



Research Article

FORMULATION AND EVALUATION OF SEED OILS FOR THEIR ANTIOXIDANT ACTIVITY AND SUN SCREENING EFFECT

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ABSTRACT

The present work was designed to formulate and evaluate topical formulation for its antioxidant properties using various fruit seed oils reported for their antioxidant effect. Products containing natural antioxidants are used for combating the deleterious effects of ultraviolet radiations thus producing photo protective effect and have the ability to heal and repair the skin giving a smooth texture and glow to the facial skin. Pomegranate seed oil (PSO), Carrot seed oil (CSO) and almond oil are widely known for its benefits in prevention of disease due to the bioactive constituents it contains such as polyphenols, flavonoids, polyunsaturated fatty acids. These seed oils are reported for their antioxidant properties, sun protecting, anti-aging and skin rejuvenating properties. In the present work these seed oils were analyzed for their poly unsaturated fatty acid content (Linoleic acid and oleic acid) using GC-MS and HPTLC. The formulation were prepared using blend of the three oil namely PSO,CSO and almond oil (3% and 5%)with carbopol, stearic acid, cetyl alcohol as emulsifying and thickening agents. Further, in-vitro antioxidant activity was carried out by using reducing power assay and DPPH assay for the oils and formulation. The sun screening effect of the seed oils and formulation was studied by using Mansur equation. The physicochemical parameters of the formulations (F1 to F4) were studied and subjected for stability studies for three months at $30 \pm 2^\circ \text{C}$ and $65\% \pm 5\%$ RH. Based on the physicochemical parameters, stability and the antioxidant activity, F4 formulation containing 10 % of seed oils can be considered as best for its use in skin care.

Key words: Seed oils, GC-MS, HPTLC, Antioxidant activity, Sun Protecting Factor

INTRODUCTION

Cosmetic formulations available for skin help in restoring youthful complexion, whitening the color of skin, prevent moisture loss and generate a protective layer on the skin. The skin is the largest organ which acts as a primary external barrier to protect the body from external environment¹. Thus, it protects the body from unfavourable stimuli, such as microorganisms, photo radiation, allergens and irritants. Ultraviolet light and environmental pollutants are known initiators of free radicals². The utilization of antioxidant in topical formulation helps in neutralization of free radicals, reactive superoxide molecules that break down skin cells and cause wrinkles leading to damage at the cellular level. Antioxidants are said to reduce the effect of dust, smoke, and pollution. Literature reports suggest the application of antioxidant cream to the skin which can slow down the aging process. Now a day; cosmetic products developed incorporate cleansing and moisturizing agents along with antioxidants which can protect the skin from oxidative stress³.

Many plant parts are used in herbal formulations as additives or are used due to their functional properties such as emollient, anti-irritant, antioxidant, moisturizing, anti-aging. Plants are affluent source of free radical scavenging molecules namely vitamins, terpenoids, phenolic compounds, lignin's, tannins⁴. The use of natural antioxidants in cosmetic products enables the nourishment of the skin in the same way as consumed orally for nourishing our body through fruits and vegetables. Oils from fruits and vegetables containing triglycerides of higher saturated and

unsaturated fatty acids⁵. Serve as a cosmetic base. The unsaturated fatty acids such as omegaseries acids like ω -9 (oleic), ω -6 (linoleic) and ω -3 (α -linolenic) reduce the trans-epidermal water loss and can improve the skin moistening, activate regeneration of damaged lipid barrier of the epidermis, heal inflammations and stabilize the skin metabolism⁶.

The PSO is characterized by a high content of polyunsaturated (n-3) fatty acids such as linolenic, linoleic, and other lipids such as punicic acid, oleic acid, stearic acid, and palmitic acid. Besides it also contains protein, crude fibers, vitamins, minerals, pectin, sugars, polyphenols, iso-flavones and is reported to stimulate keratinocyte proliferation in monolayer culture thereby regenerate the epidermal layer⁷. Almond oil is an extremely popular oil for its rich content of oleic and linoleic acid along with stearic and palmitic acid. Therefore, almond oil has been used as a lipophilic vehicle to reduce UV catalyzed degradation of retinyl palmitate to the less active *cis*-isomers⁸. It is also reported for its extensive use in traditional medicine to maintain the elasticity of the skin and its youthful appearance⁹. Carrot (*Daucus carota*) seed oil is known for its antioxidant effect. When applied topically to the skin provides natural sun protection¹⁰. Carotenoids present in Carrot seed oil have been found to enhance the body's immune response to UV rays, which can decrease skin damage from UV exposure. Based on the reported activities of the seed oils, cream formulation was prepared as oil in water emulsion taking cetyl alcohol, stearic acid and carbopol 934 as emulsifier and stabilizing agents¹¹.

