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**FORMULATION AND IN VITRO DETERMINATION OF SUN  
PROTECTIVE POTENTIAL AND PHYSICAL STABILITY OF  
POLYHERBAL LOTION CONTAINING *TRIDAX PROCUMBENS*,  
*PORTULACA GRANDIFLORA*, *MALUS DOMESTICA* AND *ALOE  
BARBADENSIS* EXTRACT**

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**ABSTRACT**

The aim of this study is to evaluate the sunscreen activity of an herbal lotion containing extract leaves of *Tridax procumbens* (coat buttons) family-asteraceae, *Portulaca grandiflora* (family-portulacaceae), juice of *Aloe vera* (family-Liliaceae) and fresh pulp *Malus domestica* (family-Rosaceae). The dried shaded leaves were removed in succession by a soaking technique using a hydrochloric solution. Three different batches of formulations such as F1, F2 and F3 have been formulated and tested for physicochemical parameters such as pH, dye testing, dilution, patch testing for irritability. Determination of SPF is a laboratory magnitude to evaluate sunscreen effectiveness. The higher the SPF, the greater the protection against UV rays. UV rays are harmful rays that cause skin cancer, sunburn, wrinkles. Therefore an enduring need for protection against UV rays. In this sunscreen formulation various herbal plants are selected because of their bioactive compounds as they contain polyphenolic compounds like alkaloids, flavonoids and tannins etc. This ensues in a non-mutagenic, non-irritating sunscreen. The efficiencies were tested with the standard formulation of SPF 55. From this study, it is noted that F2 formulation is more stable and effective as compared to F1 and F3.

**Keywords:** Herbal Sunscreen, SPF, Skin burn, *Portulaca grandiflora*, *Tridax procumbens*, *Aloe vera*, *Malus domestica*, bioactive compounds, Maceration

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## INTRODUCTION

In recent days, global warming has been the primary cause of rising temperature and depleted ozone layers. as a result, harmful UV rays penetrate the earth and the issue of sunburn occurs. Many people are more vulnerable to such radiations and thus produce high levels of sunburn, inflammation; itching [1]. The sunscreens are built to cure this problem. There are different chemical sunscreens available nowadays. Except in the case of hypersensitive individuals, they also have several other side effects on the skin. Melanoma and squamous cell carcinoma can be avoided with sunscreen formulations. Photo defense is carried out by flavonoids, polyphenols, tannins, alkaloids, anthraquinones and antioxidants [2]. The present study tries to establish a sunscreen lotion with The diverse array of anti-UV radiation at different concentrations from the extracts of *Tridax procumbens* (compositae), *Portulaca grandiflora* (portulacacea), Aloe vera (liliaceae), *Malus domestica* (rosaceae). The main ingredient is *Tridax procumbens* while *Portulaca grandiflora* acts as a secondary ingredient [3-6]. *Portulaca grandiflora* also serves as a hepatoprotective, antidiabetic, antiulcerogenic, anticancer. While aloe vera does not have any of its own sun

protection, it cures the damage caused by sunlight and it can treat sunburns also [3]. Aloe vera also helps to heal canker sores, reduces the level of blood sugar, lowers dental plaque, and decreases constipation. Quercetin, 0.2 percent tannins, flavonoids, *Malus domestica* phenolics help block UV rays and shield the skin from harmful UV radiation. Therefore, it is used to treat sunburns in the form of juice [7-15]. SPF stands for Sun Protection Factor, which is a measure of how well sunscreen protects the skin from UV B rays, a form of radiation that causes sunburn, damages the skin and can lead to skin cancer [16-26].

The SPF15 or SPF30 sunscreens are recommended by dermatologists. There is not much more protection from the higher SPF. There are several other variables that will impact the level of security as well, such as [17-30]:

- a) Amount of sunscreen applied by you
- b) The weather circumstances
- c) How much you're sweating
- d) How long you swim and how well afterward you dry off
- e) Skin type

## MATERIALS AND METHODS

### Collection and Identification

The plant material *Tridax procumbens* Linn (Compositae), *Portulaca grandiflora*

(portulacaceae), *Malus domestica* (rosaceae), *Aloe vera* (liliaceae) was collected and certified by the Dept. of Botany, Y.C.I.S, Satara, Maharashtra, India, during the month of July 2019 from the Sangli District, Maharashtra.

#### Extraction

The dried shade of *Tridax procumbens*, *portulaca grandiflora* leaves, was extracted by maceration technique with ethanol. The ethanol extract was filtered for dryness and evaporated. *Malus domestica* and *Aloe vera* were extracted by pulp removal technique.

