Formulation and Evaluation of Herbal Cream for Treatment of Melasma

[1]Bhagwan M Kale,[2]Vaishnavi K Chivte,[3]Rutuja G Dhakne,[4]Snehal S Dhewale

[1]Principal ,[2]Assistant Professor,[3]Assistant Professor
[1]Pharmaceutics, [1]Shenfadu Fakirba Sonwane Institute Of Pharmacy, Aurangabad ,India

Abstract: Herbal medicine has a product of worldwide importance of both medicinal and economical. Although usage of this herbal cosmetics has increased its quality, efficacy, safety, cultural acceptability & lesser side effects are concern in worldwide. Herbal cosmetics are getting increasing patient's conformity as they are devoid of representative after effects of biomedicine. The present research has been taken on the aim to formulate and evaluate the herbal cream containing Glycyrrhizaglabra, Calotropis Procera, Rubia Cordifolia herbal extract for the treatment of Melasma. The cream formulation was composed by using Herbal Extract, Steric Acid, Vaseline White, Cetyl Alcohol, Glycerin, Stearyl Alcohol, Methyl Paraben, Almond Oils& appropriate amount of Distilled Water. The skin phwas maintained by dropwise addition of Tri-ethanolamine. The physiochemical parameter of formulation like colour, odour, solubility, pH, Viscosity, spread ability, Extrudability, Drugs Content uniformity, In vitro drugs Release Study & stability study Were determined. The study indicates that formulation C-two containing herbal extract shows the drugs content release & stability studies which is stable. The pH of C-two Formulation was found to be compatible with the normal pH range & that way the chances of skin irritation are least.

Index Terms: Glycyrrhizaglabra, CalotropisProcera,RubiaCordifolia, Herbal Cream, Melasma, Cosmetics.

I. INTRODUCTION

The challenge of herbal cosmetics due to the availableness of modernconstituents the financial benefits for developing successful formulation and keep up of quality standard. Cosmetics are the formulation applying on the body. Herbal cream are used as cosmetic for softening and cleansing effects. The Ayurvedic system of medicine was one of the most principal systems that uses herbal plant and extract of the treatment of management of Melasma.

Glycyrrhizaglabra Linn.

Biological source:Glycyrrhizaglabra Linn.Yasti consist of dried, unpeeled, roots and stolons of GlycyrrhizaglabraLinn.belonginig to family leguminosae. Yasti contains not less than 3.0 per cent of glycyrrhinic acid.

Family-Fabaceae

Habitat- India, Iran, Italy, China, Pakisthan& England.

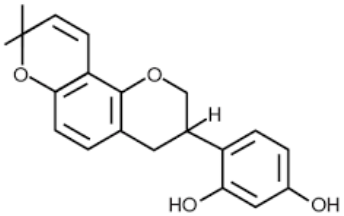
Parts used- seeds, roots etc.

Pharmacognostical characteristicss

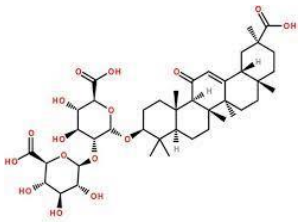
Chemical constituents-Glycyrrrhizine, isoflavoneglabridene ,Glabrene etc.

Medicinal properties & it's used- Anti-tussive& expectorant activity, Antioxidant activity , Skin lightening and skin tightening activity, Anti-inflammatory activity, Anti-viral effect, Anti-fungal activity,Anti-bacterial Activity , Anti-malarial activity, Anti hyperglycemic activity , Immunostimulatory effects, Memory enhancing activity, Hepatoprotective activity , Anticoagulant

Dose- 200-800mg/daily [33.]



Glabridin



Glycyrrhizin

Rubia Cordifolia Linn(Manjishtha)

Biological source:

It consists of dried stems of a climber known as Rasta pushpin Rubiacordidolia Linn. Sensu. Hook f. belonging to family Rubiacease. It should contain not less than 0.02 per cent of rubiadin on dried basis.

