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FORMULATION AND CHARACTERISATION OF A POLYHERBAL FORMULATION FOR TREATMENT OF PHOTOSENSITIVE SKIN DISORDERS

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ABSTRACT

Photosensitive skin disorders refer to those diseases that are caused as a result of excessive exposure to solar radiations, especially ultraviolet radiations. Natural substances extracted from plants have recently been considered as potential sunscreen resources because of their ultraviolet ray absorption in the UVA region and their antioxidant activity. Medicinal plants, which have flavonoids and other phenolics as the most important components are potential candidates for formulating into topical sunscreen preparations .The present study involves the formulation of a polyherbal suncream with herbal active ingredients and its evaluation studies. The poly herbs selected for the study were the alcoholic extracts of: *Camellia sinensis L* (Tea) , *Vitis vinefera* (Grapes) and *Sylibum marianum* (Milk thistle) with different concentration of extracts and excipients. The prepared formulations were evaluated for pH, viscosity, spreadability, drug release, antioxidant activity and sun protection factor. The formulation F4 can be used as a good photo protective agent due to its high antioxidant activity and drug content, Also its sun protection factor is high, indicating that F4 can be a good candidate for preventing UV radiation induced photosensitive skin disorders.

KEYWORDS: Polyherbal, Sunscreen, Sun Protection Factor , *Camellia sinensis L*, *Vitis vinefera*, *Sylibum marianum.*

INTRODUCTION

Herbal medicine is widely practiced in worldwide. For centuries, people have turned to natural remedies to cure common ailments such as colds, allergy, upset stomachs and toothaches and the trend is constantly increasing. Thus, there has been a shift in universal trend from synthetic to herbal medicines.[1] The World Health Organization (WHO) reported that 4 billion people (80% of the world's population) use herbal medicines for some aspect of primary healthcare. Herbal medicine has been recognized by WHO as essential components for primary health care and about 11% of the drugs are derived from plants. Phytochemicals have been reduced the risk of many human diseases include cardiovascular disease, hepato-renal diseases, diabetes, cancers and neurodegenerative disorders. Herbal medicine is effective, lesser side effect, and affordable than the medicines bought from an allopathic medicine.[2] Herbal medicines include herbs, herbal materials, herbal preparations, and herbal products that contain different parts of plants or other plant materials as active ingredients. It has been well documented that herbal plants and their derivatives play critical roles in modern drug development.[3]

Drugs via skin reach the desired area in optimum concentration, dropping the chances of side effects leading to increased bioavailability and increased patient compliance.[4] Percutaneous absorption of drug molecules is a key factor of particular importance in the case of topical drug delivery systems because the drug has to be absorbed to an adequate extent and rate to achieve and maintain uniform, systemic, therapeutic levels throughout the duration of use. In general, once drug molecules cross the stratum corneal barrier, passage into deeper dermal layers and systemic uptake occurs relatively quickly and easily. Drugs with the lipophillic character are better suited for topical delivery. These systems ensure that the drug get into the body and reach the area where it is needed.[5]

Photosensitive skin disorders refer to those diseases that are caused as a result of excessive exposure to solar radiations, especially, ultraviolet radiations. Exposure to the sun is known to be associated with various skin cancers, accelerated skin aging and possibly has an adverse effect on a person's ability to resist infectious diseases. Some of the common photosensitive skin disorders include sunburn, tanning, solar keratosis and formation of freckles or brown spots on skin.[6] Due to these facts, sunscreens are now incorporated into

everyday products such as moisturizers, creams, lotions, shampoos, mousses, and other hair and skin preparations. Sunscreens are topical applied products that absorb or reflect some of the sun's ultra violet radiation and thus helps protect against UV induced photosensitive skin diseases. They are agents which absorbs 95% or more of UV radiation within wavelength 290- 320 nm.

A number of people with sensitive skin, such as those suffering from skin hypersensitivity don't want to use chemical sunscreens due to concern about skin exposure to unknown chemicals. Although a variety of hypoallergenic cosmetic products have been introduced for customers with sensitive skin, there are still limited options in sunscreen agents. Now, however, researchers have claimed that cosmetics having herbal components are more suitable for hyperallergic skin because they are less irritant and more easily adjustable to skin. Topical cosmetic formulations are the most preferred treatments asked by patients and are also often most prescribed by family physicians and dermatologists for sun burn. Herbal cosmetics must have one or more active sunscreening agent with antioxidant properties in order to achieve good photoprotection effect.[7]

Natural substances extracted from plants have recently been considered as potential sunscreen resources because of their ultraviolet ray absorption in the UVA region and their antioxidant activity. Medicinal plants, which have flavonoids and other phenolics as the most important components are potential candidates for formulating into topical sunscreen preparations.[8] Plants with photprotective properties include Green Tea (*Camellia sinensis*), Aloe Vera (*Aloe vera*), Myrobalan (*Terminalia chebulla*), Tomato (*Solanum lycopersicum*), Carrot (*Dacus carota*), Turmeric (*Curcuma longa*) and Grapes (*Vitis vinifera L*)

For the present study, a combination of three polyherbal have been selected. They are *Camellia sinensis L* (Tea) , *Vitis vinefera* (Grapes) and *Sylibum marianum* (Milk thistle).

MATERIALS AND METHODS

The crude drugs for the present research *Camelia sinensis* extract were procured from Synthite Industries Private Limited and *Vitis vinefera* extract and *Sylibum marianum* extract from Shreedha Phyto Extracts.

Chemicals and Materials: All the chemicals used for formulation and evaluation was obtained from Nice chemicals Pvt. Ltd.

Apparatus: UV spectrophotometer - Shimadzu UV 1700, IR spectrophotometer- Bruker, Stability chamber - Remi, India.

Formulation of a Polyherbal Cream[9]

For the preparation of a polyherbal cream ethanolic extracts of CS, GS and MT were incorporated in castor oil. The sunscreen cream was prepared in 3 steps.

Step 1: Water soluble components like glycerine, methyl paraben, triethanolamine was dissolved in distilled water and heated up to 75^0 C.