#### Phytochemical Examination:

The general phytochemicals identification tests were performed on the extract.

1) *Tridax procumbens* contains high amount of flavonoids:

**Shinoda test:** To dry extract, add 5ml of 95% ethanol, few drop of concentrated hydrochloric acid and 0.5 g of magnesium turning. The finally pink color observed.

To a small quantity of extract, add lead acetate solution, it shows yellow colored precipitate formed [5]

2) *Portulaca grandiflora* contains high amount Alkaloids and Tannins -

**Dragondorff's Test:** To dry extract add 5 ml of Dragondorff's reagent (solution of potassium bismuth iodide) to gives orange colored precipitate (alkaloid present)

**Test 2-**Take 1 gm powered extract and add 10 ml of distilled water. the mixture is

boiled for five minute and add 2 drops of 5%  $FeCl_3$  to produce greenish precipitate (tannin present) [3]

#### 3) Fruits of *Malus domestica* contains high amount of flavonoids-

**Test 1-** To a small quantity of extract, add lead acetate solution, it shows yellow colored precipitate formed (lead sub acetate test)

#### 4) *Aloe Vera* contains high amount of amino acid-

**Ninhydrin Test-**To dry extract add few drops of Ninhydrin reagent to gives blue – violet positive result.

**Test 2-**Boil the test material in a test tube for 5 min with 1 ml of dilute sulphuric acid (Anthracene glycoside is hydrolyzed by boiling with acids into aglycone and sugars). Centrifuge the filter while the lower dichloromethane layer is separated by hot, filtrated, cooled and shaken with equal volume of dichloromethane (aglycone dissolves ideally in dichloromethane). Separate the lower dichloromethane layer and shake with dilute ammonia at half its volume to give the ammonia layer a rose-pink red tint [4].

#### Instruments

Instruments used for analysis were pH meter (Labline, digital pH meter), Brookfield viscometer, Microwave synthsizer, UV visible spectrophotometer (UV1280-Shimadzu Japan)

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### Formulation of Herbal Sunscreen Lotion:

**1) Oil phase-** Precise weighting of stearic acid, cetyl alcohol, starch, aluminum oil, titanium dioxide, zinc oxide. They are heated to 80°C at a constant temperature with continuous stirring.

**2) Water phase-** Accurate quantity of water was measured and taken in 500 ml beaker. Add triethanolamine in water and stirred. The water solution was heated upto a temperature of 80 °C

The oil phase is added into the water phase after both phases are ready at 80°C constant stirring for 20-25 minutes before the homogenized phase is formed. The sunscreen lotion prepared for cooling was held aside. Then the weighed quantity of Aloe extract, *Malus domestica pulp*, *Tridax procumbens ethanolic extract* and *Portulaca grandiflora* were added and well combined until all the ingredients were mixed evenly. Finally, rose water introduced a total of three formulation batches as a flavoring agent, as F1, F2 and F3 were prepared using separate formulas tabulated as **Table 3**.

#### In vitro SPF determination method:

Kaur *et al* 2011 and Ashawat *et al* 2006 studied the in vitro screening approach. The herbal lotion solution was prepared with 95% ethanol. For each sample, absorbance was measured at 290-320 nm at an interval

of 5 nm using a visible UV spectrophotometer [11, 12].

The SPF was calculated by using the following formula:

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

Where CF is correction factor,  $EE(\lambda)$  is erythrogenic effect of radiation with wavelength  $\lambda$  and  $Abs(\lambda)$  is spectrophotometric absorbance values at wavelength at  $\lambda$ . The values of  $EE(\lambda) \times I(\lambda)$  are constant. The results are summarized in the **Table 4**.

#### Evaluation of herbal sunscreen lotion:

**1. Physical parameters:** Appearance, color, texture and homogeneity of the formulations were determined [13]. The results are tabulated in the **Table 06**.

**2. pH determination:** The pH of the formulations was calculated by a digital pH meter from Labline. The pH meter has been calibrated using the pH 4, 7 and 9 phosphate buffer solution. After calculating the pH of each formulation, the electrodes were thoroughly washed with distilled water [14]. The pH of the formulations was directly measured while dilutions were also prepared. In Table No.6, the results are shown.

**3. Viscosity determination:** The viscosity of the formulations was determined by a Brookfield viscometer choosing the correct spindle (spindle no.4) at 6 rpm by maintaining a constant torque [15]. The

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formulations viscosity was measured using the following formula and the results are shown in **Table 6**.