Family- Rubiaceace

Habitat- Asia, Europe, Africa etc.

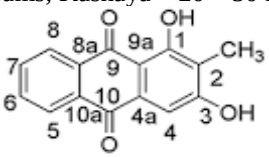
Parts used- Flower, leaves, stem, roots etc.

Pharmacognostical characteristics-

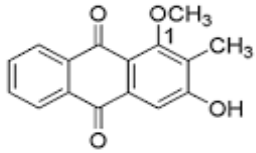
Chemical constituents-Alizarine (colourant), Anthraquinones, Iridous, Hexapeptides, Rubiprasins, Quinones, and Triterpenoids. Chemical constituents-it contains glycosides manjistin, purpurine, resin and red dye rubiadin.

Medicinal properties: Anti-inflammatory activity, oraganic compounds, as a dye, colourants, blood purifier activity, anticancer, astringent, antidysentric, antiseptic, deobstruent properties and antirheumatic, hepatoprotective.Itis used in treatment of leucoderma,

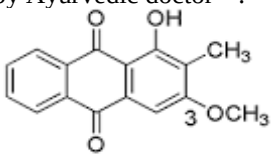
gauty arthritis & skin pigmentation. It help to gain lustre and glow of skin & aids to remove pimples, freckles & discolouratiuon.in ayurvedic medicine it is used as blood purifier & in textile industry used for dyeing of fabrics.
Dose-Powder 1 to 3grams; Kashaya – 20 – 50 ml in divided doses or as directed by Ayurvedic doctor [35].



Rubiadin



Rubiadin-1-methyl ether



Rubiadin-3-methyl ether

CalotropisProcera
Biological name - Calotropisprocera
Family- Apocynaceae
Habitat- North Africa, Tropical North Africa, Western Asia etc.
Parts used- Flower, leaves, seeds, roots etc.
Pharmacognostical characteristics-
Chemical constituents-Calotropine, calotoxinecalotropagenine
Medicinal properties: Anti-acne property, Skin lightening, Anti-arthritic property, Anti-cancer property, Anti-inflammatory activity, wound healing activity, Anti-microbial activity, Anti-convulsant Activity. Calotropisprocera is a well-known plant and has been traditional used for diarrhoea, stomatic, sinus fistula, and skin disease, and the leaf part is used to treat jaundice.
Dose- 200-300mg/ weekly[34].

II.Materials and Method

Extraction of Plant Material:
Herbal extract of Glycyrrhiza glabra,Calotropis Procera&Manjeesta.

Preparation of Herbal Cream

Incorporation of solid Ingredients into Cream Base
Oil in water emulsion-based cream was formulated. The emulsifier (stearic acid) and other oil soluble components (cetylalcohol, castor oil) dissolve in an oil phase (PartA)& heated to 70ºC. The preservatives & other water-soluble ingredients were dissolved in the aqueous phase (PartB)& heated to 70ºC. After heating the aqueous phase was added in portions to the oils phase with continuous stirring until cooling of emulsifier took place[47, 48, and 49]. The composition of herbal cream prepared from *herbal extractof Glycyrrhiza glabra, Calotropis Procera & Manjeesta*tabulated in Table 1.
Table 1: Composition of various cream formulations containing *herbal extract*

Ingredients	C1	C2	C3	C4	C5	C6
Hebal Extract	1.3	1.5	1.4	1.2	1.6	1.1
Steric acid	1.5	1.0	1.2	1.0	0.90	1.2
Vaseline white	1.5	1.2	1.2	1.0	1.1	1.0
Cetyl alcohol	-	0.275	0.225	0.300	0.300	0.290
Glycerine	-	0.275	0.225	0.300	0.300	0.290
Stearyl alcohol	-	0.275	0.230	0.300	0.300	0.300
Triethanolamine	0.135	0.160	0.135	0.160	0.135	0.135
Methyl paraben	0.05	0.05	0.05	0.05	0.05	0.05
Almond oil	0.30	0.30	0.30	0.35	0.40	0.30
Water up to (ml)	100	100	100	100	100	100

