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Formulation and *in vitro* evaluation of gel for SPF determination and free radical scavenging activity of turpentine and lavender oil

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Abstract

The present study was to investigate the *in vitro* free radical scavenging activity of Turpentine oil and Lavender oil. Antioxidant activity of oils was studied by DPPH assay method. Carbopol 934 was used as the gelling agent and the prepared gel was then evaluated for its color, odor, pH, spread ability, Viscosity etc. UV protective study i.e. SPF determination was also performed by UV-Spectrophotometer at the range of 200-400nm. The maximum absorption for the gel and oil was found between 220-300 nm. Both Turpentine oil as well as Lavender oil having good possessed *in vitro* free radical scavenging activity (Anti-Oxidant activity) and Sun Protection Activity.

Keywords: Turpentine oil (TuO), lavender oil (LvO), Gel formulation, sun protection activity and *in vitro* free radical scavenging activity etc.

Introduction

Skin is the furthest and biggest organ of the body subsequently it is most inclined to photo damage as it is specifically presented to daylight. As of late, the occurrences of bright radiation related illnesses and scatters are ceaselessly developing. At the point when the mammalian skin is presented long haul to bright radiation, it actuates the oxidative worry by creating the receptive oxygen species, which trigger the improvement of sunburn, erythema, edema, immunosuppressant, photo aging, skin tumor etc. [1, 2]

The fundamental exogenous wellspring of the UV radiation is daylight. It is made out of different wavelengths extending from bright light through infrared to noticeable light. Among all, bright light is the most destructive to the skin. Bright radiation from the sun can be additionally partitioned into three classes in view of the wavelength, long wave UVA (320-400 nm), medium wave UVB (280-320 nm) and short wave UVC (200-280nm) [3]. Exposure to UVA radiation brings about harm to the versatile and collagen fiber of connective tissue of skin, which causes untimely maturing (photograph aging).while UV-B radiation realize intense inflammation (sun consume) and escalation of photograph aging.UVC radiations sifted by the climate before achieving earth. UV-B radiation isn't totally sifted through by the ozone layer and is in charge of the skin harm due to sunburn [4].

Over 90% of sunlight based radiation that achieves the earth is UVA which infiltrates profound into the epidermis and dermis of the skin (Fig.1). It is around 1000 times more compelling in delivering a quick tanning impact when contrasted with UV B. Long haul presentation to UVA can consume touchy skin and harm the basic structures in the dermis and cause untimely photoaging of the skin. It causes skin hanging and smother some immunological capacities. It additionally triggers the oxidative changes in uncovered people which create singlet oxygen, hydrogen peroxide and hydroxyl free radicals. These can make harm cell proteins, lipids and saccharides. UV damage likewise tends to cause rot of endothelial cells, in this manner harming the dermal veins. It can deliver auxiliary changes in DNA and impede the safe framework which inturn brings about malignant condition [5].

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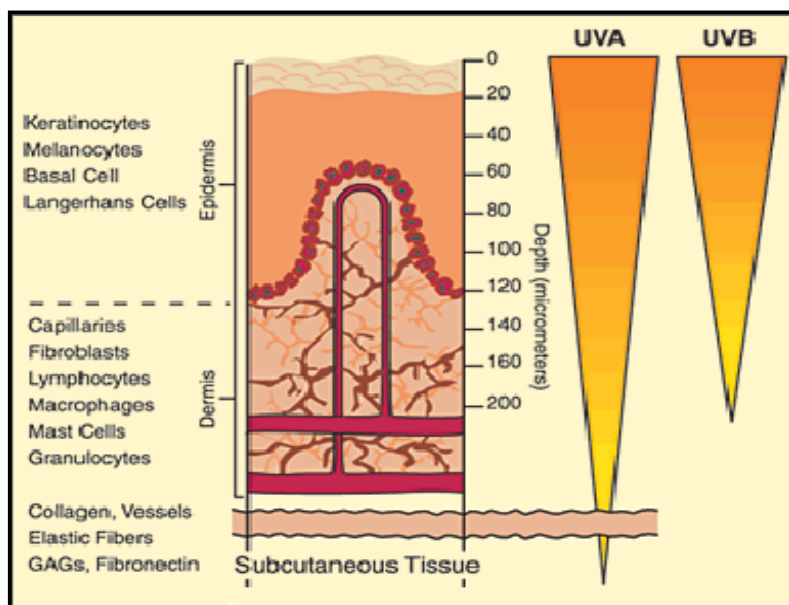


Fig 1: Penetration capacity of UV radiation on the skin

UVA radiation penetrates deep until subcutaneous tissue whereas the penetrating ability of UVB shows that it poses cutaneous damage very frequently.