Step 2: Oil phase was prepared by incorporating medicated oil, stearic acid, cetyl alcohol and this was heated to 75^0 C.

Step 3: Oil phase was added in water phase at 75°c with continuous stirring for 15-20 min. and then it was homogenized till uniform emulsion is formed.

The finished product has orange colour and cream like consistency. It was then poured into the wide mouth container and stored at temperature not exceeding 37°C.

Table 1: Six different polyherbal formulations.

Ingredients	F1	F2	F3	F4	F5	F6
CS	0.2g	0.1g	0.1g	0.1g	0.1g	0.1g
GS	0.1 g	0.1g	0.2g	0.2g	0.2g	0.1g
MT	0.1g	0.2g	0.1g	0.1g	0.1g	0.2g
Cetyl alcohol	0.2g	0.4g	0.6g	0.8g	1g	1.2g
Stearic acid	0.4g	0.8g	1.2g	1.6g	2g	2.4g
Glycerine	5 ml	5 ml	5ml	5ml	5ml	5ml
Methyl paraben	01.g	0.1g	0.1g	0.1g	0.1g	0.1g
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s
Castor oil	2ml	2ml	2ml	2ml	2ml	2ml
Water	q.s	q.s	q.s	q.s	q.s	q.s

Evaluation Studies of prepared polyherbal cream

Organoleptic Properties[17]

The prepared formulation was inspected visually for their color, homogeneity, grittiness and appearance.

Determination of pH[10]

The pH of the cream cannot to be directly measured. So, about 0.5 g of cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured with a pH meter .The pH meter was calibrated using standard buffer solution The electrode must be washed and free from any residue of acid and alkali to ensure the accurate reading.

Viscosity Determination[11]

The viscosity of the formulation was determined by Brookfield Viscometer. The viscosity measurements were done using Brookfield DV-II viscometer. The developed formulation was poured into the adaptor of the viscometer and spindle number 64 was used and

optimum operating conditions were set up. Then the viscosity was measured directly at 6 rpm speed by keeping the torque constant. Viscosity is determined in Centipoises using the equation.
Viscosity = Dial Reading × Factor for DV II at 6 RPM Factor is 1M (1000)

Spreadability Determination[11]
For the determination of spreadability, excess of sample was applied in between two glass slides and then was compressed to uniform thickness. The weight was added toa pan. Excess formulation was placed between 2 glass slides and 100g weight was placed on the upper glass slide for 10 minutes to compress the formulation to uniform thickness. Excess of cream was scrapped off the edges.100g weight was added to the pan .The time in seconds to separate the two slides was taken as a measure of spread ability. Spread ability is determined by the equation.
S=M*L/T
where,
S-Spreadablity
L-length of glass slide
M-weight tied to upper slideT-time
(Here M= 60 gm and L= 7.5 cm)

Drug Content Determination[22]
a) Preparation of Standard solution
100 mg of each extracts were dissolved in 100 ml of water (Stock solution 1).From this stock solution 1ml was taken and diluted to 10 ml [stock solution - 2]. From stocksolution - 2 take volume 1,2,3,4 and 5ml separately and made up to 10ml to get concentration range 10, 15, 20, 25 and 30µg/ml respectively. The absorbance was measured using ethanol as blank.

b) Drug Content Measurement
5g of cream was taken and dissolved in 20 ml of ethanol. The solution was transferredto a 100ml volumetric flask and made upto 100ml with ethanol. The solution was filtered using Whatmann filter paper no.41 .Transferred 1ml of the solution from this to 25ml volumetric flask and makes the volume with the water and takes the absorbance using a Shimadzu UV Spectrophotometer

***Invitro* Drug Release Study[12]**
Dialysis membrane diffusion technique was used to study in-vitro diffusion of drug from the prepared polyherbal formulations. The receptor medium used was freshly prepared phosphate buffer pH 7.4. Synethic cellulose membrane previously soaked overnight in the receptor medium was placed on the diffusion cell assembly. 1 g of formulation was placed in the donor compartment and the assembly was kept on the diffusion study apparatus at $37^oC \pm 2^oC$ and stirred at 700 rpm. Aliquots of 0.5 ml were withdrawn at pre-determined time intervals 0,1,2,3,4,5,6 and 8 hours.) andimmediately replaced by same volume of the fresh buffer solution. The aliquots were suitably diluted with the dissolution medium and analyzed by UV Spectrophotometer. The data obtained from the in vitro diffusion studies were fitted to various kinetic equations to find out the mechanism of herbal drug release from the polyherbal cream.

Release Kinetics[20]
To study the release kinetics of *in-vitro* drug release, data was applied tokinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas.
- Zero order

$Q = K_0 t$
where,
Q is the amount of drug release at time, t in hrs
K_O is the zero-order release rate constant (con/Time)
- First order

$\log Q = K_1 t$
where,
Q is the percent of drug release at time, t
K_1 is the first order release rate constant
- Higuchi

$Q = K_2 t^{1/2}$
where,
Q is the percentage of drug release at time t
K_2 is the Higuchi diffusion rate constant
- Korsemeyer Peppas

$Q = Kt^n$
where,
Q is the percent of drug release at time t
K is the diffusion rate constant
n is diffusion exponent

Total Poly Phenolic Content Determination[13]
The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin- Denis reagent or Gallic Acid Equivalence method (GAE) uses a mixture of phosphomolyb date and phospho tungstate for the colorimetric assay of phenolic and polyphenolic antioxidants. It works by measuring the amount of the substance needed to inhibit the oxidation of the reagent. Prepared calibration curve of standard Gallic acid (0.10-5 mg/ml in water) and to this added 1 milligram/ml of extract solutions. Mixed 1 ml of each sample with 1ml of Folin Ciocalteu reagent and 10 ml of 7% sodium carbonate solution. The mixture was allowed to react for 40 min at room temperature. After the reaction period, mixed the contents and measured the blue colour at 725 nm in comparison with standards. Calculated the amount of total phenols from calibration curve as a Gallic acid equivalent by the following formula.
T=C*V/M
where,
T: is total content of phenolic compounds, (mg/g of plant extract)C: The concentration of Gallic acid (mg/ml)
V: the volume of extract (ml)
M: gram weight of plant extracts (g)

