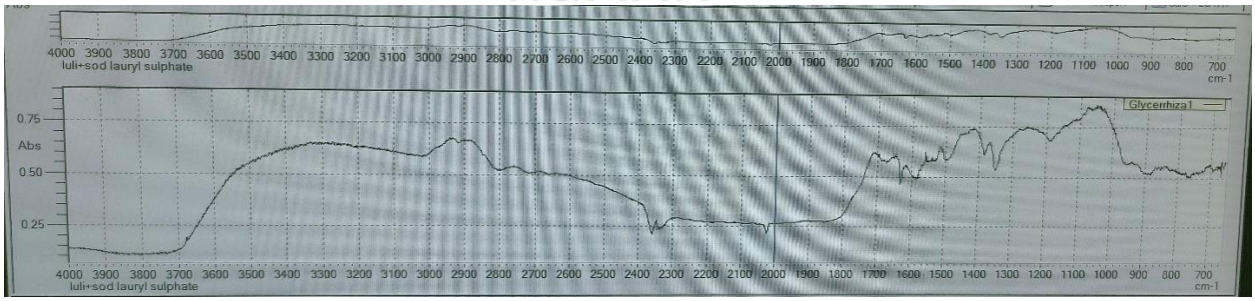

Figure No. 5: IR spectra od *Glycyrrhiza glabra* Linn

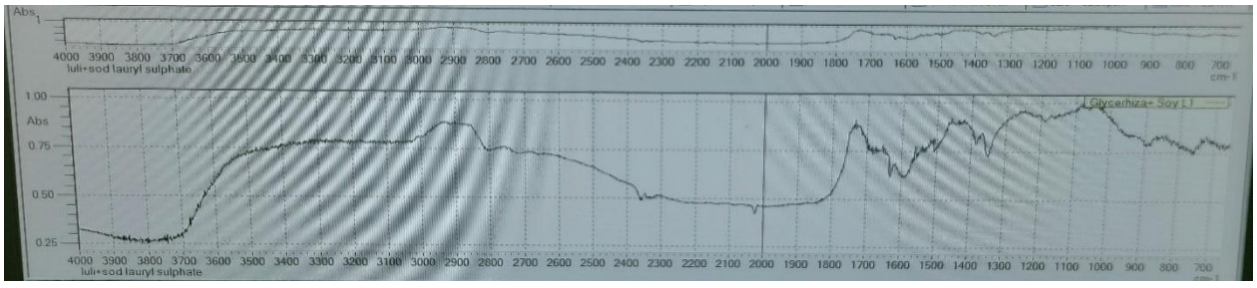

Figure No. 6: IR spectra of *Glycyrrhiza glabra* + Phosphatidylcholine

Table No. 11: Fourier Transform Infrared Spectrophotometry study

Absorption peak (In cm^{-1})	Type of Vibration	Description
3378	Stretching Vibration	Phenolic -OH group in liquorice
1674	Stretching Vibration	The sharp absorption band of the benzene ring
1724 **1228**	C=O absorption P=O absorption	In phosphatidylcholine complex
2918 **2840**	Stretching Vibration Deformation of $-CH_3$ group	In phosphatidylcholine complex
1455	Bending vibration	$-CH_2$ In phosphatidylcholine
1074	Stretching Vibration	P-O-C in phosphatidylcholine

The spectrum of the physical mixture showed an additive effect of extracts and phosphatidylcholine, in which the characteristic absorption peaks of extracts were still present at 3374 cm^{-1}, 2316 cm^{-1} (liquorice). These changes showed that extract and phospholipids formed a complex by hydrogen bonding between the OH group of the phenol rings of extracts and the P = O group of the phosphatidylcholine.

k. Development of cream Containing liquorice extract and potato juice – phosphatidylcholine Complex

Response Surface and central composite, this are study type and design type was carried out for having batches using design expert software version 13. This software gave 9 Formulation batches which show high efficacy of formulation with different concentrations of olive oil and stearic acid.