Evaluation Parameters for Photoprotectives

The sunscreen activity of plant actives can be measured by various biochemical parameters as discussed below;

- **Erythema determination:** Erythema is the slight reddening of skin after 24 hours exposure to UV radiation. It is determined by investigating the histological, ultrastructural, biochemical and immunological effects of UV radiation on the skin and its relationship to photodamage and skin cancer.
- **Lipid damage determination:** UV radiation induces the formation of reactive oxygen species resulting in damage to various components of skin like lipids which results in degradation of free fatty acids and cholesterol. It is observed that UV exposure decreases lipid melting temperature of the mouse skin and that application of sunscreen prior to UV radiation would reduce this epidermal damage.
- **Sunburn cell count:** Sunburn cells are apoptotic keratinocytes observed in humans, mice, rabbits and guinea pigs. They absorb the lethal dose of UV radiation and acts as the indicator of acute photodamage.
- **SPF determination:** The sun protective activity of sunscreens was measured as Sun Protection Factor (SPF). This *in vitro* method measures the reduction of the irradiation by measuring the transmittance after passing through a film of product [18].
- **Quantification of UV induced DNA damage:** Exposure of UV radiation in the skin results in the generation DNA lesions. The DNA damage caused by UV radiation is estimated before and after the application of test formulation and analyses whether it has any protective effect on DNA lesions.
- **Skin viscoelasticity determination:** To determine the effects of treatment with the test formulation on skin firmness.
- **Wrinkle volume determination:** To determine the decrease in wrinkle volume after treatment with test formulation.

Lavender Oil and Turpentine Oil: Skin Benefits [22]

1. **Helps to maintain healthy and flawless skin:** The natural antioxidants present in Turpentine oil as well as Lavender oil that help to protect the skin from the harmful free radicals, aid in regulating the production of sebum and help treat hormonal imbalances in the body.
2. **Helps treat skin problems:** this oil helps to treat skin problems like eczema, psoriasis and sunburns. Its potent healing qualities are helpful in repairing wounds, cuts, pimples, cracks and other break outs on the skin without leaving any scars or marks behind.
3. **Reinvigorates Skin:** The Turpentine oil and Lavender oil is by acting on skin stimulates the keratinocytes. Major cells are found in the outer layer of the skin. This helps to reverse skin damage, revive skin and reveal a more youthful appearance.

At the point when human skin is uncovered for long haul to bright radiation, skin is influenced by oxidative worry because of arrangement of receptive oxygen species, which trigger the advancement of sunburn, erythema, edema, photograph maturing, skin growth and so on. Insurance of skin from photograph harm is a dire concern. One technique for shielding the skin from UV radiation is the utilization of sunscreens to balance the receptive oxygen species by hindering the UV radiation uncovered on the epidermis.

Turpentine oil and Lavender oil are known for their cancer prevention agent property. They are accounted for to shield the skin from natural ambushes like UV beams, contamination, and stress. Carotenoids introduced in Turpentine oil have been found to upgrade the body's safe reaction to UV beams, which can diminish skin harm from UV presentation.

A reasonable gel detail of Turpentine oil and Lavender oil were arranged and assessed for standard parameters, as gels are winding up more prevalent because of simplicity of use and better percutaneous retention, than other semisolid readiness. Gel offers more prominent potential as a vehicle for topical organization of medication as they are non-sticky and require low vitality amid detailing.

Material and Methods

Turpentine oil procured from Katyani Exports New Delhi and Lavender oil procured from Natural Essential Oil, Ggenex, Pune both oils were analyzed. Carbopol 934 and Polyethylene glycol 400 procured from Himedia, Methyl paraben, Propyl paraben, Glycerine, Triethanolamine, Butyl Hydroxy Toulene, Rose oil, Oleic acid procured from Research Lab. Methanol, Ethanol from Himedia and distilled water. DPPH, Ascorbic acid procured from Research Lab.

