BioGecko

Vol 12 Issue 03 2023
ISSN NO: 2230-5807

DEVELOPMENT AND EVALUATION OF HERBAL COSMETIC SERUM ON MELASMA-AGING ACTIVITY

Sakshi Kohakade[1*], Dr. Revan Karodi[2], Shital Rajad[3], Shubham Dange[4], Varsha Jadhav[5], Kiran Andhale[6]
[1,2,3,4,5,6] Dr. D Y Patil College of Pharmacy, Akurdi, Pune

ABSTRACT
A chemical that is very concentrated based on water or oil is a cosmetic serum. Because the outcome is nearly immediate, utilizing concentrates gives us not only an immediate visual improvement but also psychological fulfilment following therapy. Along With a non-greasy finish and an effective formulation, and elevated levels of active ingredients, the serum has the potential to absorb quickly and reach the deeper layers of the skin. The objective of this study was to create a serum using polyherbal extracts based on these qualities. The goal was to do the extraction, research the phytoconstituents behind the fairness action in the polyherbal extract, and assess different physical, chemical, and biological aspects of the formulation. The Serum's super-special combination of natural active components may enter the epidermis and color cells of the skin, resulting in a fair complexion and even skin tone. It contains substances that improve skin texture and leave skin feeling silky smooth, fair, and soft. It contains Glycyrrhiza glabra and Mangifera indica extracts. There were numerous physical, chemical, and biological assessments made. Heavy metals were not present in the formulation, which was also determined to have acceptable spreadability. Studies on skin irritation showed it was non-sensitizing and cost-free to use. Within a week, fairness should be implemented.

INTRODUCTION
Aging is a common occurrence for all living organisms. It can be detected by the slow degeneration of cells and organs. In terms of humans, aging is a complex phenomenon that incorporates changes to one's physical, psychological, and social makeup. Biologists believe that aging is the culmination of all changes that occur in a living thing over time and lead to a loss of function, a decreased ability to endure stress, and other changes. Melasma, formerly known as chloasma, is a pigmentary condition that often affects the face and gradually worsens over time. UV exposure and hormonal variables are the primary causes of this illness, which primarily affects women and those with darker skin tones.
Clinically, the appearance of dentofacial, malar, and mandibular symmetric reticulated hyper melanosis often indicates the existence of melasma [1]. In 50–80% of instances, the dentofacial pattern is the predominant clinical pattern. It has an impact on the forehead, nose, and upper lip but not on the chin, cheeks, or philtrum. [2][3].
For a very long time, herbal remedies and natural sweeteners have been made from the roots and rhizomes of the liquorice (Glycyrrhiza) species. Among the ailments that are commonly treated with liquorice root, a traditional medicine, are hepatitis C, gastric ulcers, and lung and skin diseases. Anti-inflammatory, antiviral, antibacterial, antioxidant, anticancer, immunomodulatory, hepatoprotective, and cardioprotective activities are additional advantageous pharmacological traits [4]. Glabridin and isoliquiritigenin, which are present in liquorice extract and act as melasma and skin-lightening agents in pharmaceutical and cosmeceutical products [5], limit the action of T1 and T3 tyrosinase isoenzymes.
The peel, stalks, leaves, barks, kernel, and stone of the mango fruit contain significant levels of the xanthone mangiferin, as do many other parts of higher plants. It is a powerful antioxidant with incredible health advantages, such as analgesic, antiviral, anticancer, antidiabetic, antioxidant, anti-aging, and immunomodulatory actions [6]. The mango tree's leaves, mango peel, and twigs all contain the phenolics isomangiferin and homomangiferin, which account for 10% of all phenolics [7][8]. They prevent the production of hydroxyl radicals by having the ability to chelate iron in Fenton-type processes [9].
A cosmetic serum is a highly concentrated solution made of water or oil. Concentrates, also known as serums,

have around ten times as many physiologically active chemicals as creams, allowing for quicker and more effective treatment of aesthetic problems. Localized effects of serums can be seen on the face, neck, decollete, and eyelids, among other body areas. You can use them regardless of your age [10].
In this investigation, liquorice and Mangiferin extracts were mixed to produce a polyherbal serum with a rapid equitability effect.

PLANT AND EXCIPIENT PROFILE:

Glycyrrhiza glabra L.

There are more than 30 species of the Glycyrrhiza genus, which is a member of the Leguminosae family (often known to as the Fabaceae). The Greek words glykys and rhiza, which mean sweet and root, respectively, are the source of the name "glycyrrhiza" [11]. Other names for it include liquorice, glycyrrhiza, sweet wood, and Liquiritiae radix [12]. The three Glycyrrhiza species that have been studied the most and utilised as Radix Glycyrrhizae are G. glabra L., G. uralensis Fisch., and G. inflata Bat (liquorice) [13]. The Chinese Pharmacopoeia also lists them as Glycyrrhiza plants for medicinal purposes [14].