Various formulations tried for the development of stable cream are shown in Table no.4. Cosmetic cream changes its color when it is heated to a temperature of more than 40°C. Therefore, phosphatidylcholine complex was added after the temperature of the cream drops to 35°C with constant stirring.

Figure No. 7: Liquorice & potato phosphatidylcholine complex cream

l. Evaluation of cosmetic serum:

1. Organoleptic properties:

Appearance: The cosmetic cream was pale brown with a characteristic pleasant odor.

Emollience: Cosmetic cream was smooth in texture; this was determined by visual appearance and by touch.

2. Presence of Foreign Particles/Grittiness: There were no foreign particles in the cream, this was determined by visual appearance and by touch.

3. pH: The formulation of cream is meant for the topical application so the pH of the cream should be similar to that of the skin. The skin has acidic pH and skin cream should be in the range of 5-9. Based on the above points. Prepared skin whitening cream has pH was found to be in the range of 5-7.

4. Rheological properties:

a. Viscosity – Viscosity of cream was found to be in the range of 3000 -4500 cP.

ANOVA For Viscosity:

The **Model F-value** of 74.38 suggests the model is significant.

P-values less than 0.0500 shows model term is significant.

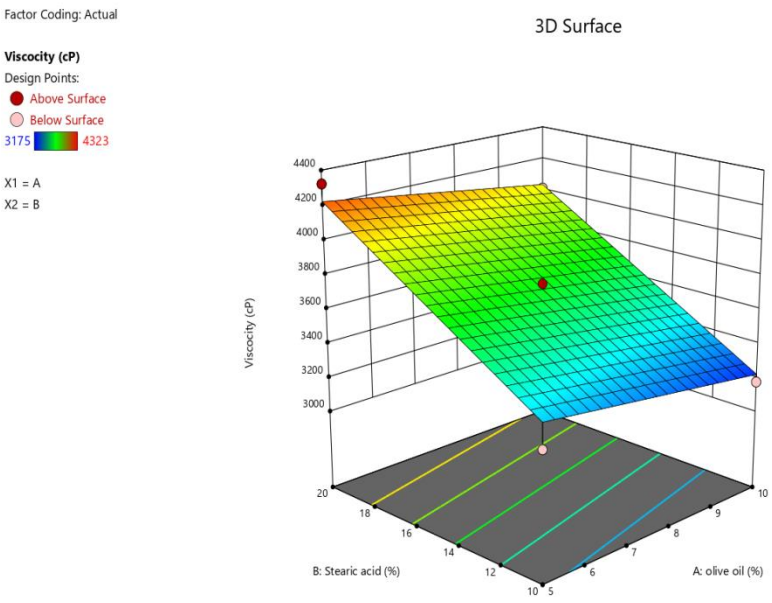

Figure No. 8: 3D Surface graph for Viscocity

b. Spreadability- The formulated cream showed good Spreadability. It was found to be 2.1-3.33 g cm s^{-1}.

ANOVA for Spreadability:

The **Model F-value** of 27.58 designates the model as significant.

P-values which are less than 0.0500 designate model term is significant.