III. Preformulations Study of Herbal Extract:
Preliminary evaluations of Herbal Extract as follows:

A) Physiochemical analysis.
Physiochemical analysis of herbal extract like colour, solubility, and pH were studied in order to check purity of extract were performed and result were reported in the table:

Table 2: Physiochemical analysis test for herbal extract

Sr no.	**Specified test**	**Result**	**Specification**
1	Colour	**Brownish red colour liq.**	Brown colour lid.
2	Solubility	**70.18**	NLT 50%
	pH buffer 7.4		
	Water	**Practically not soluble**	Not specified
3	pH of 1% V/V sol.	**6.4**	4.5-7.5

B) Solubility study of the herbal extract
Solubility of herbal extract was measured as shown in the table
Table3: Solubility of Herbal extract in various pH media.

Media	Solubility level (mg/ml)
Methanol	60.18
0.1N HCL	57.34
pH4.5 buffer	56.69
pH6.0 buffer	54.10
pH6.8 buffer	50.94
pH7.4 buffer	70.18

C) Phytochemical analysis Herbal extract

Phytochemical analysis of herbal extract including test for terpenoids, tannins, flavanoids, alkaloids etc. are given for identification of active drug & result were reported in table.

Table 4: Physiochemical test result for herbal extract

Sr no.	Test	Constituents of reagent	Observation	Result
1	Mayer's test	Mayer's reagent	Cream colour precipitate	Alkaloids is presents
2	Wagner's test	Wagner's regent	Reddish brown precipitate	Alkaloids is presents
3	Dragendroff's test	Dragendroff's reagent	Reddish brown precipitate	Alkaloids is presents
4	Hager's test	Hager's reagent	Yellow precipitate	Alkaloides is presents
5	Tannins	5ml DW+boil+cool+0.1% FeCl3	Brownish green precipitate	Tannins is present
6	Terpenoids	2ml chloroform+ 3ml H2SO4	Yellow color	Terpenoids is presents

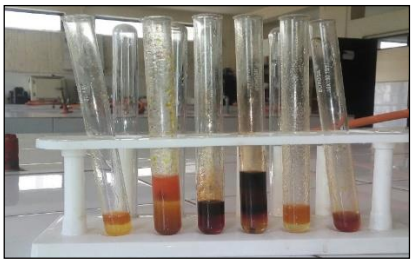


Figure 8: Physiochemical analysis

D)UV spectroscopy: Obtained sample was examined by UV Spectrophotometry & an compared with the reference standard spectrum of herbal extract. Maximum absorbance 0.323shown at 238 nm& standard range is 254nm.

Calibration curve for Herbal extract

Scanning of Herbal extract in pH buffer 7.4 by UV Spectrophotometer showed the λ_{max} 238nm (Figure). On this wavelength the standard curve followed the Beer- Lambert's law in the concentration range 10 to20 mcg/mL with $R^2 = 0.998$**.**

Table 5: Calibration curve of Herbal extract

Sr. No.	Concentration.(µg/ml)	Absorbance
1	0	0
2	10	0.394
3	12	0.499
4	14	0.566
5	16	0.642
6	18	0.715
7	20	0.793

E) Melting point determination

Melting point of Herbal extract was measured and found to be in the range of 238-242^{0}C. It was confirmed with the reported melting point of Herbal extract is 240°C

F) Thin Layer Chromatography

TLC of herbal extract was performed and found to be as shown in table and some mobile phase used Rf values are- Water selected as moving phase Rf values are 0.25; 0.29; 0.52, acetone was used as solvent Rf values are 0.33; 0.4; 0.79 combination of chloroform, benzene, ethanol was used Rf values are 0.37; 0.49; 0.68. And combination of chloroform, methane, Carbon tetrachloride is solvent Rf value is 0.36;0.45;0.56.