Fig 1

Mangifera Indica

Mangiferin was initially found in Mangifera indica's leaves and bark (the mango tree). [15] Mango peels and kernels, Iris unguicularis, Anemarrhenaasphodeloides rhizomes, and Bombax ceiba leaves are further sources. Glycyrrhizae (liquorice).

Fig 2

METHODS

Extraction of active constituents:

Aqueous liquorice extract was made by dissolving 50g of powdered, dry liquorice roots in 300ml of distilled water, letting it sit for two days, filtering it, then condensing it under decreased pressure while keeping it chilled [16].

Alcoholic extract of Mangifera Indica:

The bark was cut into tiny pieces and allowed to air dry. These were then turned into a rough powder using a blender. 250 g of plant powder and 1.5 L of methanol were extracted using a soxhlet system for 18 to 20 hours. The resultant extract was subsequently concentrated in vacuo using rotary evaporation with controlled temperature and lowered pressure. After evaporation, the concentrated extract was stored in the fridge and kept at 4°C until it was needed (Yield: 5.5 g, 2.2%).

Excipients: The following list includes the different excipients utilised in this formulation:

a) Carbopol b) Disodium EDTA c) Triethanolamine d) Glycerin e) Vitamin E f) Sodium Benzoate g) Water h) Olive oil

EXPERIEMENTAL WORK

A.Preliminary Phytochemical Evaluation: [17]

Identifying phytoconstituents using a testDrug solubility study.

a. Standard Calibration Curve of Drug
b. Determination of TPC, TFC, Antioxidant Activity
c. Drug polymer stability study by FT-IR.
d. HPTLC Fingerprint.

B. Formulation of Serum using Design of Experiments.

C. Evaluation of Serum

- Organoleptic Properties
- Presence of foreign particles
- pH
- Particle size distribution
- Rheological Properties
- Drug content.
- In-vitro diffusion study
- Stability Study.
- Anti-Microbial study.

RESULT AND DISCUSSION:

A. Primary assessment of phytochemicals:

a. Identification test for phytoconstituents

Table No.1. Test for identification of phytoconstituents In Glycyrrhzia Glabra and Mangifera Indica Extract

Sr No	Phytochemicals	Observation	Inference of Glycyrrhzia Glabra	Inference of Mangifera Indica
1	Alkaloid	Yellow Orange Ppt	+	+
2	Flavanoid	Red Color	+	+
3	Glycoside	Brick Red Ppt	+	+
5	Saponin	Persistent froth for 15 min	+	+
6	Steroid	Brown ring at the interface of the two liquids	+	-
7	Tannin	Green – Black Color	+	-

a. Drug Solubility Profile:

Glycyrrhzia glabra and Mangifera indica were tested for solubility in several solvents, including water, methanol, ethanol, and chloroform. Water was shown to have the highest solubility for Glycyrrhzia Glabra and Mangifera Indica.

c. Calibration curve of Glycyrrhzia Glabra and Mangifera Indica

Analysis Of Wavelength Maxima

For both the concentration of 10ug/ml of Glycyrrhzia Glabra and Mangifera Indica diluted in distilled water and scanned throughout a wavelength range of 200-400nm, respectively, the wavelength maxima were found to be 254-255 nm.