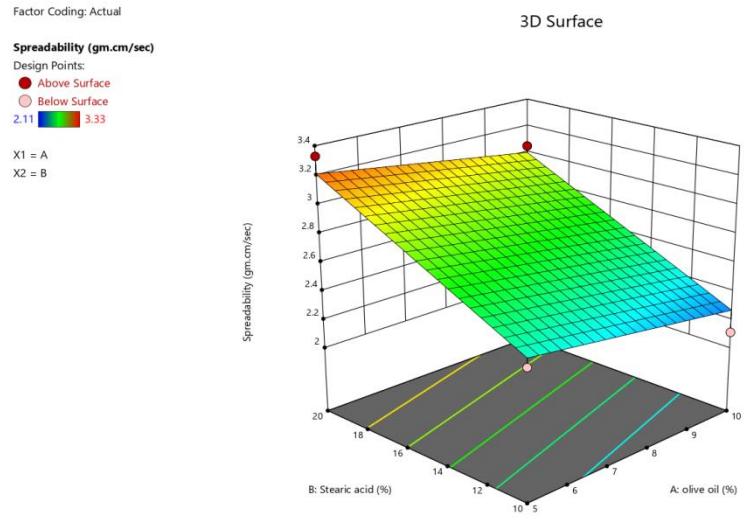

Figure No. 9: 3D Surface graph for Spreadability

5. In -vitro drug diffusion study:

The formulated skin whitening cream showed good drug release through the cellophane membrane and it was found to be 70-85 % drug release from the formulation.

ANOVA for Drug release:

The **Model F-value** of 147.38 shows the model is significant.

P-values that are less than 0.0500 shows model term is significant.

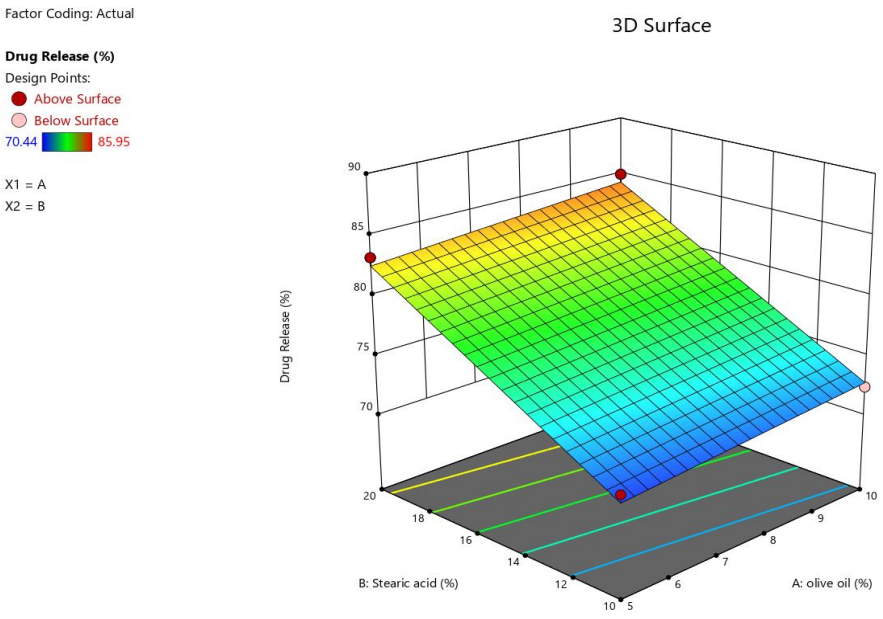

Figure No. 10: 3D Surface graph for Drug release

Table No.12: Consolidated data for all the batches for viscosity, Spreadability, and drug diffusion study.

Batch	Viscosity (In cP)	Spreadability (In gm.cm/sec)	Drug Release (In %)
1	3891	2.84	75.22
2	4323	3.33	83.22
3	3175	2.11	72.35
4	3754	2.6	76.45
5	3239	2.45	70.44
6	4012	3.05	84.96
7	3641	2.66	78.36
8	4201	3.21	85.95
9	3265	2.42	71.65

m. Optimization of Skin whitening cream:

According to the Design expert (file version 13.0.3.0.) showed batch 2 was optimized as it showed optimum results for Viscocity, Spreadability, Drug diffusion study. Batch 2 was further evaluated for the parameters like drug content and sun protection activity.

1. Drug Content (31):

1gm of cream was dissolved in 30 ml methanol, and the solution was kept aside intact for half an hour to attain homogenous equilibrium. The solution was withdrawn, the absorbance was assessed by a UV spectrometer at 255nm, and the concentration of phenolic compounds was measured. The experiment was performed in triplicate.