Antioxidant activity[14]
Antiradical activity was measured by a decrease in absorbance at 517 nm of a solution of colored DPPH in methanol brought about by the sample. A stock solution

of DPPH (1.3 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml methanol gave an initial absorbance of 0.9. Decrease in the absorbance in the presence of sample extract and standard at different concentrations was noted after 30 minutes. EC50 was calculated from% inhibition. A blank reading was taken using methanol instead of sample extract. Absorbance at 517 nm is determined after 30 min. using UV-Visible Spectrometer (Systronic double beam- UV-2201), and IC50 (Inhibitory concentration to scavenge 50% free radicals) is also determined. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. IC50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The capability to scavenge the DPPH radical was calculated using the following equation.

% inhibition = C-T/C X 100

where,

C = Absorbance of DPPH alone

T= Absorbance of DPPH along with different concentrations of extract

IC_{50} was calculated from equation of line obtained by plotting a graph of concentration versus percentage inhibition.

Determination of SPF by UV Spectrophotometer[15]

1 gm quantity of formulated cream was weighed, transferred to 100 ml volumetric flask and diluted to volume with ethanol. Discarded the initial 10 ml. Afterwards 5 ml of solution was transferred to 25 ml volumetric flask and the volume was adjusted with ethanol. The absorption spectra of samples in solution were taken in the range of 290-450 nm using ethanol as blank. The absorption data obtained in the range of 290-320 nm every 5 nm interval. SPF of formulated cream was calculated by the application of equation.

$SPF = CF^{290\text{-}320}$ EE (λ)*I (λ)*Abs (λ)

Where,

C: Correction factor

E: Erythmogenic effect of light with wavelength

I: Solar intensity Spectrum

Abs: Spectrometric absorbance values at 290-320 nm

The value of EE*I is a constant. The aliquot prepared were scanned between 290-320 nm and the obtained absorbance values were multiplied with the respective EE (λ) and I (λ) values. Then, their summation was taken and multiplied with the correctionfactor.

Stability studies[16]

The stability of the polyherbal cream was evaluated by accelerated stability studies. It was carried out according to ICH guidelines by storing the samples at 25±2°C with 60 ±5% RH and 40 ±2°C with 75 ±5% RH for 3 months using Stability chamber (Remi, India). The samples are collected at 0, 1, 2, 3 months. It is then evaluated at 0, 1, 2, 3 months for drug content as well as any changes in their physical appearance, chemical stability during the storage period (after 3 months) was checked.

RESULTS AND DISCUSSION

Organoleptic Properties

Table No 2: Organoleptic Properties of Formulations.

Formulation	Colour	Phase separation	Grittiness	Homogeneity
F1	Yellowish brown	None	None	Good
F2	Yellowish brown	None	None	Good
F3	Creamy orange	None	None	Good
F4	Creamy orange	None	None	Good
F5	Light brown	None	None	Good
F6	Light brown	None	None	Good

Formulation F1 and F2 have yellowish brown colour. F3 and F4 have creamy orange colour, F5 and F6 have light brown colour.No formulations showed any phase separation or grittiness. Particles were distributed homogenously in all formulations without any settling of particles.

Determination of the pH

Table 3: pH of Formulations.

Formulation	pH
F1	6.06
F2	5.98
F3	5.98
F4	5.81
F5	6.04
F6	6.10

pH values of formulations were found in range of 5.81-6.10.So these formulations could not produce any local irritation to the skin.

Determination of Viscosity

Table 4: Viscosity of Formulations.

Formulation	Viscosity (Cps)
F1	25678
F2	27767
F3	28987
F4	30321
F5	33654
F6	36553

Viscosity of the formulation depends on polymer concentration. Viscosities of the formulation are given in the table below. They were found in the range of 25678-

36553.Changes in the concentration of stearic acid and cetyl alcohol affect viscosity.

Spread ability Determination

Table 5: Spreadability of Formulations.

Formulation	Spread ability (gcm/sec)
F1	18.38
F2	18.20
F3	20.23
F4	20.89
F5	18.77
F6	19.58

The value of spread ability indicates that the cream is easily spreadable by small amount of shear. Spread ability of the formulation were in the range of 18.20-20.89.A short interval indicates better spreadability.

Drug Content Determination.

Table 6: Drug Content of CS.

Formulation	Drug content (%w/w)
F1	70.87
F2	75.09
F3	78.25
F4	82.97
F5	76.67
F6	79.82

Table 7: Drug Content of GS.

Formulation	Drug content (%w/w)
F1	77.86
F2	81.31
F3	85.27
F4	86.44
F5	82.94
F6	81.08

Table 8: Drug Content of MT.

Formulation	Drug content (%w/w)
F1	75.96
F2	77.53
F3	79.91
F4	80.78
F5	78.60
F6	77.07

From the data obtained it was found that formulation F4 contains maximum amount of drugs than all other formulations. It was found that F4 formulation contains 82.97%w/w of CS, 86.44 %w/w of GS and 80.78 % w/w of MT respectively, which makes it a very good photoprotective agent.

***Invitro* Drug Release Study**

Table 9: *Invitro* Drug release of CS.

Time(hrs)	Percentage drug release (%w/w)					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	18.76	16.43	22.56	24.89	20.78	21.55
2	25.89	23.67	26.54	29.65	24.55	25.87
3	30.56	28.65	33.43	35.87	29.78	31.87
4	36.89	35.87	41.65	42.66	36.87	38.30
5	44.54	42.31	47.89	50.66	44.12	45.97
6	50.65	49.34	54.89	56.87	51.23	52.87
8	66.47	63.56	71.34	75.77	68.76	63.78

Table 10: *Invitro* Drug release of GS.