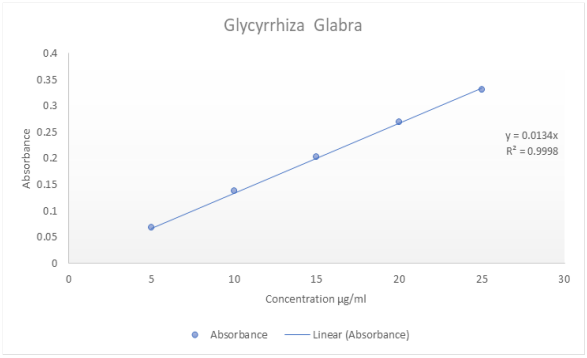


Fig No.3: Calibration Curve of Glycyrrhiza Glabra

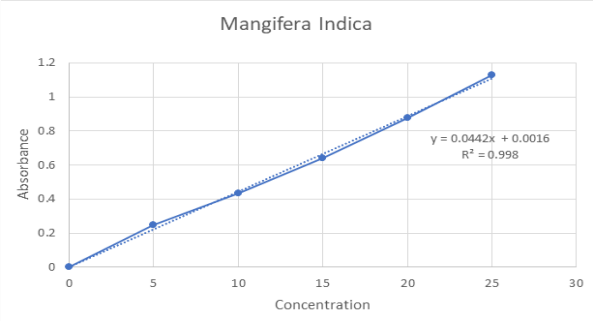


Fig No.4: Calibration Curve of Mangifera Indica

d. Determination of TPC, TFC, Antioxidant Activity

i. **Total Phenolic Content:** By using the Folin-Ciocalteu method, the TPC in Glycyrrhiza glabra and Mangifera indica was identified and is expressed as gallic acid equivalent. The values discovered for the total phenol concentration are given as milligrammes of GAE per gramme of extract. Glycyrrhiza glabra and Mangifera indica extracts were reported to have total phenolic contents of 98.502mg/g GAE and 185.19 mg GAE/g, respectively.

ii. **Total amount of flavonoids:** The TFC in Glycyrrhiza glabra and Mangifera indica was discovered using the AlCl3 process and is given in units of rutin equivalent. The results for total flavonoid concentration are given as milligrammes of RUE per gramme of extract. Glycyrrhiza glabra and Mangifera indica extracts were reported to have total flavonoid contents of 59.43 mgRUE g-1& 234.57 mgRUE g-1, respectively.

iii. **Antioxidant Study:** Based on Maisarah et al. (2013) with changes, the scavenging efficacy of extracts against DPPH radical was tested. Each well plate with various extract concentrations 100 L of the DPPH solution are added to (2,4,6,7,8,10ug/mL). The mixture was kept at room temperature in the dark for 30 minutes. Ascorbic acid served as the reference standard. The absorbance was measured at 517 nm.

Table No.2. Antioxidant Study on Glycyrrhiza Glabra

Sample Concentration	Sample Absorbance	% Inhibition
2 ug/ml	0.257	**71.39%**
4 ug/ml	0.295	**67.05%**
6 ug/ml	0.308	**65.62%**
8 ug/ml	0.320	**64.28%**
10 ug/ml	0.337	**62.38%**

Table No.3. Antioxidant Study on Mangifera Indica Sample

Sample Concentration	Sample Absorbance	% Inhibition
2 ug/ml	0.189	**79.54%**
4 ug/ml	0.258	**72.07%**
6 ug/ml	0.307	**66.77%**
8 ug/ml	0.324	**64.93%**
10 ug/ml	0.368	**60.17%**

e. Drug polymer stability study by FT-IR.

It was determined to characterise any potential interactions between the drug and the excipient using Fourier Transform Infrared Spectroscopy. With the use of an FT-IR spectrophotometer, it was captured. The spectra's frequency range was examined between 4000 and 400 cm-1.