Viscosity = Dial reading = Factor

**4. Spreadability test:** The two glass slides of the 20-5 scale have been chosen. On one slide, a particular amount of lotion was put and another slide was placed over the 1st slide so that between these two slides the lotion was sandwiched. The 100gm weight was placed on the slide and, with the aid of a spatula, the excess lotion was scrapped off. The weight was removed afterward. The lower slide was tightly held and a weight of 70 gm was tied and put on the slide. For each formulation, the test was repeated 3 times, and the results were reported [15]. The formula used for spreadability was  $S = ML/T$  and **Table 06** shows the results.

Where S = spreadability, M = weight tied to the upper slide, L = length of the slide and T = time. Here, M = 70 and L = 7.5cm we used.

**5. Thermal stability:** This test was carried out at 60-70 percent relative humidity (RH) and at a temperature of  $37 \pm 10^\circ\text{C}$  in the humidity chamber. In the beaker, the specified quantity of the mixture was taken and put in the humidity chamber [15]. There should not be phase separation to pass the examination. The findings are summarized in **Table 06**

**6. Rancidity:** Rancidity is the decomposition of fats, oils and other lipids by chemicals. Rancidity affects the product's appearance, odor and flavour [13]. These fatty acids showed pink color in the presence of hydrochloric acid and phloroglucinol solution, as tabulated in Table No. 06

**7. Dye test:** It combined the scarlet red dye with the lotion. On a microscopic slide, a drop of this mixture was mounted, covered with a cover slip and examined under a microscope.<sup>20</sup> If the backdrop is colorful and scattered globules are colorless, then the substance is o/w type and vice versa.

**8. Irritancy test:** The examination on the left hand was conducted. On the left hand dorsal surface, an area of 1sq.cm was numbered, the lotion was applied and time was noted. Irritancy, erythema and edema were tested and reported at regular intervals of up to 24 hours.<sup>16</sup> The findings are shown in **Table 06**

**9. In vitro antioxidant activity:** The DPPH (1, 1 diphenyl 2 picryl hydrazyl) method calculated this activity. 1 ml of each formulation was taken and added to the vial; 5 ml of DPPH methanol solution was added. The vials were put at  $37^\circ\text{C}$  for 30 minutes in an incubator. After 30 minutes, the absorbance at 516 nm was measured against blank methanol. The absorption of DPPH was used to monitor

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[17, 18]. The % antiradical activity was calculated by using the following formula:

$$\% \text{ antiradical activity} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

The results are shown in **Table 06**

#### 10. Stability study by centrifugation test:

Sunscreens were centrifuged at 500 rpm for an interval of 10 minutes at 4000-12000 rpm and evaluated for phase separation [19]. The results are recorded in **Table 06**.

### RESULTS AND DISCUSSION

Sunscreens are used for the treatment of sunburns; erythema is caused by UV rays and often protects the skin from UV B rays. The sunscreen's efficiency is dependent on the SPF number. It is also used on the basis of the form of skin, which is clarified in **Table 1**.

The most important aspect in the formulation is pH. The skin's pH is acidified. Acidic pH is lost as a result of washing the skin with soap. Therefore, by providing a moisturizing effect, the formulation should be acidic and also normalize the skin. Any change in the product's pH can cause chemical reactions to occur. Sunscreen viscosity was measured by a Brookfield viscometer at 100 rpm using spindle no.7. The viscosity should not be too high to make it tough to extract the substance from the container and also not too low to make the formulation more aqueous. One of the essential parameters is viscosity, too. The temperature is a critical

factor as well. The substance should remain stable at various temperature ranges. The formulations were placed for a specified period of time in a humidity chamber at 60-70 percent RH and 37°C. As a consequence, all formulations were found to be thermally stable and no phase separation was found. As a consequence, all formulations were found to be thermally stable and no phase separation was found. It is mandatory for any pharmaceutical product to achieve thermal stability. As the formulation involves the oil process, there is a possibility of reaction to oxidation. Any alteration of color, odor, and texture causes rancidity that is not approved pharmaceutically. The potency of the formulation diminishes because of rancidity. In this analysis, phloroglucinol and hydrochloric acid solutions determined the rancidity of the sunscreen. The production of the pink color indicates that rancidity is present. The formulations against rancidity were observed, and no pink color was detected. It is also said that the formulations are free from rancidity. The shape of the formulation is also a required factor that deals with the type of skin directly. It is given in table no. It was verified by performing the dye test that the