2. Sun protection activity (33):

Determination of the SPF value was carried out in vitro using a UV-Vis spectrophotometer using skin lightening Cream preparation on the solvent used, then measured and obtained absorbance. The absorbance of each preparation is calculated by the Mansur equation,

$$SPF = CF \times \sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times Abs\,(\lambda)$$

Where,

CF = correction factor (10),

EE (λ) = Erythrogenic result of radiation with wavelength λ,

Abs (λ) = Absorbance values at given wavelength λ.

The values of Entrapment Efficacy x λ are constants.

According to Wasitaatmadja, the categories for each sunscreen preparation based on SPF indigo given as a protection factor against sunlight are as follows (34)

1. Minimum, if the SPF ranges between 2-4.

2. Medium, if the SPF ranges between 4-6

3. Extra, if the SPF ranges between 6-8

4. The maximum, if the SPF ranges between 8-15

5. Ultra, if the SPF is greater than 15

- **Results for optimized batch evaluation:**

1. Drug content:

Batch 2 was evaluated for drug content and it shows 80.15 % drug in phosphatidylcholine complex.

2. Sun protection factor determination:

Table No. 13: Observation study for SPF activity determination

Wavelength	EE×I	Absorbance
290	0.015	2.520 ± 0
295	0.0817	2.520 ± 0
300	0.2874	2.475 ± 0
305	0.3278	2.447 ± 0
310	0.1864	2.438 ± 0
315	0.0837	2.334 ± 0
320	0.0180	2.143 ± 0

The skin whitening cream formulation showed the **ultra-SPF value of 25.20 ± 3.19.**

The amount of Vitamin E added to the formula influences the SPF value, the higher the concentration of vitamin E added to the Blemish Balm Cream preparation, the higher the SPF value, the rise in the SPF value is due to vitamin E can offer absorbance in the range of UV light wavelengths so that it can upsurge longer protection from sun exposure.

Vitamin E or tocopherol is a reduced form photoprotective ingredient so that when UV light interacts with sunscreen it will be blocked by vitamin E. Vitamin E does not only play a role in growing SPF values *in-vitro* but also stops photodegradation in UV light.

- **Stability studies:**

The stability studies were carried out for ICH guidelines. The cream-filled in the bottle and kept in a humidity chamber which was maintained at 30 ± 2 °C/ 65 ± 5 % R_H and 40 ± 2 °C / 75 ± 5 % R_H for two months. At the end of the research study, samples were examined for their physical properties and viscosity. The results are listed in Table No.14. Stability studies were done on optimized batch 2, as it shows no instability in the formulation then it is regarded as a stable batch and which can be concluded as final formulation.

Table No. 14: Stability study data for optimized batch

	Evaluation parameters	**stability data for 1 month**	**stability data for 2 months**	**stability data for 3 months**
30 ± 2 °C/ 65 ± 5 % RH	pH	5.56	5.52	5.45
	Viscocity (in cP)	3175	3645	4120
	Spreadablity (gm.cm/sec)	2.54	2.46	2.39
	Drug content (in %)	75.65	71.21	65.17
	In-vitro drug diffusion (in %)	83.17	82.46	81.79
40 ± 2 °C / 75 ± 5 %	pH	6.12	6.15	6.16
	Viscocity (in cP)	4323	4564	4786
	Spreadablity (gm.cm/sec)	3.33	3.32	3.29
	Drug content (in %)	80.27	88.24	80.21
	In-vitro drug diffusion (in %)	83.22	83.2	83.19