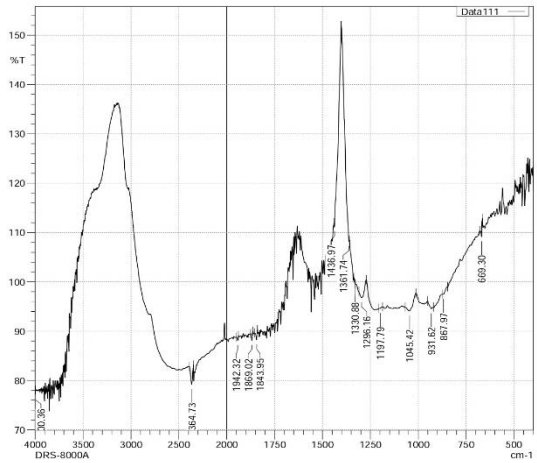


Fig No.5: FTIR of Glycyrrhiza Glabra Extract

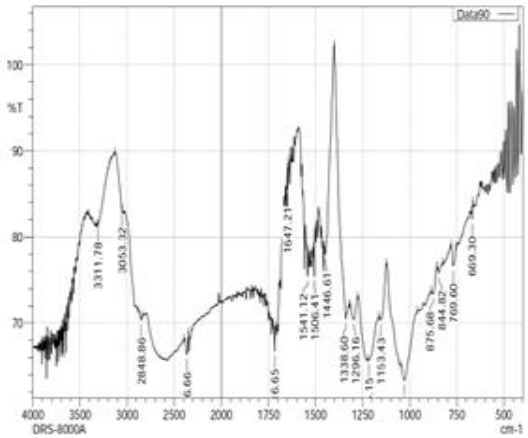


Fig No.6: FTIR of Mangifera Indica Extract

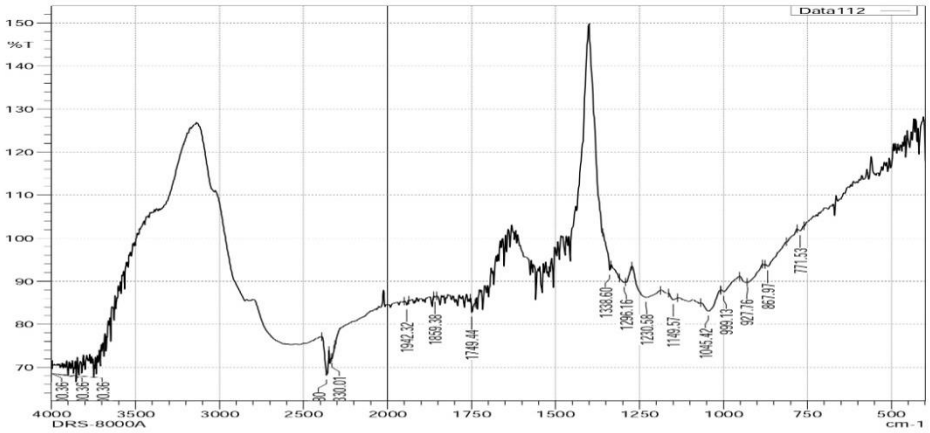


Fig No.7: FTIR of Glycyrrhiza Glabra & Mangifera Indica Extract

f. HPTLC Fingerprint

Analysis using fingerprints was done on the sample that was submitted. Following the development, the photos were taken in R white, R 254 nm, and R 366 nm. Derivatization is followed by documentation and scanning of images at R White and R 254nm.

Table No.4: Parameters For HPTLC

SR NO	PARAMETER	DESCRIPTION
1	Software	Vision CATS (3.1)
2	Phase of stationary	TLC Al Plates Silica Gel 60 F254
3	Plate Layout	100x100 mm
4	Phase of Mobile	GlycyrrhziaGlabra-nBuOH:AA:WATER(7:2:1) Mangifera Indica-EA:GAA:FA:WATER(7:1:1:1)
5	Lamp	Deuterium and Tungsten
6	Sensitivity Time	20 min
7	Scanner Type	Multiple ƛ

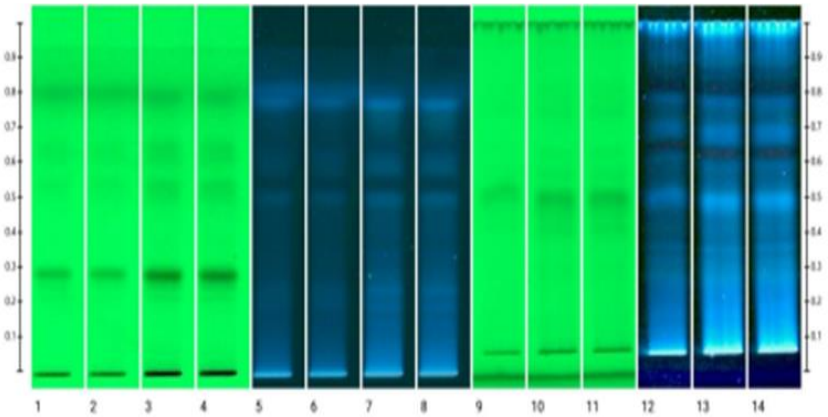
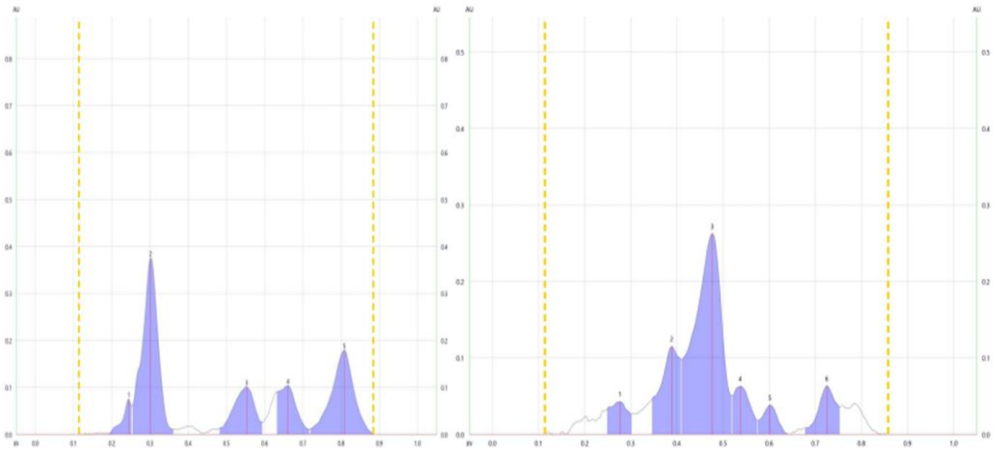