MATERIAL AND METHOD

Chemicals

Pomegranate seed oil, Carrot seed oil, Almond oil and Rose oil procured from Allin exports. Excipients were procured from Loba Chem and Research Lab. All chemicals and solvents used were of analytical grade.

Equipment

Mechanical stirrer, pH meter equipped with magnetic stirrer (Make: Equiptronic, EQ-614A) for pH measurement, Viscosity was recorded using Brookfield RVDV-II + Pro with small sample volume adaptor spindle (S95), HPTLC (Camag), GCMS (Shimadzu, Model: TQ8030).

Characterization of Seed Oils

Physicochemical parameters of Seed oil¹²

Following physicochemical parameters of Seed Oils (Pomegranate seed oil, Carrot seed oil, Almond oil) were analyzed like Appearance, color, odour, texture, Refractive index, Density, Specific gravity, Acid value, Saponification value, Iodine value as per procedure mentioned in I.P.

GCMS of oil samples

For the GC-MS study, seed oil samples were esterified using the procedure described by Christie W.W¹³. Oleic acid and Linoleic acid (25 mg) and seed oil samples (100mg) were dissolved in Toluene (5 mL) in round bottom flask fitted with condenser, and 1% Sulphuric acid in methanol (10 mL) was added. Mixture was refluxed for 2 hours. Water (25 mL) containing sodium chloride (5%) was added and the required esters were extracted with hexane (2 × 25 mL), using separating funnel to separate the layers. The hexane layer was washed with water (20 mL) containing potassium bicarbonate (2%) and dried over anhydrous sodium sulphate. The solution was filtered and the solvent was removed under vacuum. The esterified samples were further sent to Central Instrumentation Facility, Savitribai Phule Pune University Pune for GCMS study.

Estimation of Linoleic acid in oil by HPTLC

Preparation of standard solution¹⁴

About 1 mg of standard sample of Linoleic acid was taken in 1 ml Ependorff tube 1.0 ml of methanol was added to it and mixed in vortex mixture till the material got completely dissolved (1000ng/ μ l).

Preparation of sample solution

About 10 mg of PSO and CSO was dissolved in 10 ml methanol in a volumetric flask and further 1ml of the oil sample stock solution was diluted to 10ml (100ng/ μ l) It was then mixed in vortex mixture and subjected to ultrasonication till the material completely dissolved. Then it was filtered through 0.45 μ syringe filter.

Preparation of calibration curve of linoleic acid and analysis of oil sample

HPTLC analysis was performed using isocratic technique as reported by Chakraborty A. The TLC plates were prewashed with methanol and activated at 110°C for 15 min, prior to chromatography. The linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (Camag, Muttentz,

Switzerland). A Camag HPTLC system containing Camag Linomat 5 sample applicator, Hamilton syringe (100 μ l), Camag TLC Scanner-3 with Wincats software version 1.3.0 was used. The standard solution was applied on Silica Coated aluminum plates (10cm × 10cm) in the concentration ranging from 2 to 10 μ l. The mobile phase was optimized with n-hexane and ethyl acetate in a ratio of 5:4 v/v. All the procedures were done at ambient temperature. After development the plate was dried and sprayed with sulphuric acid-anisaldehyde reagent. The plate was kept at 110°C for 5 minute in hot air oven and evaluation was carried out at 540nm. Calibration curve of linoleic acid was obtained by plotting peak areas versus concentrations of linoleic acid applied. Sample solution was applied consequently 20 and 40 μ l and analyzed using the optimized chromatographic condition. Peak areas of sample peak was determined for estimating content of Linoleic acid in oils.

Formulation of cream¹⁵

Preparation of Base

Accurately weighed amount of Carbopol 934 was slowly added in weighed amount of water with continuous stirring using mechanical stirrer (Part I: Water soluble). Weighed amount of Cetyl alcohol and Stearic acid was added (Part II: Oil Soluble) and allowed to stand for 15 min. in a water bath at 50-60 °C till it melts. Part II was immediately added in Part I with continuous stirring followed by glycerin and pH of the base was adjusted using triethanolamine (Table 1)

Preparation of Formulation

The base was used to formulate the cream containing seed oils namely PSO, CSO and almond oil. All the oils were mixed together in a beaker and added to the base with continuous stirring followed by the addition of preservatives, and rose oil for fragrance. pH of the cream formulation was adjusted using triethanolamine (Table 2)¹⁶

Evaluation of formulated batches

Physicochemical parameters of oil and formulations¹⁷

All the formulated batches were tested manually for: Appearance, color, odour and texture, consistency, greasiness, homogeneity, grittiness etc

pH determination¹⁸

pH measurement was done using digital pH meter. pH of the topical formulation is required to be in the range of 6.0-7.5 for better absorption in the skin. The electrode prewashed with double distilled water and dried was dipped in 20gm formulation and the pH recorded at ambient condition.