Table 6: Rf values by TLC

Drugs	**Spot**	**Rf value**	**Std. spot**	**Std. .Rf value**
Glycyrrhiza glabra	2.2±0.36	0.36±0.062	2.1±0.36	0.33
Rubiacordifolia	2.7±0.20	0.45±0.062	2.8±0.20	0.46
Calotropisprocera	3.4±0.20	0.56±0.032	3.2±0.20	0.49

G) Stability study of herbal extract

Stability of herbal extract was carries out under ICH guideline- 30±2 ºC & RH 65% as shown in table

Table7: Stability study of herbal extract

Sr no.	**Parameter's**	**0 Days**	**30 Days**	**90 Days**
1	Appearance	Slightly greasy	Slightly greasy	Slightly greasy
2	PH	6.5±0.3	6.3±0.3	6.4±0.3
3	Colour	Brownish red	Brownish red	Brownish red
4	Odour	Characteristics	Characteristics	Characteristics
5	Nature	Viscous liq.	Viscous liq.	Viscous liq.

IV. Evaluation of Herbal Cream

A) Physiochemical Proproperties

Table 8: physiochemical properties of cream

Parameter	C1	**C2**	**C3**	C4	C5	C6
Colour	creamy	**Creamy white**	**Creamy white**	Creamy	Creamy	Creamy
Odour	characteristics	**characteristics**	**characteristics**	characteristics	characteristics	Characteristics
Appearance	Greasy	**Non greasy**	**Non greasy**	greasy	greasy	changes
Nature	Viscous	**smooth**	**smooth**	smooth	smooth	smooth
pH	changes	**6.5**	**6.8**	Change	change	changes

B) pH study

The pH of all the formulations was determined by using digital pH meter. 1.5gm of cream was accurately weighed and dispersed in 15ml of distilled water and stored for two hours. The measurement of pH of each formulation was carried outin triplicate and the average values are represented in Table 9. The pH of dispersions was measured using pH meter.[47,48]

Table 2: pH values of Herbal Cream.

Sr.no.	Formulation	pH value
1	C1	7.0
2	**C2**	**6.5**
3	**C3**	**6.8**
4	C4	7.2
5	C5	6.7
6	C6	6.9

The pH of C2 and C3 formulations was found in the range. On the basis of pH the batches (C2 and C3) were found to be satisfactory that way C2 and C3 formulations are suitable for further evaluations study.

C) Viscosity study

The apparent viscosity values were measured for formulation using Brookfield viscometer DV-I with S94 at different speeds i.e., 2.5rpm, 4rpm, 5rpm and 10rpm. As the speed increases viscosity get decreased. From result it was observed that C2 and C3 herbal cream formulation showed maximum viscosity than C1, C4, C5 and C6 formulations. Viscosity standard range of semisolids dosage form is 8500 to 20000cps. Out of 6 formulations C2 herbal cream formulation showed maximum viscosity than other cream formulations. Values of viscosity (cps) of cream shown in table.

Table 10: Viscosity data of Herbal Cream

	Viscosity (cps) at rpm			
Formulations	2.5	4	5	10
C1	8300±0.20	7700±0.22	6800±0.21	6000±0.23
C2	**9800±0.21**	**9100±0.20**	**8400±0.22**	**7500±0.23**
C3	**8400±0.21**	**7800±0.22**	**6900±0.21**	**6000±0.23**
C4	8300±0.21	7600±0.20	6700±0.21	5800±0.23
C5	8300±0.20	7700±0.22	6800±0.20	6000±0.22
C6	8100±0.22	7600±0.20	6500±0.21	5800±0.23

D) Spreadability

All formulation was subjected to spread ability study with the help of the apparatus suggested by Mortimer et al. Spread ability is an important parameter for semisolid preparation. If spread ability is good then there will be ease of application of medicaments and ultimately absorption of medicaments will be good as medicament is spread properly. From result it was observed that C2 and C3 herbal cream formulation showed maximum spread ability than C1, C4, C5 and C6 formulations. Order of spread ability of herbal cream was found to be C2 >C3 >C4> C5> C6> C1. Out of 6 formulations C2 herbal cream formulation showed maximum spread ability than other cream formulations. Values of spread ability (mm) of cream shown in table.