Fig No.8: HPTLC of Glycyrrhzia Glabra Extract image at 254 nm



a) **b)**

Fig No. 9: HPTLC Densitogram of Glycyrrhzia Glabra at 254 nm

a) 5 bands observed at 254 nm at Rf = 0.243, 0.301, 0.554, 0.661, 0.812
b) 6 bands observed at 254 nm at Rf = 0.276, 0.389, 0.476, 0.538, 0.601, 0.725

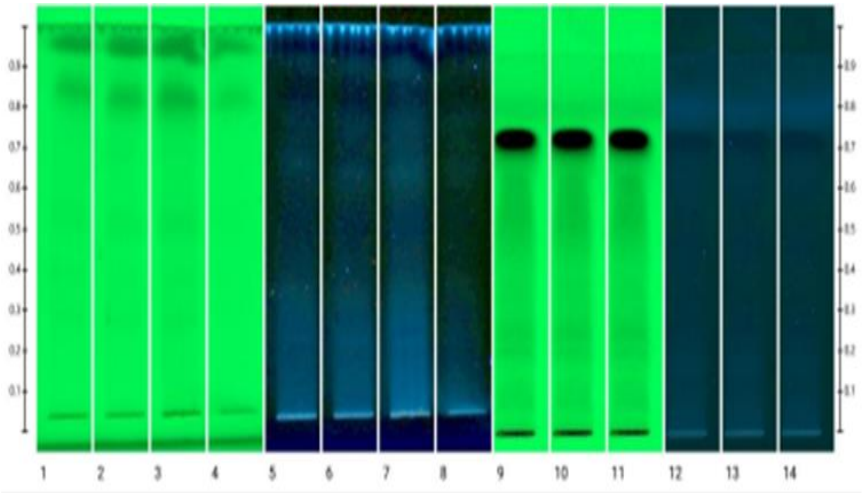
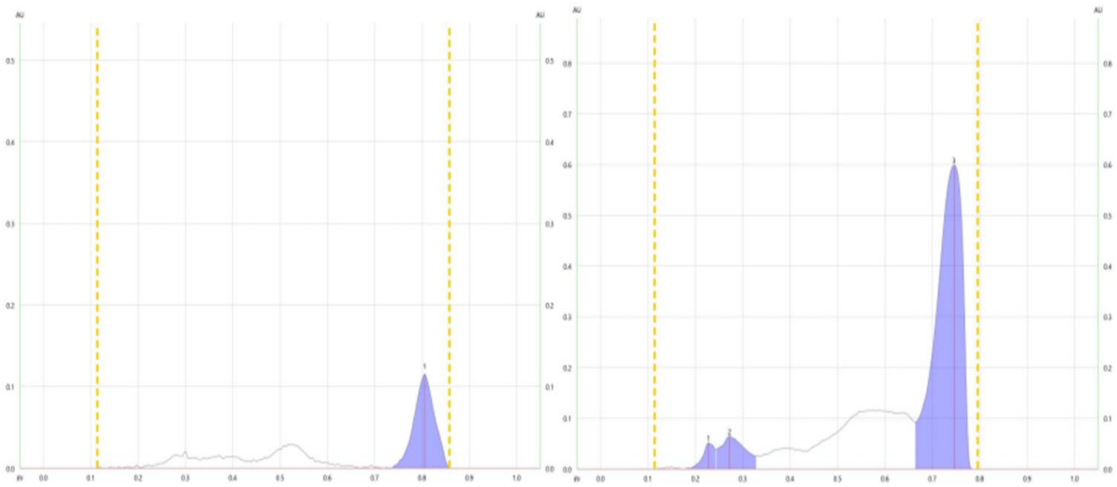


Fig No.10: HPTLC of Mangifera Indica Extract image at 254 nm



a)b)

Fig No.11: HPTLC Densitogram of Mangifera Indica Extract at 254 nm

a)1 band observed at 254 nm at Rf = 0.812
b)3 bands observed at 254 nm at Rf = 0.224, 0.276, 0.764

B. Formulation of Serum using Design of Experiments.

A core composite design was employed in the development of the formulas. Nine confirmatory runs' parameters underwent evaluation. By building a model in Design Expert to estimate various parameters, interactions, and to assess the quadratic impacts of components on Glycyrrhiza glabra & Mangifera indica serum, the formulation was improved using a central composite design with two independent variables (version 8.0.0, Stat-Ease Inc., Minneapolis, Minnesota).

For the answers shown in Table 1 as F1 to F9, nine runs were made, with factor concentrations ranging from minimum to maximum.