Time (hrs)	Percentage drug release (%w/w)					
0	0	0	0	0	0	0
1	21.55	25.76	28.98	29.54	24.67	23.32
2	26.67	29.34	35.44	38.90	29.74	25.65
3	31.22	33.87	41.76	44.60	38.76	37.33
4	38.44	35.67	46.89	50.44	42.19	44.87
5	45.89	42.67	55.71	59.64	48.23	48.56
6	52.55	48.74	62.67	66.71	55.87	56.02
8	64.65	62.32	74.55	78.12	68.09	66.11

Table 11: *Invitro* Drug release of MT.

Time(hrs)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	16.44	20.31	23.12	25.06	23.44	24.07
2	23.56	24.07	27.56	29.41	26.21	29.25
3	26.87	28.66	34.22	36.40	32.07	34.11
4	32.43	35.21	41.88	44.72	38.98	39.85
5	39.07	40.39	49.41	52.54	45.12	44.41
6	47.87	48.26	56.71	60.69	48.05	49.02
8	61.32	62.11	71.73	74.71	63.16	61.05

Figure 1-3 shows graphical representation of *in vitro* drug release data.

In vitro drug release studies show that maximum amount of drug release was shown by formulation F4.The percentage w/w of drug release for CS, GS and MT from formulation F4 was 75.77, 78.12, and 74.71 respectively. Maximum drug release occurred after 8 hours.

From the data obtained from *in vitro* release studies it was found that the polyherbal formulation F4 showed maximum drug release after 8 hours. So the release kinetics studies were performed for F4 formulation. The release kinetics for formulation F4 was plotted into different models like zero order, first order, Higuchi model and Kosmayer –Peppas plot

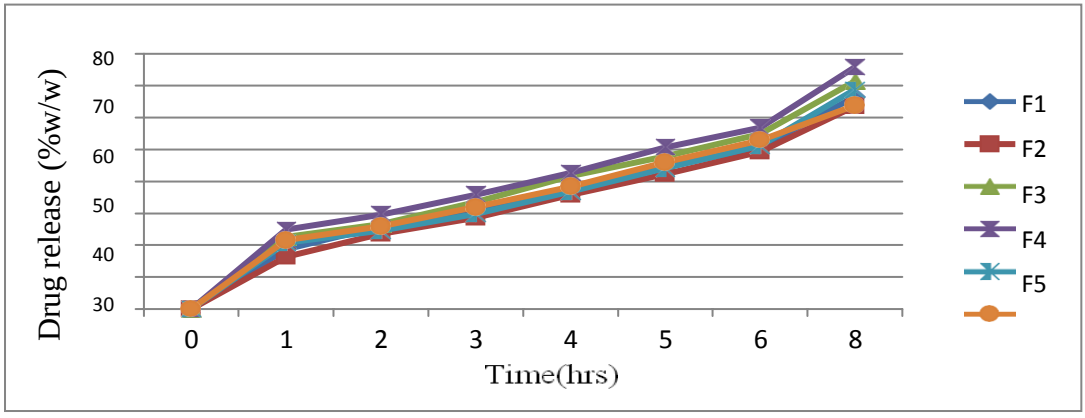

Figure 1 : *Invitro* drug release of CS.

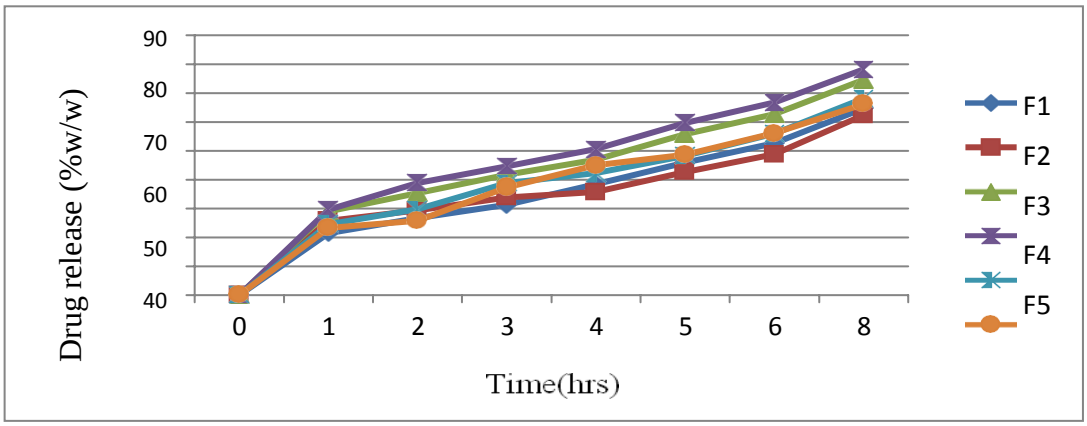

Figure 2: *Invitro* drug release of GS.

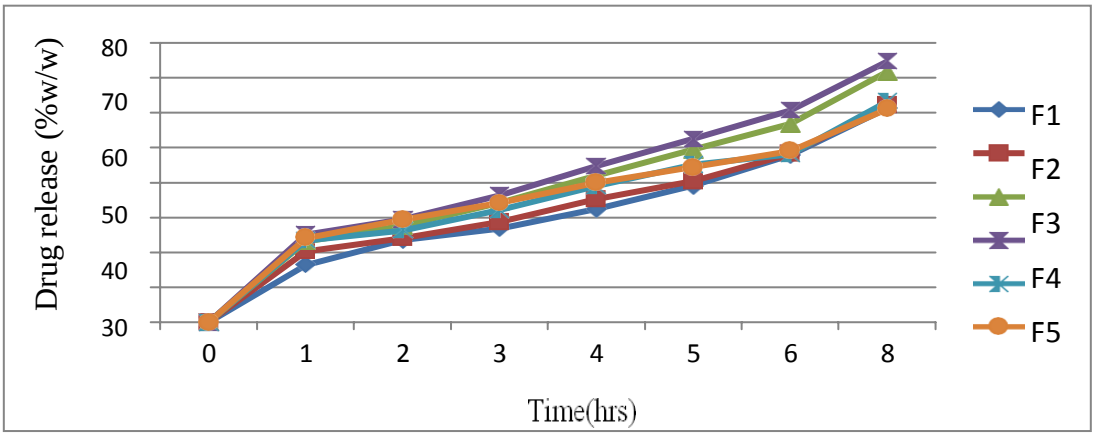

Figure 3: *Invitro* drug release of MT.