Table 11: Evaluation of Spread ability

Sr. No	**Formulation Code**	**T1**	**T2**	**T3**	**Mean Time**	**Spread ability (mm)**
1	C1	6.8	6.7	6.6	6.7	45.55mm± 0.22
2	**C2**	**5.2**	**5.1**	**5.2**	**5.2**	**56.58mm± 0.22**
3	**C3**	**5.0**	**5.1**	**4.9**	**5.0**	**52.25mm± 0.22**
4	C4	5.4	5.7	5.7	5.6	50.35mm± 0.22
5	C5	5.8	5.6	5.7	5.7	47.45mm± 0.22
6	C6	6.4	6.5	6.4	6.5	46.15mm± 0.22

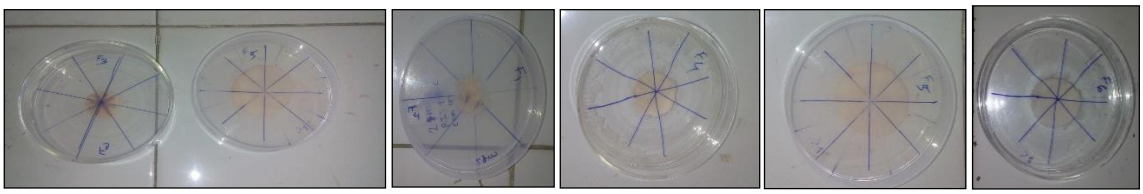


Fig 12: Spread ability of Herbal cream formulations.

E) Tube Extrudability

All tubes are subjected for evaluation of extrudability. More hard the formulation less will be extrudability as more force required to extrude the formulation. Extrudability study of C1 to C6 formulation were carried out as shown in table

Table 12: Evaluation of Tube Extrudability

Formulation Code	**Net wt. of formulation in the tube (g)**	**Wt. of Cream Extruded (g)**	**Extrudability amount (%)**	**Grade**
C1	2		85.00± 0.19	++
C2	**2**		**97.16± 0.15**	++++
C3	**2**		**86.00± 0.17**	++++

C4	2		88.00± 0.16	++
C5	2		89.00± 0.11	++
C6	2		86.00± 0.15	++

F) Drug Content of Herbal Cream

The drug content of the formulations was carried out on the UV Spectrophotometry.

The drug content of the formulations was carried out on the UV Spectrophotometry. The drug content of the C2 formulation 98.10% and that of the C3 formulation was found to be 79.30%.

Table 13: Percentage drug content of Herbal Cream

Sr. No.	Formulation Code	Drug Content (%)
1	C1	54.20± 0.15
2	**C2**	**98.10± 0.10**
3	**C3**	**79.30 ± 0.14**
4	C4	63.30± 0.16
5	C5	76.40± 0.14
6	C6	75.50± 0.13

G) *In vitro* drug release study

In vitro release studies of formulations were performed using the Franz diffusion cell with dialysis membrane. Phosphate buffer of pH 7.2 was used as diffusion media. The release of medicaments from the cream was linear. The graph between percentage drug release and square root of time showed almost linear relationship after the initial period. Initial slow release of the medicament from the cream may be due to the base i.e. hydrophilic &lipophilic thus the medicament partition between the two phases which does not allow the partitioning of the medicament.

Table 14: Percentage Drug Release of Herbal Cream

Sr. no.	Time (hr)	C1	C2	C3	C4	C5	C6
1	1 hr	1.20	**9.80**	**4.91**	2.35	4.91	3.31
2	2 hr	19.73	**19.73**	**22.73**	21.62	22.73	12.33
3	3 hr	25.18	**48.58**	**24.61**	28.25	24.61	34.41
4	4 hr	32.56	**52.76**	**39.32**	39.36	39.32	46.52
5	5 hr	35.61	**65.51**	**51.79**	41.70	51.79	50.29
6	6 hr	40.17	**80.67**	**64.35**	44.54	64.35	54.55
7	7 hr	46.36	**82.33**	**70.65**	50.56	67.65	57.85
8	8 hr	49.17	**89.71**	**72.32**	52.32	68.32	62.42
9	9 hr	51.65	**98.11**	**77.16**	55.36	70.68	65.36
10	10 hr	55.62	**98.20**	**77.31**	58.36	73.12	68.65

H) Stability study

The stability studies carried out on optimized formulation C2 at 30ºC temperature and 65% RH for 30 days, 90 days. The C2 formulation was showing good stability with no remarkable change in drug content, Spread ability, Extrudability and *in vitro* drug release profile as shown in table.