Table No.5: Formulation design of Serum

Sr no.	**Ingredient**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1.	**Carbopol 940 (gm)**	**0.3**	**0.3**	**0.3**	**0.3**	**0.4**	**0.44**	**0.2**	**0.158**	**0.4**
2.	Glycyrrhiza Glabra (gm)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
3.	Mangifera Indica (gm)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
4.	Triethanolamine (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
5.	Disodium EDTA (gm)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
6.	**Tween 20 (ml)**	**0.75**	**0.396**	**1.103**	**0.5**	**0.75**	**1**	**0.75**	**1**	**0.5**
7.	Glycerine (ml)	3	3	3	3	3	3	3	3	3
8.	Sodium Benzoate (gm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
9.	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

10.	Olive oil	1	1	1	1	1	1	1	1	1
11.	Vitamin E	1	1	1	1	1	1	1	1	1
12.	Rose Water	1	1	1	1	1	1	1	1	1

Using design expert software version 13, the research type and design type for batches were response surface and central composite. This software generated 9 batches, each of which shown high formulation efficacy using various carbopol 940 and tween 20 concentrations. The many formulations that were tested to generate a stable serum are displayed in table no.

C. Evaluation of serum

1) Organoleptic Properties:

Appearance- The cosmetics serum was brownish in color with a chracteristics pleasant odor.

Emolliency- The texture of the cosmetic serum was smooth, as seen by appearance and felt by touch.

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

2) pH: Since the serum is designed to be applied topically, its pH should be comparable to that of the skin. The pH of the skin is acidic, and a skin serum should be between 5 and 9. based on the earlier points. As the pH of the skin serum for melasma anti-aging activity is neither too acidic nor too basic, it was determined to be in the range of 4-5.5.

3) Particle size of distribution:

Characterization of Optimized Formulations: The formulas were developed using a central composite design. Malvern zetasizer was used to determine globule size and dispersion. In order to achieve homogeneous dispersion, ethanol was used to dissolve a 1.0 gramme sample. A sample was inserted into the zetasizer's photocell. The distribution and mean globule diameter were determined. The formulation's average globule size was determined to be 366 nm, and the zeta potential was 22.35 mv.

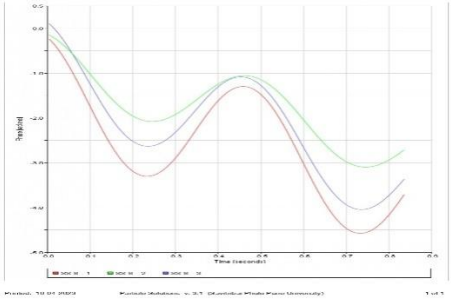


Fig No.12: PALS Graph

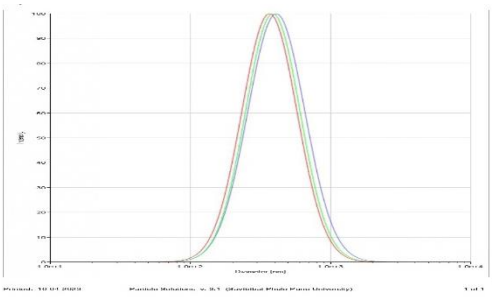


Fig No.13: DLS Graph

4) **Rheological Properties:**
   a. **Viscosity-** The viscosity of serum is the most important parameter in the evaluation of cosmetic product. As viscosity is affected by many factors such as change in temperature, change in manufacturing condition, quality of excipients.Viscosity of serum was between 78 to 16380 Cp.
   b. **Spredability:** The formulated serum showed good spredability. It was found to be 16-30 g cm $^{-1}$.

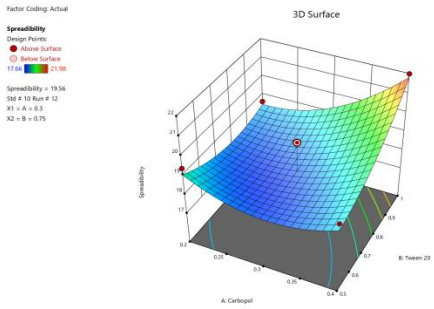


Fig No.14: 3D Surface graph for Spredability

5) **Drug Content:** Add 10 milligrammes to the serum equivalent. In a 100 ml volumetric flask with 15 ml of methanol added, Glycyrrhzia Glabra and Mangifera Indica were added. After stirring for 30 minutes, the remaining volume was made up with phosphate buffer, and the necessary dilutions were prepared. A 0.45 m filter was used to filter the final solution. A spectrophotometric measurement of the solution's absorbance was made at 268 nm [18]. Every formulations' medication content was tested in accordance with industry standards. Drug content was discovered to be displayed on a graph.