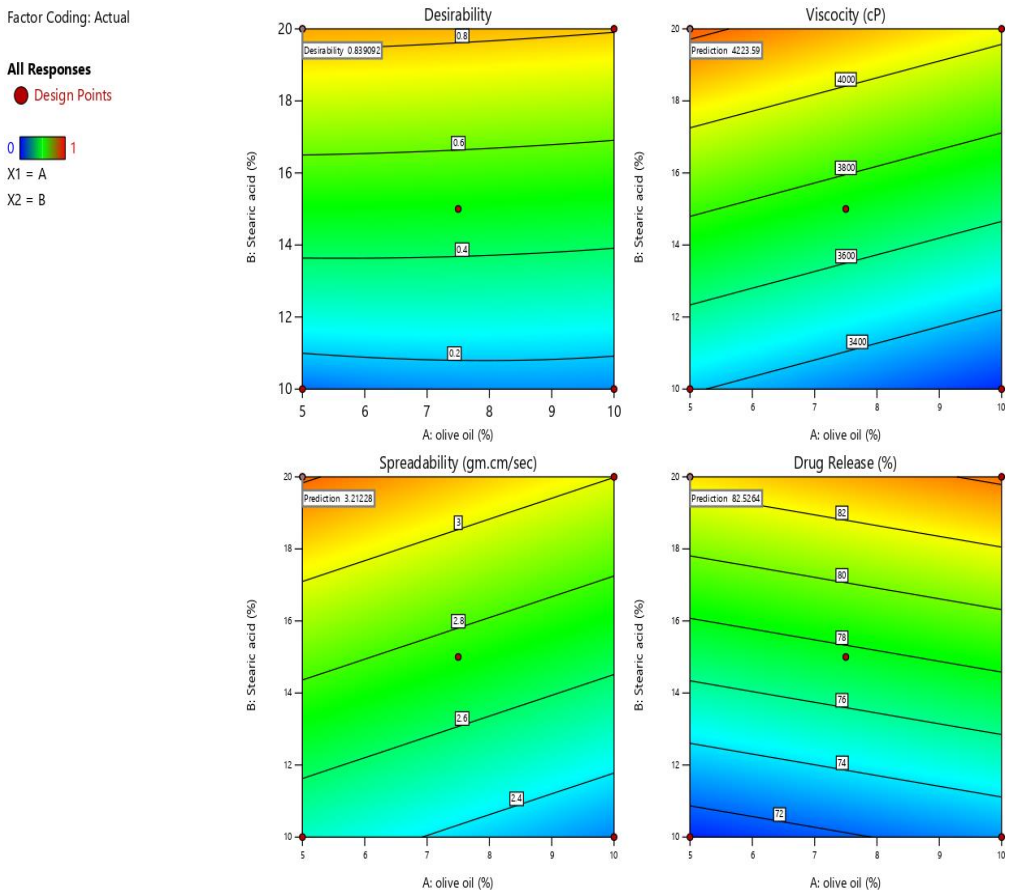

Figure No. 11: Effect of stearic acid and olive oil on viscosity, Spreadability & drug release by Design-Expert software.

CONCLUSION:

Liquorice extract & potato juice Phosphatidylcholine complex cream showed good TPC, TFC, and anti-oxidant activity. By combining both extracts, collaborative anti-oxidant & skin Lightning activity was achieved. The solvent evaporation method was chosen for phosphatidylcholine complex preparation because it gave a good % yield and EE. Out of the obtained complexes of extract: phospholipid, ratio 1:2 exhibited the highest EE. Evaluation parameters of the phosphatidylcholine complex confirmed the formation of the complex. In-vitro diffusion profile studies for phosphatidylcholine complex cream showed that there was the release of polyphenols through cellophane membrane for a longer time in phosphatidylcholine complex cream. This could increase the duration of the activity as the complex slowly releases the active constituent. Based on the results of the research that has been done, it can be concluded that the phosphatidylcholine complex in cream prepared was found to be stable and homogeneous and gave the most effective results. Thus, the Phosphatidylcholine complex of liquorice extracts and potato juice exhibited good potential

as skin Lightning and can either be used alone or as an additive to skin bleaching formulation.

ACKNOWLEDGMENT:

The authors wish to express their gratitude to Amsar private limited, Indore, India, for the generous gift of *G. glabra*. We thank the Botanical Survey of India, Western Regional Center, Pune-411001 for authentication of the *S. tuberosum* L. sample. We are also grateful to Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune, for the provision of expertise, technical support, and necessary guidance regarding the project.

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