Table 16: Stability Study Data of C2 formulation.

Sr. No.	**Parameters**	**Storage period (Days) at 30±2ºC Temperature and 60±5% RH**		
		0	**30**	**90**
1	**Appearance**	Creamy white	Creamy white	Creamy white
2	**Drug content (%)**	98.10%	97.90%	97.66%
3	**Extrudability (%)**	97.16%	97.00%	97.10%
4	**Spread ability (mm)**	56.58	56.35	56.00
5	**%Drug release**	98.20%	97.47%	97.00%

V. Result and Discussion

Herbal cream has emerged in the last 50 years to improve complexion. Fairness is considered equal to attractiveness. Herbal cream blocks sun rays and prevent secretion of melanin, which gives dark color to skin. Herbal medicine is being used by about 80% of the world population primarily in the developing countries for primary health care.

Glycyrrhizaglabra, Calotropis Procera & Rubiacordifolia were selected for the preparation of herbal cream. Plant materials containing polyphenol organic substance which imparts potent antioxidant activity. The antioxidant substance scavenges the reactive oxygen species and inhibits the production of melanin in the human skin. Moreover, it also absorbs the UV rays and prevents the formation free radicals in the skin. Therefore, we tried to make an herbal fairness cream containing the extract of ***Glycyrrhizaglabra, CalotropisProcera&Rubia Cordifolia*** in different concentration along with almond oil. The prepared cream formulations were subjected to stability study as per ICH guidelines for the period of three month. ***Glycyrrhizaglabra,CalotropisProcera&RubiaCordifolia are*** well known for its medicinal and cosmeceuticals value in Indian traditional system of medicine. Present work, was based on extraction of these herbs and formulate herbal cosmetic cream. The tyrosinase inhibitors substances are used in cosmetic products as skin whitening agent to reduce skin pigmentation by decreasing the melanin production. The liquorice extracts a glabrene reported to exhibit antityrosinase activity, and also inhibited melanin production in cell culture. It has reported that glabrene is present in liquorice extract as bioactive compound. Our study indicated that the formulation C2 and C3 found to be more stable, while remaining formulations were not stable and resulted in breakdown of the emulsion when stored for long time. These formulations C2 and C3 had almost constant pH, homogeneous, emollient, non-greasy and easily removed after the application. The optimized formulation **C2** is the best formula which gives accurate result. The % drug content of herbal extract was found to be 97.66%. The pH was 6.5 color was white creamy semisolid cream, viscosity was 9800 cps, spread ability was 56.53mm and tube extrudability was 97.16. It was concluded that the herbal cream can be formulated by fusion method, for the treatment of Melasma disease which minimizes the black color of the patches. The findings of present investigation exhibited that the prepared herbal creams containing extracts of ***Glycyrrhizaglabra,CalotropisProcera&RubiaCordifolia*** were safe to use in skin. Our study also indicated that the formulation use highest concentration (30%) of extract found to be more stable, while remaining marketed formulations.

VI.CONCLUSION

This research work was carried out to develop a new topical Herbal cream formulation for topical application. The prepared Herbal cream was further evaluated for colour, odour, solubility, pH, Viscosity, spread ability, Extrudability, Drugs Content uniformity, In vitro drugs Release Study & stability study were determined. The optimized formulation C2 complies with all the parameters.

VII.ACKNOWLEDGMENT

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VIII. CONFLICT OF INTEREST

We, authors declare that we have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper

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