Release Kinetics of F4 formulation

Release Kinetics for F4 containing Camelia sinensis extract data is given in the table below. From the data graphs for first order, zero order, Higguchi and Kosmayer- Peppas were obtained.

Table 12: Release Kinetics of CS in F4.

Time (hrs)	%CDR	Log % CDR	%DRTR	Log % DRTR	Log time	√t
1	24.89	1.39	75.11	1.87	0	0
2	29.65	1.47	70.35	1.84	0.30	1.41
3	35.87	1.55	64.13	1.80	0.47	1.73
4	42.66	1.63	57.34	1.75	0.60	2
5	50.66	1.70	49.34	1.69	0.69	2.23
6	56.87	1.75	43.13	1.63	0.77	2.44
8	75.77	1.87	24.23	1.38	0.90	2.82

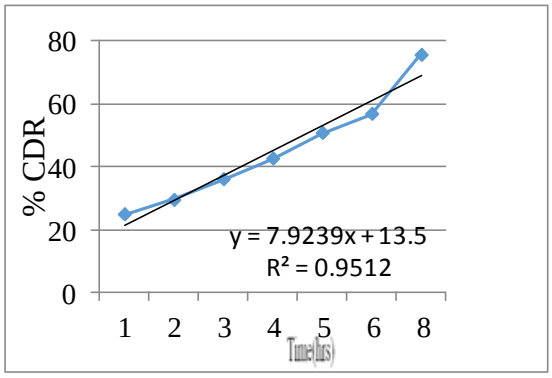

Figure 4: Zero order plot for CS in F4.

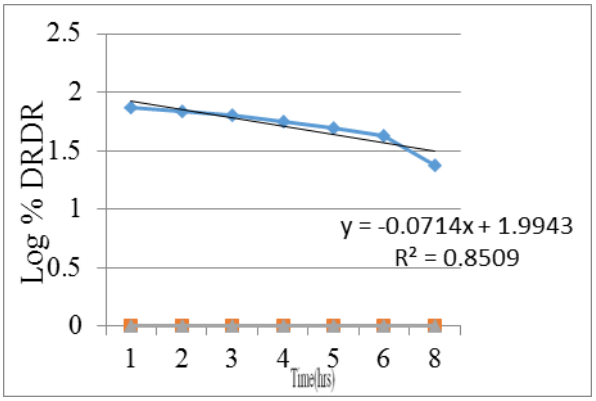

Figure 5: First order plot for CS in F4.

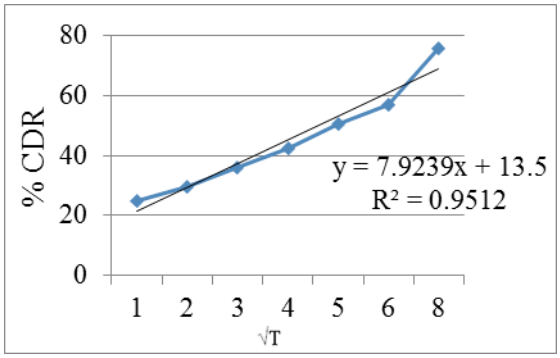

Figure 6: Higguchi plot for CS in F4.

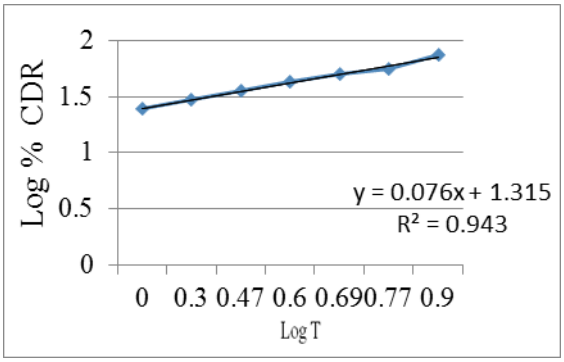

Figure 7: Kosmayer-peppas for CS in F4.

Release Kinetics for F4 containing Vitis vinefera extract data is given in the tablebelow. From the data graphs for first order, zero order, Higguchi and Kosmayer- Peppas were obtained.

Table 13: Release Kinetics of GS in F4.

Time (hrs)	%CDR	Log % CDR	%DRTR	Log %DRTR	Log time	√t
1	29.54	1.47	70.46	1.84	0	0
2	38.90	1.58	61.1	1.78	0.30	1.41
3	44.60	1.64	55.40	1.74	0.47	1.73
4	50.44	1.70	49.56	1.69	0.60	2
5	59.64	1.77	40.36	1.60	0.69	2.23
6	66.71	1.82	33.29	1.52	0.77	2.44
8	78.12	1.89	21.88	1.33	0.90	2.82

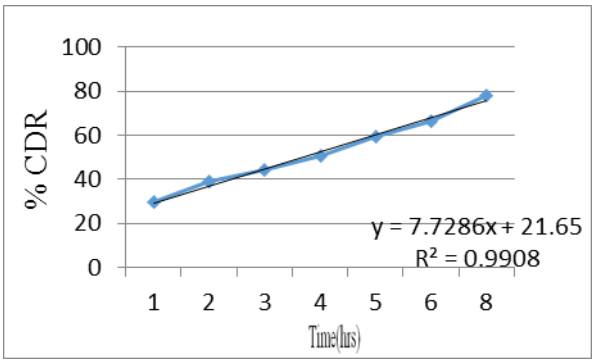

Figure 8: Zero order plot for GS in F4.