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formulation is of the type w/o. A small amount of formulation was combined with the methyl red dye and a drop of this mixture was placed on the microscopic slide, covered with the cover slip and examined under the microscope. It was noticed that colorful and scattered globules were colorless in the background. Every sunscreen or pharmaceutical product should be irritant-free. Skin reactions such as redness, itching, swelling, erythema, etc. do not occur. The formulation was added to the left hand dorsal surface and left for a particular period of time. No discomfort was shown in the formulation. And it was therefore found that the sunscreen is safe to use even in sensitive individuals. The *Malus domestica* extract and aloe vera have antioxidant activity. Antioxidant action, which is beneficial to the skin, is important. The adverse chemical reaction which produces free oxygen radicals and damages the skin is oxidation. The test was conducted using the method of DPPH and the results were reported. There is antioxidant activity in the prepared sunscreen. The UV defense activity is further improved by the antioxidant properties of flavonoids and phenolic compounds. Antioxidants provide endogenous photo security and are important for the protection and maintenance of healthy skin. The product

should be stable with respect to any factor. It has already been established that the formula is thermally stable. But to achieve the necessary stability, the centrifugation test must be carried out. The sunscreen passed the exam by conducting the centrifugation stability test. The determination of SPF values is influenced by many things, such as the use of various solvents in which the sunscreen is dissolved, the chemicals used in the formulation, the combination and concentration of the ingredients, the pH system, the vehicle's contact with the skin, the addition of other active ingredients, and several other factors. The sunscreen should have The diverse array of absorption between 290 nm and 400 nm to evaluate the correct prevention of sunburn and other skin damage. The proposed spectrophotometric UV method is clear, fast, and economical which is used in many other cosmetic formulations for in vitro determination of SPF values. The lotion formulation has strong sunscreen activity with SPF in the current analysis. Extracts rich in flavonoids, phenolics, tannins and quercetin have outstanding properties and are much more essential for sun protection. The presence of antioxidants prevents oxidative damage and structural changes in the skin as well. Not only are good SPF levels considered for sunscreen UV safety,

but the SPF of other ingredients, such as diluents, should also be considered. In order to manufacture non-irritable, non-toxic, healthy sunscreen, the concentration

of phytoconstituents and associated details should also be known.

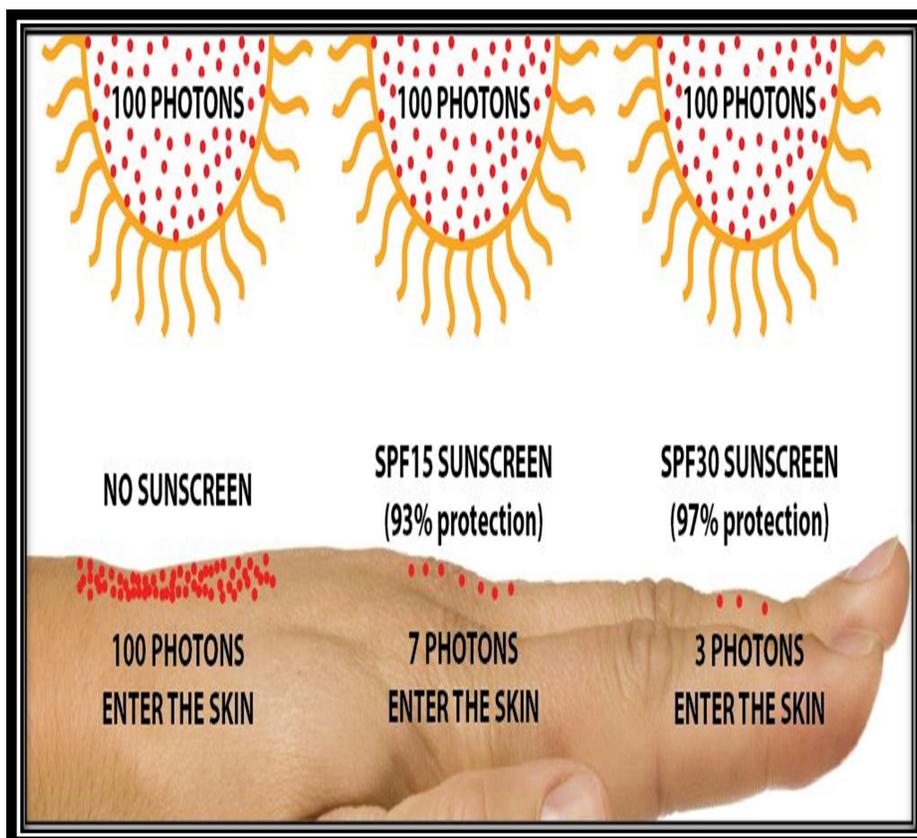


Figure 1: Effect of SPF Number on Skin

Table 1: SPF and Blockage of UV rays

SPF	Blockage of UV B rays
15	93%
30	97%
50	98%
100	99%

Table 2: Skin type and SPF

Skin complexion	Sun's effect on the skin	Recommended SPF
Very fair	Always burns easily, never tans	30-50+
Fair	Always burns easily, tans minimally	30-50+
Light	Burns moderately, tans eventually	15-30
Medium	Burns minimally, always tans well	6-15
Dark	Rarely burns, tans readily	2-10
Very dark	Never burns, becomes deeply pigmented	2-10