Spreadability

The spreadability of formulation was determined using spreadability apparatus. The apparatus consisted of two glass slides (7.5 × 2.5 cm), one of which was fixed onto the wooden board and the other was movable, tied to a thread which passed over a pulley, carrying a weight. 0.5 gm of gel was placed between the two glass slides. 100 gm weight was allowed to rest on the upper slide for 1 to 2 minutes to expel the entrapped air between the slides and to provide a uniform film of the gel. The weight was removed and the top slide was subjected to a pull of 5 gm. The time necessary for top slide to travel pre-marked 6.5 cm distance was noted. This gave an idea of relative spreadability of

the formulation. Spreadability was calculated by following formula;

$$S = M \times L / T$$

Where, S= Spreadability, M= Mass attached with the slide, L = Length moved by the glass slide, T = Time required to travel a distance to slide.

Viscosity

The prepared topical formulation (5gm) was transferred in sample cell and placed carefully within the adaptor. The guard leg was placed around the adaptor and the sample was stirred slowly using motor driven stirring element at rotation speed of 100 rpm/min.

Type of emulsion under dye test¹⁹

The scarlet red dye was mixed with the cream in watch glass. A drop of the cream was placed on a microscopic slide and then it was covered with a cover slip and examined under a microscope. If the disperse globules appear red and the ground is colourless, the cream is O/W type. The reverse condition occurs in W/O type cream i.e. the disperse globules appear colourless in the red ground.

Irritancy Test²⁰

An area (1 cm²) on the dorsal left hand surface was marked. The cream was applied to the specified area and the time was noted. Irritancy, erythema, edema was checked for regular intervals up to 24 hours and reported.

Determination of Antioxidant activity

Antioxidant activity of seed oils and formulation was evaluated using Reducing Power assay and DPPH assay method and compared with ascorbic acid as standard.

Preparation of standard solution

Stock solution (1.0 mg/ml) was prepared for Ascorbic Acid in distilled water. Again 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml was withdrawn from stock solution and each diluted to 10.0 ml with distilled water to get final concentration of 50.0 µg/ml, 100.0 µg/ml, 150.0 µg/ml, 200.0 µg/ml and 250 µg/ml respectively.

Preparation of sample solution

100.0 mg of oils were accurately weighed and diluted up to 100 ml with methanol. The solution was sonicated for 10 min. Again 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml was withdrawn from stock solution and each diluted to 10.0 ml with distilled water to get final concentration of 50.0 µg/ml, 100.0 µg/ml, 150.0 µg/ml, 200.0 µg/ml and 250 µg/ml respectively

Reducing Power assay²¹

The reducing power assay of standard, PSO and gel was carried out using the same dilutions (as mentioned for DPPH assay in the range of 50-250µg/ml. Sample solution (1ml) each was incubated with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide (K₃Fe [CN]₆) at 50°C for 20 minutes. The reaction was terminated by adding 2.5 ml of 10% TCA solution and the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant (1.0 ml) was mixed with 2.5 ml of distilled water and 1.0 ml of 0.1% ferric chloride (FeCl₃) solution and absorbance was measured at 700 nm after incubation at room temperature for 10 min.

Radical Scavenging activity by DPPH assays method

A set of test tubes were labeled for control, standard dilution, formulation dilutions. 2.0 ml of DPPH solution (0.135mM) was added to all test tubes. Standard and sample dilutions (1ml each) of various concentrations were added to the separate test tubes. For the control, 1ml of methanol was used. Further 2.0 ml of methanol was added to all the test tube and the mixture was allowed to stand in dark for 30.0 minutes. The absorbance of these solutions was measured at 517 nm against methanol and radical scavenging activity was determined by following formula:

$$\% \text{ Scavenging Activity} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where, A_{control} = Absorbance of blank DPPH solution,
A_{sample} = Absorbance of sample solution

Determination of Sun Protection Factor by using Mansur equation²²

Accurately about 1.0g of the sample was weighed and transferred to 100 ml volumetric flask; ethanol was added up to 3/4th volume of the flask. The contents were sonicated for about 10 minutes and volume made up to the mark using ethanol and solution was filtered through Whatman No.1 filter paper and filtrate collected by rejecting the first few ml of the filtrate. 5ml of the aliquot was taken in a 50ml volumetric flask and volume made up to the mark using ethanol. Then 5ml of the diluted solution was taken in to 25ml volumetric flask and volume made up to the mark using ethanol. The absorption spectra of sample solution were obtained in the range of 250 to 400 nm using 1 cm quartz cell, and ethanol as blank. The absorption data were obtained in the range of 290 to 320. The SPF of the samples were calculated using the below equation (a mathematical expression derived by Mansur)

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

Where, CF is correction factor (=10), EE (λ) - Erythral effect of radiation with wavelength λ, I(λ) -solar intensity spectrum, Abs (λ) - Absorbance of sunscreen product

Stability study²³

Stability study was determined by placing the formulation into the stability chamber controlled at temperature 30 ± 2°C and 65% RH for three months. Formulations were evaluated for their organoleptic and physicochemical parameters at interval of 30 days for three months.