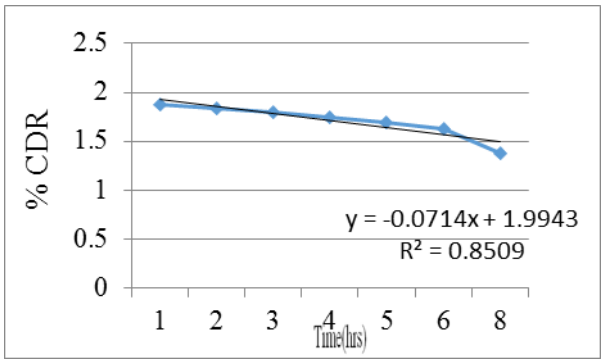

Figure 9: First order plot for GS in F4.

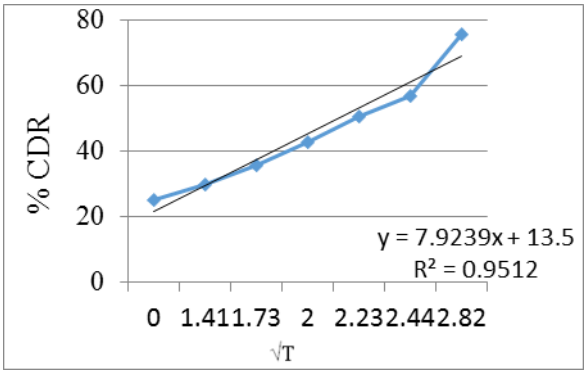

Figure 10:- Higguchi plot for GS in F4.

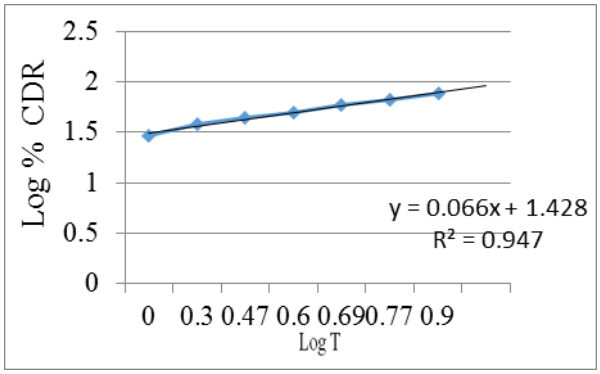

Figure 11: Kosmayer Peppas plot for GS in F.

Release Kinetics for F4 containing Sylibum marianum extract data is given in the table below. From the data graphs for first order, zero order, Higguchiand Kosmayer-Peppas were obtained.

Table 14: Release Kinetics of MT in F4.

Time (hrs)	%CDR	Log % CDR	%DRTR	Log % DRTR	Log T	√t
1	25.06	1.39	74.94	1.87	0	0
2	29.41	1.46	70.59	1.84	0.30	1.41
3	36.40	1.56	63.60	1.80	0.47	1.73
4	44.72	1.65	55.28	1.74	0.60	2
5	52.54	1.72	47.46	1.67	0.69	2.23
6	60.69	1.78	39.31	1.59	0.77	2.44
8	74.71	1.87	25.29	1.40	0.90	2.82

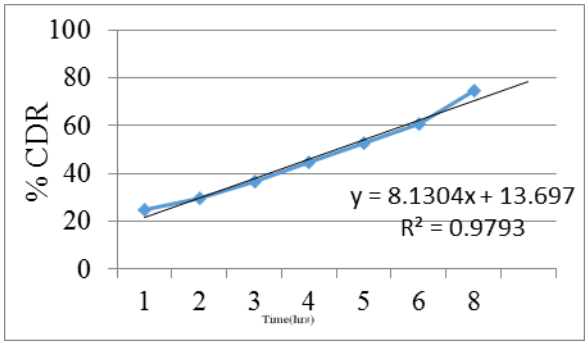

Figure 12: Zero order plot for MT in F4.

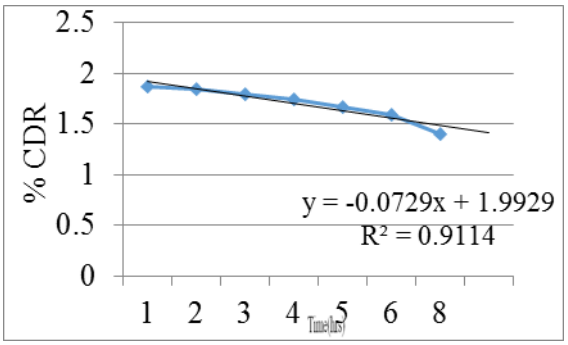

Figure 13 : First order plot for MT in F4.

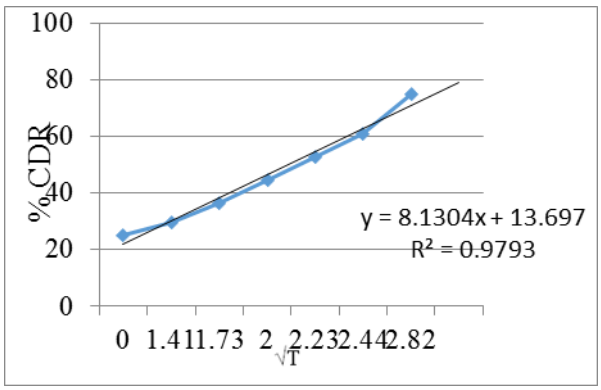

Figure 15: Higguchi plot for MT in F4.

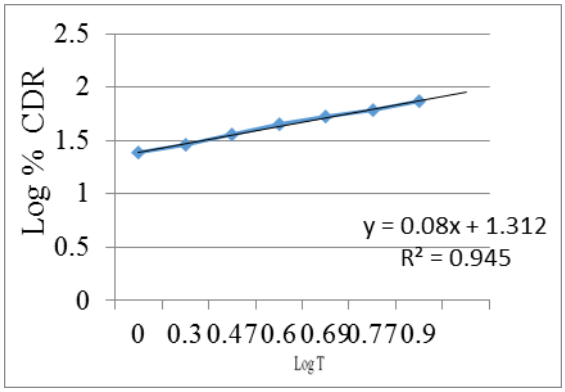

Figure 16: Kosmayer Peppas plot for MT in F4.