Experimental Methods

Characterization of Oils

Physicochemical parameters

- Refractive index
- Density
- Specific gravity
- Iodine value
- Acid value

- Saponification value (as per procedure mentioned in IP)

Formulation of Sunscreen Gel

Step-I Preparation of Carbopol gel base

Weighed amount of Glycerin was added to distilled water and stirred with mechanical stirrer for incorporation of Glycerin in water. Weighed amount of Carbopol 934 was added slowly to the mixture of water and glycerine and continuously stirred with the mechanical stirrer for 1 hr. Preservatives also added in the gel base and again stirred with the mechanical stirrer.

Step-II Preparation of gel formulation

BHT is oil soluble antioxidant so it was solubilised in Turpentine oil and Lavender oil. Final mixture of BHT, PEG 400, oil TuO and LvO was added in carbopol gel base, then perfume was added and finally pH was adjusted with Triethanolamine.

Table 1: Formulae for Gel Formulation

Ingredient	TLI	TLII	TLIII	TLIV	TLV	TLVI	TLVII	TLVIII	TLIX
TuO	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Carbopol 934	1.0%	1.0%	1.0%	1.5%	1.5%	1.5%	0.5%	0.5%	0.5%
LvO	0.5%	1.0%	1.5%	0.5%	1.0%	1.5%	0.5%	1.0%	1.5%
Glycerin	2%	2%	2%	2%	2%	2%	2%	2%	2%
PEG 400	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%
Methyl paraben	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%
Propyl paraben	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%
BHT	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
TEA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Perfume	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Anti- Solvent	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Evaluation of Sunscreen Gel

Organoleptic parameters

In organoleptic parameters appearance, color, odor, transparency and smoothness of formulation was studied.

pH determination

pH measurement is measured by digital pH meter with magnetic stirrer. PH of the gel should be within 6-7 for better absorption in the skin. In this method, electrode was washed with double distilled water, dried with the help of tissue paper and then dipped in 30 gm. gel formulation. The average pH (n=3) of the gel formulations were recorded at ambient condition.

Rheological Study/ Viscosity

Type of equipment – Brookfield RVDV-II + Pro with small sample volume adaptor spindle (S21)

Sample volume –35 GM

Speed of rotation of stirring element – 500-1000 rpm.

The specified volume of prepared gel was transferred in sample cell was placed carefully within the adaptor. The guard leg was placed around the adaptor and the volume of sample was stirred slowly using motor driven stirring element. The viscosity values were recorded from the display window.

Spredabiliy

The spread ability of the gel was determined using a spread ability 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 pully, 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 premarked 6.5 cm distance was noted. This gave an idea of relative spread ability of the different gels Spread ability was calculated by following formula;

$$S = \frac{M \times L}{T}$$

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.

Percentage Moisture loss

Percentage moisture loss for the different batches was determined. The accurately weighed quantity (5 gm) of formulation was kept in a desiccators containing 50gm anhydrous calcium chloride. After three days, the formulation was weighed and the percentage moisture loss was calculated using the formula:

$$\text{Percentage Moisture Loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Final Weight}} \times 100$$

Spectroscopical Study of Oils

FTIR study TuO and luO

Determination of infrared absorption spectrum of TuO and luO

IR absorption spectrum was recorded by using chloroform. Oil sample was dissolved in chloroform and placed in sample holder and infrared spectrum was recorded using FTIR spectrophotometer.

In-Vitro Radical Scavenging Activity

Antioxidant activity of TuO and luO is studied by DPPH assay method using Ascorbic Acid as a control.

DPPH assay method

Preparation of standard and sample solution:

Stock solution (1.0 mg/ml) was prepared by dissolving 25.0 mg/ml of Ascorbic Acid in 25ml of distilled water. Again 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 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 Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Solution (0.135mM)

13.31mg of DPPH was dissolved in 5.0ml of methanol and transferred to a 25.0ml volumetric flask and volume was made up to the mark with methanol. 10.0 ml of the stock solution was pipetted out and further diluted to 100.0ml in a volumetric flask to get 0.135 mM solution which was used for the estimation.