RESULT

Characterization of oils

Physicochemical parameters²⁴⁻²⁶: The seed oils were tested for their physical parameters i.e. appearance, color, odour and texture as given in Table 3. Physicochemical parameters of oils tested namely Refractive index and specific gravity and compared with the reference values. The procedures given in Indian Pharmacopoeia for acid value, iodine value and saponification value as given in Table 4.

GCMS study of methyl ester of fatty acids in oils

GCMS data of Pomegranate seed oil

Chromatogram of esterified sample of PSO showed peak at retention time of 35.03, 35.31 and 35.78 (Figure 1) Further the

mass spectra obtained using EI for the component separated at 35.319 shows the presence of methyl esters of Linoleic acid (Figure 1A) and the eluent at 35.407 shows that oleic acid is present in the oil. (Figure 1B)

GCMS data of Carrot seed oil

Chromatogram of esterified sample of CSO showed peak at retention time of 35.23, 35.38. (Figure 2) Further the mass spectra obtained using EI for the component separated at 35.23 (Figure 1A) shows the presence of methyl esters of Linoleic acid and the eluent at 35.38 (Figure 1B) shows that oleic acid is present in the oil.

GCMS data of Almond oil

Chromatogram of esterified sample of Almond oil showed peak at retention time of 36.02, 36.43. Further the mass spectra obtained using EI for the component separated at 36.02 shows the presence of methyl esters of Oleic acid (Figure 3A) and the eluent at 36.43 shows that Stearic acid is present in the oil. (Figure 3B)

HPTLC analysis

HPTLC method for analysis of linoleic acid in seed oils using optimized chromatographic conditions with n-hexane and ethyl acetate in a ratio of 5:4 v/v as mobile phases followed by spraying with sulphuric acid-anisaldehyde reagent and evaluation was carried out at 540nm. The densitogram of standard and PSO and CSO were scanned 540nm as shown in Fig.4, 5a and 5b respectively. The Rf value for standard linoleic acid was 0.53±0.02. The calibration curve of standard linoleic acid in the concentration range from 100 ng/band to 500ng/band was plotted (Figure 6) Various dilution of the oil samples were applied on the TLC plates and content of linoleic acid estimated against the calibration curve (Table 5).

Evaluation of base and formulations

Physical Parameters

All the developed formulation was found to be homogenous, non-greasy, non-gritty, light buff in color with pleasant odour (Table 6).

pH Determination: The pH of formulation for topical use should be neutral in the range of 6.0 to 7.2. Triethanolamine was added to the base and formulation to make it effective for application on skin. The pH of formulation was maintained in the range of 6.30 to 6.95 (Table 7).

Viscosity: Viscosity of all base formulations showed variation at 1RPM/min due to different concentration of carbopol 934, stearic acid and cetyl alcohol in base formulation. However as the shear is applied with increasing speed (100RPM) leads to decrease in viscosity. Presence of stearic acid and cetyl alcohol gave viscous formulation B3 and B4 as compared to B1 and B2 (57033±12433 cps at 100rpm). Viscosities of all the cream formulations were measured. Viscosity of cream decreases with an increase in shear stress (Figure 7).

Spreadability: Spreadability is measured to see the extent to which the cream applied spread easily on the skin and is thereby having good absorption and efficacy. It was observed that Spreadability decreased for B3, B4, F3 and F4 base and formulation respectively as it contain stearic acid and cetyl alcohol (Figure 8).

Type of emulsion under dye test

The dye test confirmed that all formulations were O/W cream. But formulation F3 and F4 showed more stability in O/W type emulsion.

Irritancy Test

All the formulations when applied to the dorsal surface of the hand in the specified area and observed for irritancy, erythema, edema up to 24 hours, it was seen that none of the formulation showed any sign of redness, edema, inflammation and irritation. It indicates that the formulation were safe.

Antioxidant activity

Reducing Power Assay

Antioxidant activity of standard Ascorbic acid and seed oils with their formulation was determined using Reducing Power Assay method. Dilutions in the range of 50 to 250µg/ml were taken for standard and seed oil sample.

As shown in Figure 9 and 10, a stronger absorbance indicates a higher reducing power. The activity increased in dose dependent manner for the seed oils and formulation and is comparable to standard.