Table 15: Regression Coefficient Values (R^2) For Different Kinetic Models forFormulation F4.

Extracts	Kinetics of Drugrelease		Mechanism of Drugrelease		
	Zeroorder	Firstorder	HiguchiModel	Peppasmodel	
				R^2	n
CS	0.951	0.850	0.951	0.943	0.076
GS	0.990	0.850	0.951	0.947	0.066
MT	0.979	0.911	0.979	0.945	0.08

Release kinetics studies of formulation F4 follows zero order release kinetics. From the data obtained it was found that value of r^2 for Higguchi model is greater compared to Kosmayer Peppas .So the release of the formulation is best fit with Higguchi model. Since Higguchi equation explains the diffusion release mechanism, it can be said that formulation follows diffusion mechanism of drug release. Thus formulation F4 was found to have higher drug release and better release mechanism Estimation of total Polyphenolic Content of F4.

Table 16: Standard plot for Gallic acid.

Concentrationof gallic acid (µg/ml)	Volumeof sample (ml)	Volumeof Distilled water(ml)	Volume of Ciocalttue Reagent(ml)	Volumeof 7% Na2CO3	Absorbance
Blank	-	10	1	10	0.000
100	1	9	1	10	0.0312
200	1	9	1	10	0.0682
300	1	9	1	10	0.1011
400	1	9	1	10	0.1286
500	1	9	1	10	0.1532
600	1	9	1	10	0.1828
700	1	9	1	10	0.2180
1000	1	9	1	10	0.3210
2000	1	9	1	10	0.603
3000	1	9	1	10	0.998
4000	1	9	1	10	1.524
5000	1	9	1	10	2.325

Table 17 : Standard plot data for formulations F3 and F4

Concentrationof sample (mg)		Volumeof sample (ml)	Volumeof distilledwater (ml)	Volume of Ciocalttue Reagent(ml)	Volumeof 7% Na_2CO_3	Absorbance
F3	10	1	9	1	10	0.750
F4	10	1	9	1	10	1.012

The calibration curve shows that it gives absorption of 0.75 which corresponds to 2.30 mg of gallic acid. The concentration of sample applied was 10 mg. So 10 mg of the sample gives an absorption equivalent to 2.30 mg of gallic acid. So the quantity of phenolic compounds present in the sample can be expressed as the % mg Eq of Gallic acid. So the quantity of Phenolics present in the sample F3 is 23.50 % mg Eq of Gallic acid which was calculated using the equation. Fig 17 shows the calibration curve of gallic acid for F3.

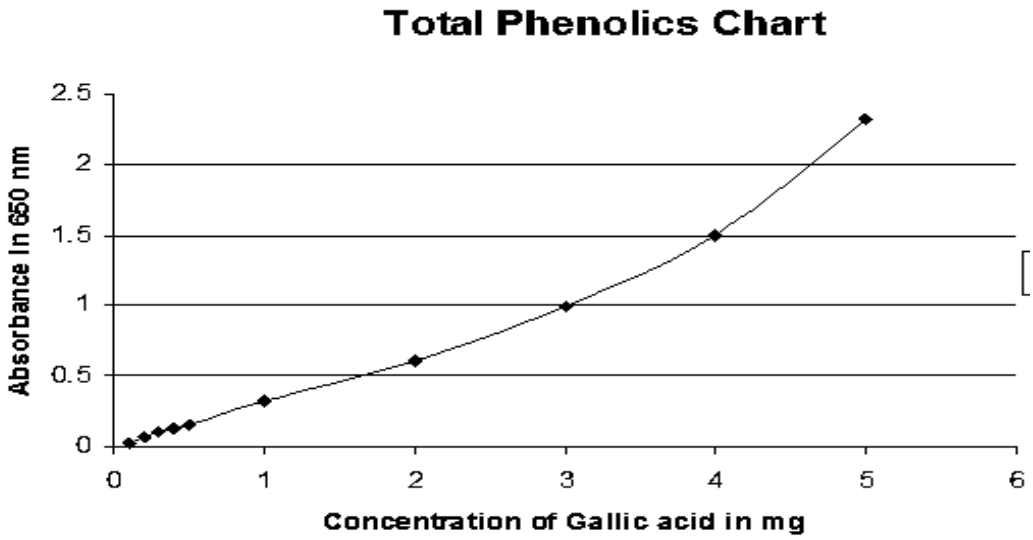

Figure No. 17: Calibration curve for F3.

Similarly, the calibration curve shows that it gives absorption of 1.012 which corresponds to 3 mg of gallic acid. The concentration of sample applied was 10 mg. So 10 mg of the sample gives an absorption equivalent to 3 mg of gallic acid. So the quantity of phenolic compounds present in the sample can be expressed as the % mg Eq of Gallic acid. So the quantity of phenolics present in the sample is 30.0 % mg Eq of Gallic acid which was calculated using the equation. This indicates the presence of higher amount of polyphenolic content in F4, which indicates its higher antioxidant potential.