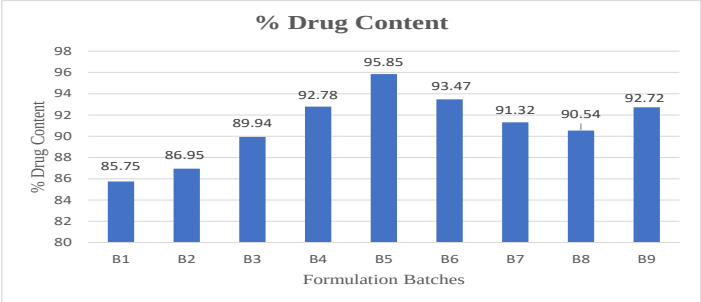


Fig No.15: % Drug Content

6) **In-vitro diffusion studies:** The diffusion cell apparatus was used to conduct the in vitro diffusion investigation. Total drug release is shown by the dissolution profile of serum within 8 hours. While other drug release profiles had much lower rates of dissolution, the drug release profile in formulation F9 was significantly higher. The concentration, polymer, surfactant, and carbopol 940, which lends viscosity to the formulation and creates a gel matrix, all had an impact on the drug release profile of serum. The serum's formulation displays good drug release through a treated cellophane membrane, demonstrating the serum's effective drug release

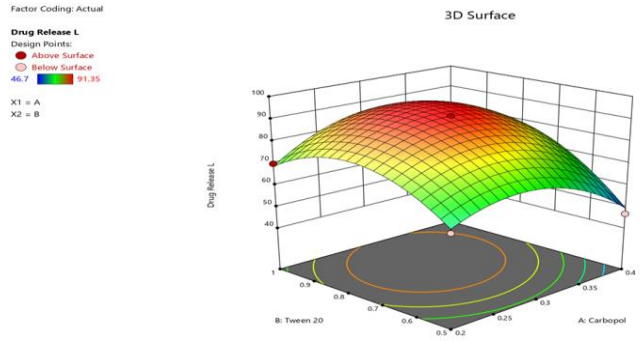


Fig No.16:3D surface graph for In-vitro drug diffusion of Glycyrrhzia Glabra

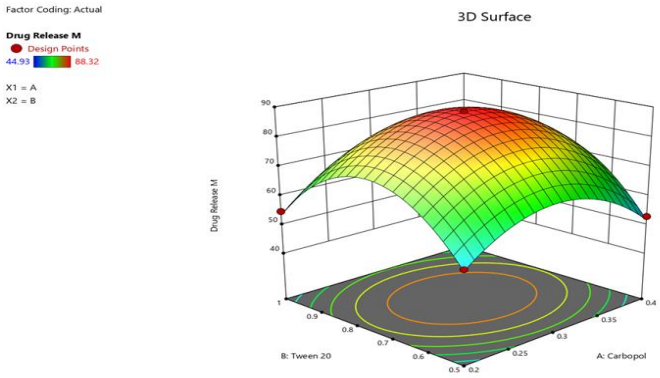


Fig No.17:3D surface graph for In-vitro drug diffusion of Mangifera Indica

7) **Stability study:** All the prepared Serum formulations were found to be stable upon storage for 3 months, no change was observed in their physical appearance, pH, rheological properties and drug content.
8) **Antimicrobial Study:** The formulated serum was inoculated on the plates of agar media by streak plate method and control was prepared by excluding the serum the plates were placed inside the incubator and kept there for 24 hours at 37 °C. Plates were removed from the incubation period and examined for microbial growth by comparing them to the control. After the incubation period, plates were taken out and no microbial growth was found.

The F5 batch was chosen from among all the batches, and sun protection work was done for the selected batch.

The SPF of the extract was calculated using the Mansur equation and a UV-visible spectrophotometer (HALO DB-20). After determining the absorbance at wavelengths between 290 and 320 nm with a spectrophotometer, the SPF was calculated using the Mansur equation.

SPF = CF x 290Σ 320EE (λ) x I (λ) x Abs (λ)

Where EE - erythemal effect spectrum; I-solar intensity spectrum; Abs-Absorbance of sunscreen product; CF-correction factor (=10).

F5 Batch formulation were evaluated for SPF activity. F5 shows a **14.8** SPF value

CONCLUSION

In terms of total phenolic content, total flavonoid content, and antioxidant activity, liquorice extract and mangiferin extract performed well. Antioxidant co-activity was attained by mixing the two extracts. The study's objective was to manufacture and assess the antioxidant activity of several plant extracts in a serum. Hexane, acetone, chloroform, ethanol, and water were successful in extracting liquorice roots and mangiferin bark.

Qualitative phytochemical screening, which reveals the presence of flavonoids, phenols, and carbohydrates in aqueous extract, was used to identify the phytochemicals present in the liquorice and Mangiferin extracts. The results of stability experiments showed that the physical and chemical characteristics did not alter significantly. Thus, licorice extract and mangiferin extract demonstrated good potential as anti-aging cosmeceuticals and may be utilised either alone or as a component of melasma anti-aging formulations.