Procedure

- 2.0 ml of DPPH solution was taken in the test tubes.
- 1.0ml of the test drug and standard ascorbic acid of various concentrations were added to the separate test tubes. For the blank 1ml of methanol was taken.
- Further 2.0ml 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 the free radical scavenging activity was determined by following formula;

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

Where,

A_{control} = Absorbance of blank DPPH solution.

A_{sample} = Absorbance of sample solution.

Determination of Sun Protection Factor of Oil and Gel

The efficacy of a sunscreen is expressed by the Sun Protection Factor (SPF). An *in vitro* method of determining SPF of the sunscreens is by using Mansur equation.

Procedure

Weigh about 2.0g of the sample in a 100 mL volumetric flask and add methanol about 3/4th volume of the flask. Sonicate the contents for about 15-20 minutes and make up to the mark using methanol. Filter the solution through Whatman No1 filter paper and collect the filtrate by rejecting the first few mL of the filtrate. Take 5mL of the aliquot in a 50mL volumetric flask and make up to the mark using methanol. Then take 5mL of the diluted solution in to the 25 mL volumetric flask and made up to the mark using methanol. The absorption spectra of sample solution were obtained in the range of 250 to 400 nm using 1 cm quartz cell, and methanol as blank. The absorption data were obtained in the range of 290 to 320, every 5 nm, and 3 determinations were made for each samples. The SPF of the samples were calculated using the below equation (a mathematical expression derived by Mansur)

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

Where,

CF is correction factor (=10);

EE (λ) - erythmal effect of radiation with wavelength λ;

I(λ) -solar intensity spectrum;

Abs(λ)-Absorbance of sunscreen product.

The values of EE (λ) × I (λ) are constant as given in Table 1. The obtained absorbance values Abs (λ) were multiplied with the respective EE (λ) × I (λ) values and then summation was taken and multiplied with the correction factor 10

Table 2: Relationship between Erythemogenic Effect and Radiation Intensity

Wavelength	EE X I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Result and Discussion

Characterization of Oil

All parameters are within the range of literature value. So it was confirmed that sample of oil is pure. Table No.3 shows all results for physicochemical parameters.

Table 3: Physical constants of oils

Parameter	Turpentine oil	Lavender oil
Appearance	Clear liquid	Clear liquid
Colour	amber	pale Yellow
Odour	Characteristic	Characteristic
Texture	Clear	Clear

Table 4: Physicochemical parameters of Oils

Parameter	Turpentine oil		Lavender oil	
	Reference value	Observed value	Reference value	Observed value
Refractive index	1.4880-1.4940	1.4521	1.4590-1.4700	1.4600
Density	0.910-0.940	0.922	0.888-0.910	0.902
Specific gravity	0.934	0.942	0.939	0.940
Iodine value g/100g	101.20	101.31	74.2	73.2
Acid value mg KOH/g	5.0	4.8	8.4	8.2
Saponification value	173.91	167.9	188.1	187.6

FTIR study TuO and luO

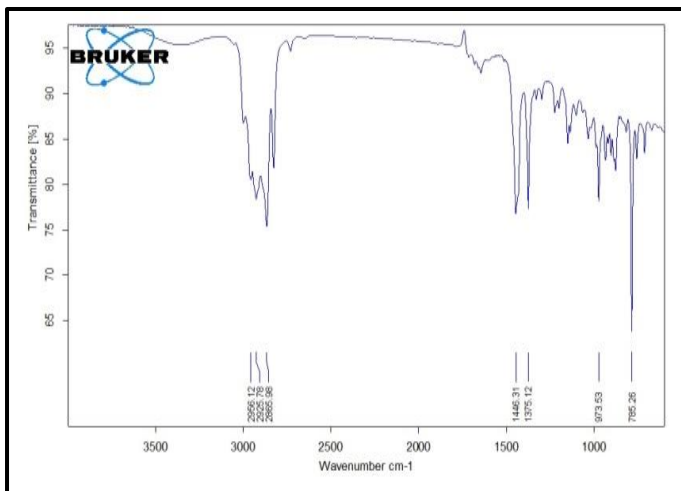


Fig 2: FTIR study of Interpretation of FTIR of Carbopol 934 + TuO

Table 5: Observed FTIT band of Carbopol 934 + TuO

Sr. No.	IR Bands (cm ⁻¹)	Functional group
1.	3026.55	=C-H alkyl
2.	2958.04	C-H saturated
3.	1757.92	C=O Acid
4.	1230.98	C-O Acid