DPPH assay method

The DPPH assay has been extensively used to study free radical scavenging activity of various natural products. The free radical scavenging activity of seed oils and cream formulation was compared with the standard antioxidant ascorbic acid are shown in Figure 11. The % scavenging activity increased with increased in concentration of seed oils. At 250µg/ml the seed oils and exhibited more than 70% scavenging effect while F3 and F4 formulation showed maximum scavenging effect (Figure 12).

Determination of Sun Protecting Factor (SPF)²⁷

Formulation containing Carrot seed oil are reported to have SPF of more than 38 to 40. Based on the literature available the formulations were evaluated for their sun protecting factor using Mansur equation. Based on the equation the absorbance is measured at wavelength ranging from 290 to 320 representing the UVB radiations. UVB radiation are directly absorbed by the keratinocytes in epidermal layers causing DNA damage. The antioxidant property of the formulation protects the skin from UVA radiation which is major cause in generating the oxidative stress. Formulations with SPF > 8 recommended. F4 showed an SPF of 11.12 (Table 8).

Stability study

Stability study was determined by placing the formulation into the stability chamber controlled at a temperature 30 ± 2°C and 65% RH for three months (Table 9). The stability study shows that the organoleptic parameters and physicochemical parameters of the cream were examined at the same temperature (30 ± 2°C), lighting and stability (65% RH) conditions to assess variations in appearance, color, texture, pH, spreadability, viscosity and moisture loss. Samples were evaluated for 30, 60, 90 days. From above study, it was concluded that F4 formulation has good organoleptic and physicochemical properties along with good antioxidant and sun protection factor.

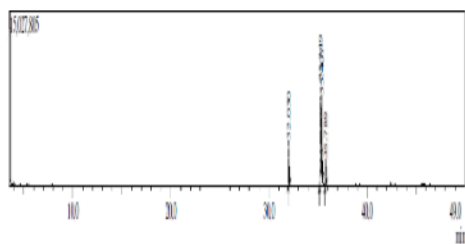


Figure 1: Chromatogram (GCMS) of linoleic acid & oleic acid in PSO

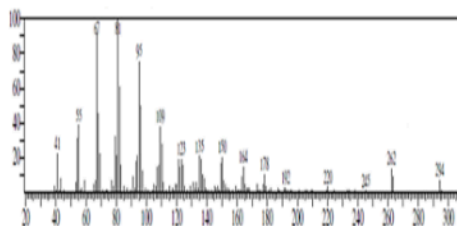


Figure 1A: MS spectra of methyl ester of linoleic acid in PSO sample at Rt-35.319 and Carrot seed oil at Rt : 35.231, Base peak obtained:81.10

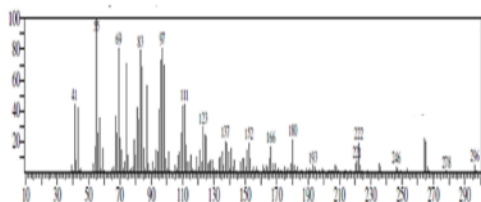


Figure 1B: MS spectra of methyl ester of Oleic acid in PSO sample, Rt-35.407, and Carrot seed oil at Rt : 35.38; Base peak obtained: 55.05

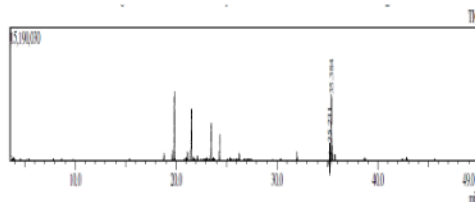


Figure 2: Chromatogram (GCMS) of linoleic acid & oleic acid in CSO

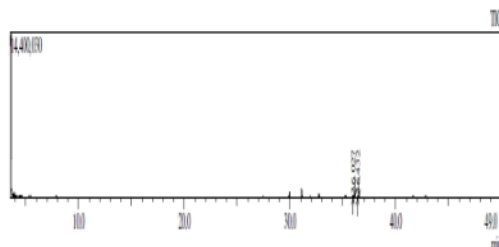


Figure 3: Chromatogram (GCMS) of Oleic acid & Stearic acid in Almond oil

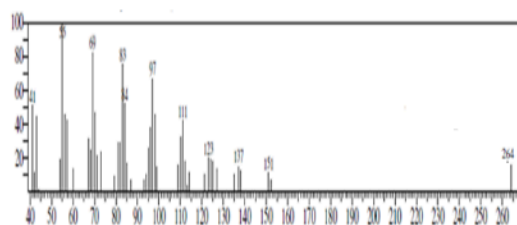


Figure 3A: MS spectra of methyl ester of Oleic acid in Almond oil

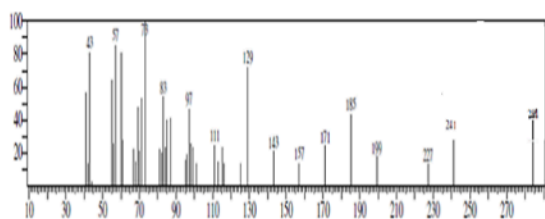


Figure 3B: MS spectra of methyl ester of Stearic acid in Almond oil sample, Rt- 36.432, Base peak obtained: 73.00