REFERENCES

Sanchez NP, Pathak MA, Sato S, Fitzpatrick TB, Sanchez JL, Mihm MC., Jr Melasma: a clinical, light microscopic, ultrastructural, and immunofluorescence study. *J Am Acad Dermatol.* 1981;4(6):698–710. doi: 10.1016/S0190-9622(81)70071-9.

Guinot C, Cheffai S, Latreille J, Dhaoui MA, Youssef S, Jaber K, and others 197 Tunisian patients participated in a prospective study on aggravating variables for melasma. 1060-1069 J EurAcad Dermatol Venereol 2010;24(9)

Tamega Ade A, Miot LD, Bonfietti C, Gige TC, Marques ME, Miot HA. Clinical patterns and epidemiological characteristics of facial melasma in Brazilian women. *J EurAcad Dermatol Venereol.* 2013;27(2):151–156. doi: 10.1111/j.1468-3083.2011.04430.x.

Marjan Nasiriya Asl, HosseinHosseinzadeh .Phytother. Res, 2008; 22(1): 709–724

Sanjay Saxena.NPR,2005; 4(5): 358-367

Dar A, Faizi S, Naqvi S, Roome T, Zikr-ur-Rehman S, Ali M, Firdous S, Moin ST. Analgesic and antioxidant activity of mangiferin and its derivatives: the structure activity relationship. *Biol Pharm Bull.* 2005;**28**(4):596–600. doi: 10.1248/bpb.28.596.

Muruganandan S, Lal J, Gupta PK. Immunotherapeutic effects of mangiferin mediated by the inhibition of oxidative stress to activated lymphocytes, neutrophils and macrophages. *Toxicology.* 2005;**215**(1-2):57–68. doi: 10.1016/j.tox.2005.06.008

Sánchez GM, Re L, Giuliani A, Núñez-Sellés AJ, Davison GP, León-Fernández OS. Protective effects of Mangifera indica L. extract, mangiferin and selected antioxidants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice. *Pharmacol Res.* 2000;**42**(6):565doi: 10.1006/phrs.2000.0727

Matkowski A, Kuś P, Góralska E, Woźniak D. Mangiferin: A bioactive xanthonoid, not only from mango and not just antioxidant. *Mini Rev Med Chem.* 2013;**13**(3):439–55.

Skin care article - http://www.ost-cosmetics.com/about-skin-care/anti-aging-serum.html

Batiha, G.E.; Beshbishy, A.M.; El-Mleeh, A.; Abdel-Daim, M.M.; Devkota, H.P. Traditional Uses, Bioactive Chemical Constituents, and Pharmacological and Toxicological Activities of Glycyrrhiza glabra L. (Fabaceae). Biomolecules 2020, 10, 352.

Pastorino, G.; Cornara, L.; Soares, S.; Rodrigues, F.; Oliveira, M. Liquorice (Glycyrrhiza glabra): A phytochemical and pharmacological review. Phytother. Res. 2018, 32, 2323–2339.

Cheng, M.; Zhang, J.; Yang, L.; Shen, S.; Li, P.; Yao, S.; Qu, H.; Li, J.; Yao, C.; Wei, W.; et al. Recent advances in chemical analysis of licorice (Gan-Cao). Fitoterapia 2021, 149, 104803

Simayi, Z.; Rozi, P.; Yang, X.J.; Ababaikeri, G.; Maimaitituoheti, W.; Bao, X.W.; Ma, S.J.; Askar, G.; Yadikar, N. Isolation, structural characterization, biological activity, and application of Glycyrrhiza polysaccharides: Systematic review. Int. J. Biol. Macromol. 2021, 183, 387–398.

K. Gorter (April 1922). "Sur La Substance Mère du Jaune Indien", Bulletin du Jardin botanique de Buitenzorg, (in French). Volume 4 Series 3 Issue 2: p. 260–267; [J.C.S. (20 April 1923). "The precursor of Indian-yellow", Chemical Abstracts, (in English), Volume 17 Issue No. 8: p. 1472] – via archive.com

Hemraj Vashist, Diksha Sharma. Asian J Pharm Clin, 2013; 6(4): 55-59

Mohdaly,;AHassanien,M.;Mahumod,A,;Sarhan,M,;Smetanska ,I .Phenolics extracted from potato,sugar beet, and sesame processing by-products. Int.J.Food Prop.2013,16,1148-1168.

Wissing SA, Müller RH. The influence of solid lipid nanoparticles on skin hydration and viscoelasticity - In vivo study. Eur J Pharm Biopharm. 2003;56(1):67–72.