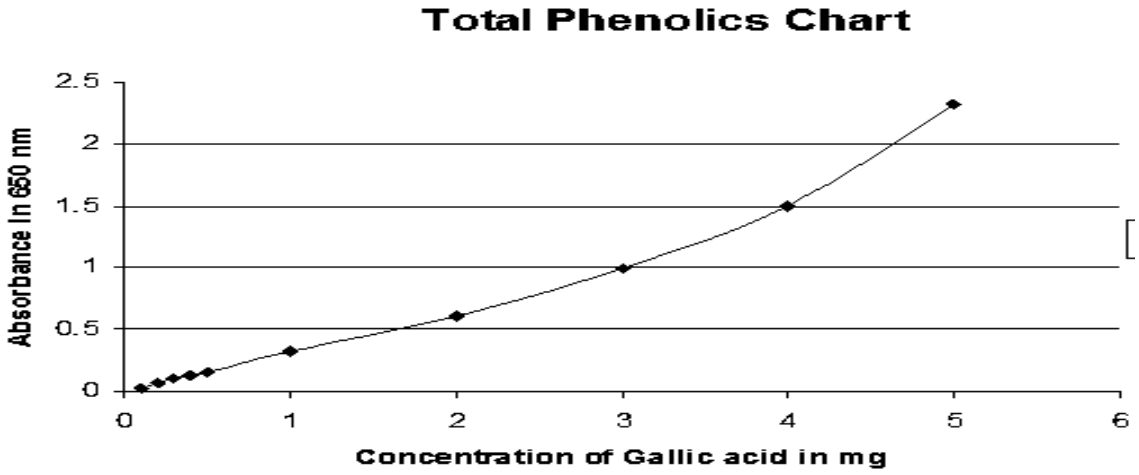

Figure 18: Calibration curve for F4.

In vitro antioxidant activity

Table 18: Free radical scavenging ability of F4.

No	Concentration (µg/ml)	DPPH Assay	
		OD	% inhibition
1	Control(DPPH alone)	1.065	0
2	100	0.983	49.97
3	200	0.781	60.25
4	300	0.619	68.49
5	400	0.598	69.56

The combined polyphenolic phyto chemicals incorporation in formulation F-IV shows synergistic antioxidant activity. As the optimized cream F-IV shows excellent antioxidant activity thus it can be efficient as photo protective. IC50 value denotes concentration of sample required to scavenge 50% of the DPPH free radicals. It can be obtained from equation of a line obtained by plotting a graph of concentration versus % inhibition. The IC50 value for F4 was found to be higher than F3.Thus F4 has higher antioxidant potential. Thus it can be used as a good photoprotective agent.

Determination of SPF

Table 19: Invitro determination of SPF of F4.

Wavelength (nm)	EE(λ)*I(λ)	EE(λ)*I(λ)* Abs(λ)
290	0.0150	0.0434
295	0.0817	0.221
300	0.2874	0.728
305	0.3278	0.735
310	0.1864	0.569
315	0.0837	0.196
320	0.0180	0.046

By summing the EE (λ)*I (λ)* Abs (λ) and by multiplying it with correction factor (which was 10), we can get the SPF. The SPF of formulation F4 was found to be 25.384 which was equivalent to the claimed SPF of synthetic formulations which contain avabenzone, which is the active constituent in synthetic sunscreen formulation. In addition the polyherbal formulation has good free radical scavenging property, so it can be used to reduce the risk of skin cancer. So, the polyherbal formulation is a having good photoprotective effect.

Stability Studies

Table 20: Stability studies.

Temperature	Evaluation parameters	Observation(months)			
		0	1	2	3
40± 20C RH = 75%	Physical appearance	Creamy o range	No colour change	Slight colour change	Slight colour ch ange
	Drug content (%w/w)				
	CS	82.97	81.06	80.44	78.97
	GS	86.44	85.62	83.77	80.59
	MT	80.78	78.21	76.43	74.77
	pH	5.81	5.75	5.69	5.64
	Viscosity(cps)	30321	30118	30004	29912

Results of the stability study show slight color change in the appearance and change in drug content of polyherbal cream after exposing to stability condition. Similarly, a decrease in pH and viscosity to a smaller extent were observed. However, since there is no considerable reduction in drug content, pH and viscosity, the formulation can be said stable on storage under accelerated conditions.

CONCLUSION

Studies were performed on formulation and characterisation of a polyherbal cream for the treatment of photo sensitive skin disorders. Conducted various preformulation studies. FTIR studies showed that there is no incompatibility between drug and excipients. Using UV spectrophotometer, the λmax of the extracts was determined. The λmax of CS, GS and MT were 273nm, 327nm and 284 nm respectively. The polyherbal formulation was prepared by incorporating herbal

extracts in castor oil. Stearic acid was used as an emulsifying agent along with cetyl alcohol. Methyl paraben was used as preservative, triethanolamine as buffering agent. Six formulations were prepared and evaluation studies were conducted.

The formulations had a creamy orange colour and showed no grittiness. All formulations were homogenous and did not show any phase separation. pH values of formulations were found in range of 5.81-6.10. So these formulations could not produce any local irritation to the skin. Viscosity of the formulations was in the range of 25678-36553. Spread ability of the formulation was in the range of 18.20-20.89, which indicate good spreadability. Determination of drug content in the formulations showed that F4 contain maximum amount of drug. The amount of CS, GS and MT present in F4 was 82.97 %w/w, 86.44 %w/w and 80.78 % w/w. In vitro drug release studies show that maximum amount of drug release was shown by formulation F4.The percentage w/w of drug release for CS, GS and MT from formulation F4 was 75.77, 78.12, and 74.71 respectively. Maximum drug release occurred after 8 hours. Release kinetics of the formulation showed that F4 follow zero order kinetics.

The release of formulation is best fit with Higguchi model and so the formulation follows diffusion mechanism for drug release. The total poly phenolic content in formulation was determined and it was found F4 contain 30 % mg equivalent of Gallic acid, which indicate for its higher antioxidant property. Determination of invitro antioxidant activity showed that IC50 values were highest for F4 which shows that it has good antioxidant potential. The combined polyphenolic phyto chemicals incorporation in formulation F-IV shows synergistic antioxidant activity. As the optimized cream F-IV shows excellent antioxidant activity thus it can be efficient as photo protective. The invitro SPF of formulation was determined using UV- Visible spectrophotometer. The SPF of formulation F4 was found to be 25.384. In addition to this the polyherbal formulation has good free radical scavenging property, so it can be used to reduce the risk of skin cancer. So, the polyherbal formulation is having good photoprotective effect. During accelerated stability studies conducted according to ICH guidelines, the formulation only showed smaller changes in their properties. Hence the formulation is stable on storage. Based on invitro drug release data, antioxidant activity, invitro SPF determination, and stability studies F4 can be used as a good photo protective agent.

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