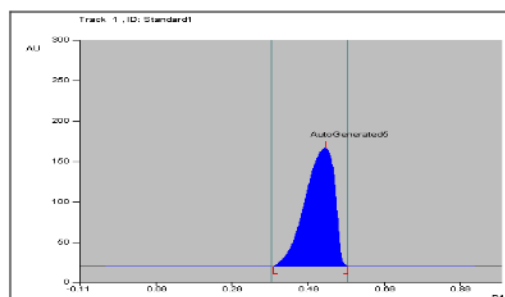


Figure 4: Densitogram of standard Linoleic Acid (200ng/band)

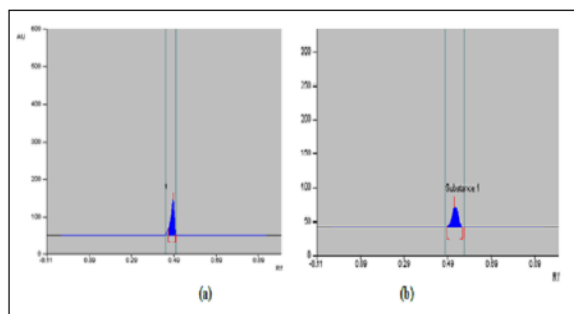


Figure 5(a): Densitogram of Linoleic Acid in Pomegranate seed oil, (b) Densitogram of Linoleic Acid in Carrot seed oil