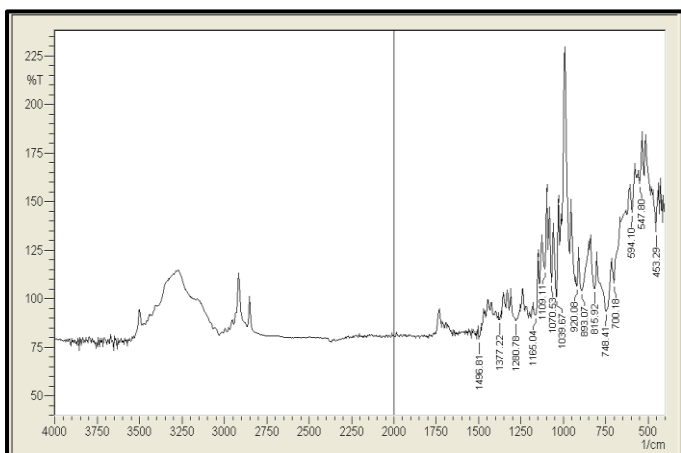


Fig 3: Interpretation of FTIR of Carbopol 934 + luO

Table 6: Observed FTIT band of Carbopol 934 + TuO

Sr. No.	IR Bands (cm ⁻¹)	Functional group
1.	3510.75	-OH acid stretch
2.	3170.75	=C-H stretch
3.	2951.74, 2954.63	-CH Acid stretch
4.	1681.66	C=O acid
5.	1263.78	-C-O acid

Table 9: SPF Determination of Oil and Gel

Wavelength	EF × I	TuO		LuO		Gel Formulation	
		Abs	EF×I×A	Abs	EF×I×A	Abs	EF×I×A
295	0.081	3.32	0.0380	0.078	0.0011	0.821	0.0132
300	0.287	3.18	0.9187	0.084	0.0255	0.730	0.2300
305	0.327	3.11	1.0287	0.083	0.0266	0.780	0.2580
310	0.1864	3.10	0.5841	0.085	0.0187	0.740	0.1555
315	0.083	3.02	0.2621	0.086	0.0052	0.778	0.06305
320	0.018	2.68	0.0600	0.089	0.0022	0.776	0.0180
SPF		29.96		0.740		7.907	

Each values is expressed as mean ± SD of three determinations.

From FTIR of Carbopol 934 + TuO and Carbopol 934 + luO, it was observed that same functional groups were present in both oil and gel. So it was confirmed that oil was incorporated in the gel formulation. Also, no additional peaks were observed in FTIR spectrum of physical mixture of oil and excipients. Thus confirming no interaction between them i.e. the mixture was compatible.

**Radical Scavenging Activity
DPPH assay method**

Table 7: Absorbance's of Ascorbic acid and oils

Concentrations (µg/ml)	Absorbance [Mean ± S.D (n=3)]		
	Ascorbic acid	TuO	luO
25	0.048±0.022	0.057±0.002	0.061±0.005
50	0.039±0.001	0.055±0.004	0.057±0.003
100	0.033±0.002	0.046±0.003	0.047±0.007
150	0.023±0.003	0.048±0.004	0.045±0.006
200	0.014±0.007	0.041±0.006	0.031±0.008

Table 8: % Radical scavenging activity of Oils

Concentrations (µg/ml)	Ascorbic acid (% scavenging activity) Mean ± S.D (n=3)	(% Scavenging activity) Mean ± S.D (n=3)	
		TuO	LuO
25	62.81±0.003	54.02±0.050	57.28±0.070
50	80.32±0.007	56.25±0.005	60.19±0.007
100	76.77±0.005	62.27±0.011	64.96±0.015
150	83.33±0.003	68.40±0.005	69.93±0.007
200	88.85±0.001	75.55±0.031	78.37±0.023