Oily skin : sunscreens with lighter base ( lotion or base )  
 Dry skin : sunscreens with moisturizing base ( cream or ointment )

Table 3: Composition of various sunscreen formulations

SR.NO	INGREDIENTS	F1	F2	F3	ROLE
1	Stearic acid	0.85 gm	0.85 gm	0.85 gm	Emollient, co emulsifier
2	Cetyl alcohol	0.35gm	0.35gm	0.35gm	Emollient, co emulsifier
3	Starch	0.15gm	0.15gm	0.15gm	Diluent, binder
4	Almond oil	1.5ml	1.5ml	1.5ml	Moisturizer
5	Benzoic acid	0.002gm	0.002gm	0.002gm	Preservative
6	Triethanolamine	0.1gm	0.1gm	0.1gm	Surface active agent
7	SLS	0.1gm	0.1gm	0.1gm	Solubilising agent
8	Rose water	q.s	q.s	q.s	Flavouring agent
9	Water	2 ml	3 ml	2 ml	Vehicle
10	EX .Malus domestica	3 ml	1.5 ml	3ml	Herbal Drug
11	EX. Aloe vera	1.5 ml	2 ml	1.5 ml	Herbal Drug
12	Ex. <i>Tridax procumbens</i>	0.3 gm	0.2gm	0.3gm	Herbal Drug
13	Ex. <i>Portulaca grandiflora</i>	0.2 gm	0.3 gm	0.2 gm	Herbal Drug

Table 4: Sun Protection Factor of the formulations

Wavelength ( $\lambda$ ) nm	EE ( $\lambda$ ) $\times$ I ( $\lambda$ ) (normalized)	EE ( $\lambda$ ) $\times$ I ( $\lambda$ ) $\times$ Abs ( $\lambda$ )		
		F1	F2	F3
290	0.015	0.02178	0.02643	0.01884
295	0.0817	0.18812	0.15507	0.13875
300	0.02874	0.07791	0.73298	0.69059
305	0.3278	1.14589	1.18562	0.94973
310	0.1864	0.67803	0.72832	0.58759
315	0.0839	0.34474	0.38711	0.33628
320	0.018	0.07666	0.09076	0.07505
Total	0.74154	2.53313	3.30629	2.79684
		SPF = 25.33	SPF = 33.06	SPF = 27.96

Table 5: Absorbance of the formulations at various wavelengths ( $\lambda$ )

Wavelength ( $\lambda$ ) nm	EE ( $\lambda$ ) $\times$ I ( $\lambda$ ) (normalized)	Absorbance ( $\lambda$ )		
		F1	F2	F3
290	0.015	1.452	1.762	1.2561
295	0.0817	2.3029	1.898	1.6983
300	0.02874	2.7108	2.5504	2.4029
305	0.3278	3.4957	3.6169	2.8973
310	0.1864	3.6375	3.9073	3.1523
315	0.0839	4.1089	4.6139	4.0081
320	0.018	4.259	5.0423	4.1695

Table 6: Summary of diverse pharmaceutical evaluation parameters and Antioxidant Activity

Parameters	Formulated Batches		
	F1	F2	F3
Appearance	Lotion	Lotion	Lotion
Color	Light green	Light green	Light green
Homogeneity	Uniform and homogenous	Uniform and homogenous	Uniform and homogenous
Texture	Smooth	Smooth	Smooth
Consistency	Good	Good	Good
pH	5.43	6.08	5.67
Viscosity	30400	32500	28700
Spreadability	Good	Good	Good
Thermal stability	Thermally stable (No phase separation)	Thermally stable (No phase separation)	Thermally stable (No phase separation)
Rancidity	No pink color, No rancidity	No pink color, No rancidity	No pink color, No rancidity
Irritation	No irritation	No irritation	No irritation
% In vitro antioxidant test	23.57	50.63	46.3
Centrifugation test	No phase separation	No phase separation	No phase separation

	(Stable)	(Stable)	(Stable)
SPF by UV spectrophotometer	25.33	33.06	27.96

## CONCLUSION

The outcome of the current research work concludes that, compared to F1 and F3, the formulated lotion F2 offers effective protection against UV rays and also has antioxidant activity and emollient action. The method used for SPF determination is quick, simple and cost-effective and requires fewer reagents.

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