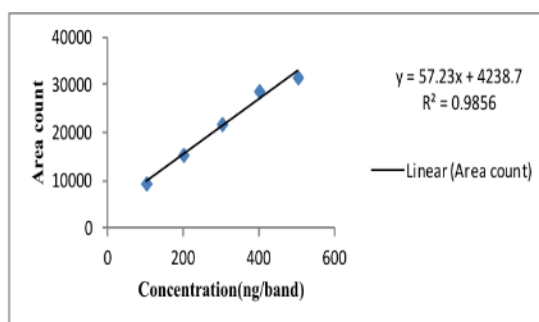


Figure 6: Calibration curve for standard Linoleic acid at 540nm

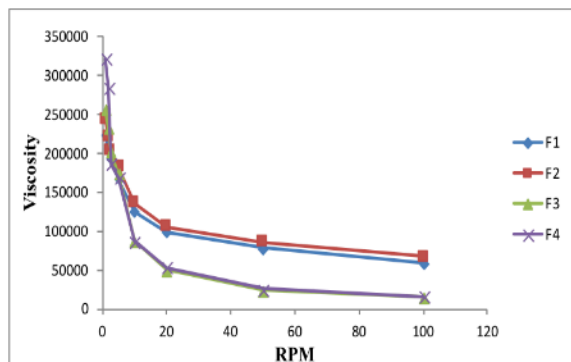


Figure 7: Viscosity of formulation

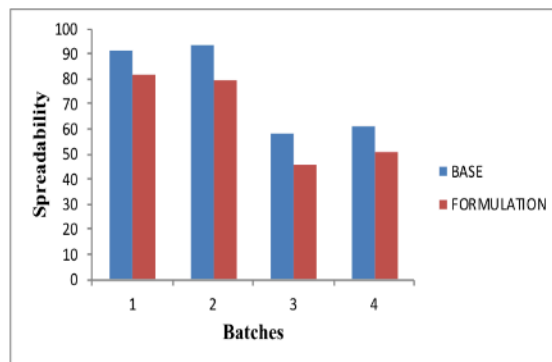


Figure 8: Spreadability of base & Formulation

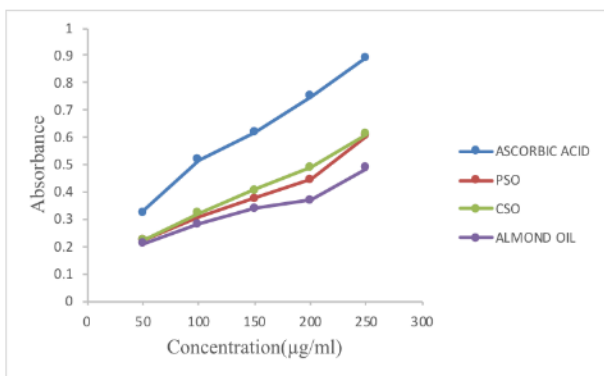


Figure 9: Antioxidant activity of seed oils using reducing power assay method

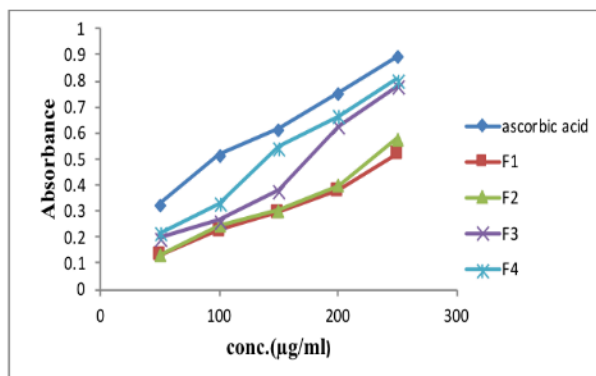


Figure 10: Antioxidant activity of formulation using reducing power assay method

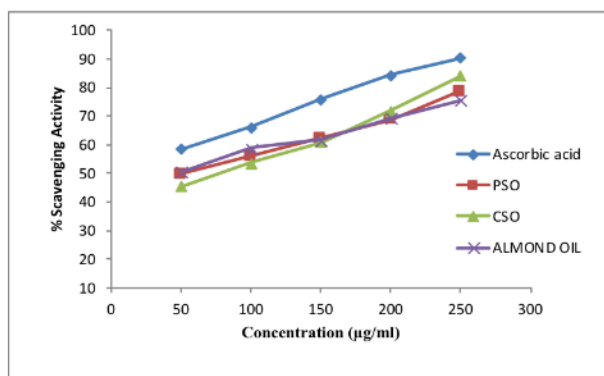


Figure 11: Antioxidant activity of seed oils using DPPH assay method

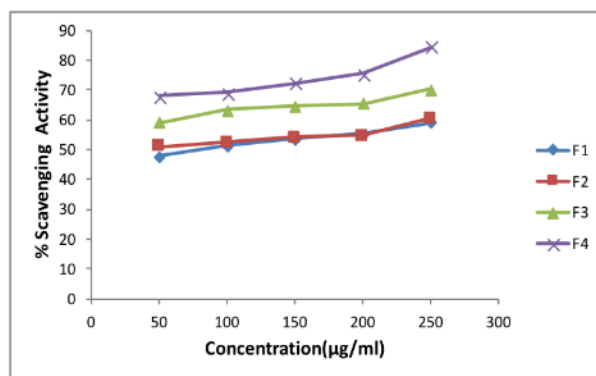


Figure 12: Antioxidant activity of formulations using DPPH assay method

Table 1: Composition of Base formulation

Ingredients	B1	B2	B3	B4
Carbopol 934	0.50%	0.75%	0.50%	0.75%
Cetyl alcohol	-	-	0.25	0.25
Stearic acid	-	-	0.25	0.25
Glycerin	3%	3%	3%	3%
Water	Q.S	Q.S	Q.S	Q.S

Q. S. Quantity Sufficient

Table 2: Composition of formulation

Ingredients	F1	F2	F3	F4
PSO	3%	5%	3%	5%
CSO	0.5%	0.5%	0.5%	0.5%
Almond oil	3%	5%	3%	5%
Carbopol 934	0.50%	0.75%	0.50%	0.75%
Cetyl alcohol	-	-	0.25%	0.25%
Stearic acid	-	-	0.25%	0.25%
Glycerin	3%	3%	3%	3%
Propylene Glycol	0.5%	0.5%	0.5%	0.5%
Methyl paraben	0.18%	0.18%	0.18%	0.18%
Propyl paraben	0.02%	0.02%	0.02%	0.02%
EDTA	1%	1%	1%	1%
Coffee	0.1%	0.1%	0.1%	0.1%
TEA	Q.S	Q.S	Q.S	Q.S
Water	Q.S	Q.S	Q.S	Q.S

Q. S. Quantity Sufficient

Table 3: Physical parameters of oil

Parameters	Pomegranate seed oil	Carrot seed oil	Almond oil
Appearance	Clear liquid	Clear liquid	Clear liquid
Color	Colorless	Yellow	Colorless
Odor	characteristics	Characteristics	characteristics
Texture	clear	Clear	clear

Table 4: Physicochemical parameters of oils

Parameters	Pomegranate seed oil		Carrot seed oil		Almond oil	
	Observed value	Reference value	Observed value	Reference value	Observed value	Reference value
Refractive index	1.4690	1.45-1.47	1.473	1.48-1.49	1.345	1.405
Specific gravity	0.940	0.939	0.945	0.900-0.943	0.911	0.910-0.920
Iodine value mg KOH/g	71.0	74.2	100.31	101.20	44.415	105.0
Acid Value	8.1	8.4	4.9	5.0	1.00	1.5-3.8
Saponification value	176.042	188.1	163.60	173.91	112.2	139