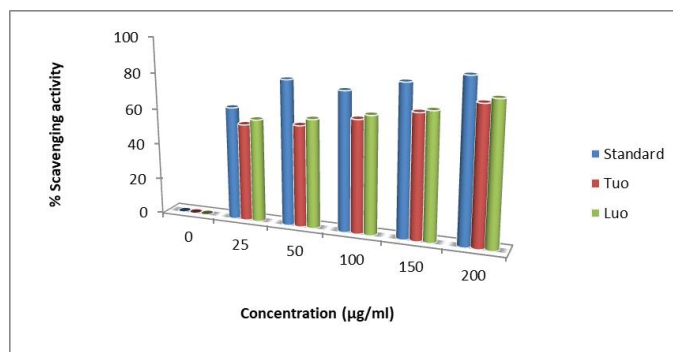


Fig 4: Radical scavenging activity by DPPH assay method

SPF Determination of Oil and Gel

SPF of oils and gel formulation was checked by absorption spectroscopy using Mansur equation method. Absorbance's obtained in spectrum were considered for SPF calculations and results are shown in Table No. 05

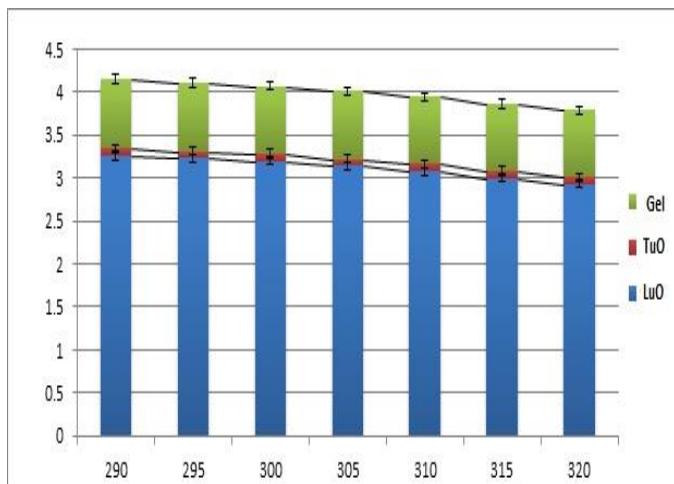


Fig 5: UV spectral absorbance readings of oils and gel formulation

SPF determination of oils and gel formulation was performed by UV-Spectrophotometer at the range of 200-400nm. The maximum absorption for the gel and oils were found between 220-300nm. SPF of TuO, LuO and Sunscreen gel formulation was found to be 29.96, 0.740 and 7.907 respectively.

Summary and Conclusion

Characterization of Turpentine oil and Lavender oil were finished by concentrate distinctive parameters. TuO and LuO passed all physicochemical assessment parameters which are inside cutoff regarding standard esteems. Cell reinforcement movement of oils were contemplated by radical rummaging action by DPPH strategy in the focus scope of 50-250 $\mu\text{g/ml}$. Cell reinforcement action of oils were contrasted and the Ascorbic corrosive. Sunscreen gel detailing was produced by utilizing carbopol 934 as a gelling operator. Detailing created by changing the grouping of Carbopol 934, TuO and LuO. At last enhanced cluster TLVIII was having 0.5% of Carbopol and 1.0% of TuO and 1.0% of LuO. Every one of the details has been considered for pH, thickness, spread ability, % dampness misfortune, and SPF assurance. Results are appeared as Ph 6.89 ± 0.005 , Spredabiliy 0.97 ± 0.005 , % Moisture loss 6.20 ± 0.052 , Viscosity 11654 ± 0.2 at 200 RPM and SPF 7.907.

Optimized TLVIII batch was found in range for physicochemical parameters. All the gel formulations have shown good consistency with no degradation of herbal actives. FTIR analysis of TuO, LuO and gel was showing incorporation of TuO and LuO in the gel formulation. Finally it is concluded from present findings that TuO and LuO containing formulation may contribute as a cosmetic ingredient for protection from UVB induced skin damage. Both Turpentine oil as well as Lavender oil having good possessed SPF (Sun Protection) and *in vitro* free radical scavenging activity (antioxidants activity).

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