Table 5: Estimation of Linoleic acid in oils

Samples	Amount taken(ng/band)	Area count	% Estimation of Linoleic acid
PSO	2000	1979.6	12.68
	4000	3722.2	12.96
CSO	2000	1792.1	11.48
	4000	3139.5	10.90

Table 6: Physical parameters of formulations

Parameters	F1	F2	F3	F4
Consistency	++	++	+++	+++
Homogeneity	Yes	Yes	Yes	Yes
Greasiness	No	No	No	No
Grittiness	No	No	No	No

Excellent: +++, Very good: ++

Table 7: pH of Base & formulations

Batches	pH of Base	pH of formulation
1	6.12±0.005	6.30±0.085
2	6.25±0.03	6.79±0.036
3	6.66±0.02	6.92±0.041
4	6.76±0.020	6.95±0.045

Table 8: Sun Protecting Factor (SPF) of Formulations

Wave-length	F1		F2		F3		F4	
	Abs	EF×I×Abs	Abs	EF×I× Abs	Abs	EF×I×Abs	Abs	EF×I× Abs
290	0.610	0.009	0.79	0.011	0.961	0.012	1.47	0.022
295	0.585	0.047	0.655	0.053	0.821	0.066	1.34	0.109
300	0.569	0.163	0.600	0.172	0.800	0.229	1.20	0.344
305	0.545	0.178	0.543	0.177	0.792	0.258	1.11	0.363
310	0.427	0.079	0.498	0.092	0.751	0.139	0.97	0.180
315	0.386	0.032	0.445	0.037	0.710	0.059	0.91	0.076
320	0.368	0.006	0.437	0.007	0.690	0.012	0.86	0.015
SPF	5.17		5.53		7.80		11.12	

Table 9: Stability Studies

Batches	Parameters	0 Month	1 Month	2 Month	3 Month
F1	Appearance	+++	+++	++	++
	Color	Buff	Sight dark	Sight dark	Dark brown
	Texture	Smooth	Smooth	Slight sticky	Slight sticky
	pH	5.50	5.49	6.0	6.12
	Spreadability	84.26	83.33	76.53	72.11
	Moisture loss	-	1.029	1.699	2.695
	Viscosity	25500	23500	22000	20500
F2	Appearance	+++	+++	++	++
	Colour	Buff	Sight dark	Sight dark	Dark brown
	Texture	Smooth	Smooth	Slight sticky	Slight sticky
	pH	5.60	5.45	5.50	5.65
	Spreadability	86.20	85.22	78.12	75.0
	Moisture loss	-	1.0	1.450	2.225
	Viscosity	15900	13500	12000	11500
F3	Appearance	++++	++++	+++	+++
	Color	Buff	Buff	Buff	Buff
	Texture	Smooth	Smooth	Smooth	Smooth
	pH	6.45	6.28	6.29	6.40
	Spreadability	65.78	64.65	65.21	66.63
	Moisture loss	-	0.985	1.00	1.14
	Viscosity	34000	34600	34700	34600
F4	Appearance	++++	++++	+++	+++
	Colour	Buff	Buff	Buff	Buff
	Texture	Smooth	Smooth	Smooth	Smooth
	pH	6.46	6.38	6.48	6.47
	Spreadability	66.96	66.37	67.56	68.80
	Moisture loss	-	0.889	0.790	0.765
	Viscosity	45500	46000	46700	46150

Excellent: +++++, Very good: +++, Good: ++

DISCUSSION

Human skin when exposed to UV radiation leads to oxidation of cellular bio molecules. Chronic exposure to environmental stress and UV irradiation leads to photo-aging. Preventive measures include the use of antioxidant treatment. Many plant products are used in formulations due to their functional properties (emollient, anti-irritant, antioxidant, moisturizing, anti-aging). The use of natural antioxidants in cosmetic products enables the nourishment of the skin^{28, 29}. Pomegranate seed oil, Carrot seed oil and Almond oil are well known for their various beneficial effects on skin. Therefore, the present work was carried out to formulate and evaluate a cream with seed oils reported for their antioxidant activity and sun screening effect. The study indicated that a blend of oils with antioxidant activity (5%) in F4 formulation was stable, non-greasy and easily spread on application. The

formulation had no side effects including itching, redness, irritation etc. From above results, it is confirmed that the formulation can be one of the best antioxidant cream with Sun screening effect.

CONCLUSION

The demand for herbal products in the world market is exponentially growing as herbal and natural products are safe to use and do not have any side effects. An attempt was done to formulate a cream with mixture of seed oil and almond oil having antioxidant effect. From all the batches, F4 batch with 5% of PSO and Almond oil showed best antioxidant activity with sun protecting factor (SPF) of 11.12. considered as satisfactory for sun screening effect along with antioxidant effect.

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