Determination of Sunscreen activity of *Viola odorata* (Banafsha) ethanolic extract and its formulated Gel by UV Spectroscopy

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**Article History:**
Received on: 26.09.2019
Revised on: 05.11.2019
Accepted on: 10.11.2019

**Keywords:**
Viola odorata, Banafsha, SPF, herbal gels, Natural sunscreen

**ABSTRACT**

In the present study, ethanolic extract of Banafsha i.e., *Viola odorata* (Violaceae) and its gel formulations was evaluated for its sun protection activity by ultraviolet spectroscopy method. The phytoconstituents like flavonoids, phenolics present in *Viola odorata* may be responsible for scavenging action on UV-induced reactive oxygen species. Extraction of aerial parts of the plant was done in the Soxhlet apparatus using ethanol as solvent. 1ml, 2ml, and 4ml of ethanol extract (200 µg/ml) of the plant were used to formulate three gels (F1-F3) using Carabopol 940, 0.5% Methyl paraben, 0.2% Propyl paraben, Propylene glycol 400 and Triethanolamine. Mansur *et al.* UV spectrophotometric method was used to investigate the *in vitro* SPF of the *Viola odorata* ethanolic extracts (100&200 µg/ml) and its formulated gels (F1-F3). Physical parameters like color, appearance, spreadability, pH, homogeneity, viscosity were determined to evaluate formulated gels. Based on the calculated SPF value, F3 formulation was chosen for the stability study. Ethanolic extract of *Viola odorata* (100 µg/ml and 200 µg/ml) have SPF value 4.05±0.07 and 11.66±0.04, respectively. Gel formulations (F1-F3) containing 1, 2,4ml of 200 µg/ml of ethanol extract have SPF values about 2.89±0.11, 4.20±0.08, and 5.63±0.07, respectively. The SPF values determined to confirm the capacity of gels to absorb UV radiation. No significant alterations in physicochemical parameters and SPF values were observed during the stability evaluation of F3 gel. Results proves that *Viola odorata* is a safe alternative which can be used over harmful chemical sunscreens used now a days in the industry and in the future plant can be explored for active component for better protection against sun rays.

**INTRODUCTION**

Sunlight includes the major portion of electromagnetic radiation, and these solar radiations comprises 50% of visible light (400-800 nm), 40% of infrared radiation (1300-1700 nm), and 10% of ultraviolet radiation UV (10-400 nm) (*Donglikar and Deore, 2016*).*UV* radiation are portion of electromagnetic radiation lying between X-rays and visible (200-400 nm) and are divided into 3 parts on the basis of the wavelength (*Jou et al., 2012*).

1. **UV-A region** (320 nm - 400 nm) responsible for skin darkening i.e., tanning due to excess of melanin production.
2. **UV-B region** (280 nm - 320 nm) the rays emitted from this part produces 1000 times more sunburn as compared to another region hence also called as burning rays.
3. UV-C region (200 nm – 280 nm) the rays of this region are less harmful.

UV radiation exposure foster skin inflammatory responses accompanied by pigmentation, hyperplasia, erythema, immune-suppression, photocarcinogenesis, and photoaging (Narayanan et al., 2010). The mutagenic effect of Ultraviolet radiation is the major factor responsible for skin cancer. Sunscreens are cosmetic preparations which protect skin from damage caused by the harmful effect of sunlight radiation and are of two types, i.e., oral/systemic sunscreens and topical sunscreens. Topical sunscreens includes organic compounds (Benzophenones, Avobenzone, Cinnamates, Salicylates, and PABA derivatives); inorganic compounds (kaolin, titanium dioxide, zinc oxide, magnesium oxide) and natural compounds (Polyphenols, curcumin, lycopene, and volatile oils). Systemic sunscreens on oral administration show photoprotective action. Phenolics, flavonoids, tannins, carotenoids, vitamins present in plants provide sun protection due to their antioxidant effect (Donglikar and Deore, 2016).

The ideal sunscreen product must provide protection to skin from adverse consequences of UV rays. It should be non-allergic, non-toxic, non-irritating with good skin permeation. The Sun protection factor (SPF) is used to investigate the efficiency of topical sunscreen preparations, which is defined as the UV energy required to produce a minimal erythemal dose (MED) in protected skin, divided by the UV energy required to produce a MED in unprotected skin and given by the following equation,

\[
SPF = \frac{\text{MED in sunscreen protected skin}}{\text{MED in non - sunscreen protected skin}}
\]

MED is a minimum time interval or dosage of UV light irradiation sufficient to produce a minimal perceptible erythema on unprotected skin (Sayre et al., 1979). Many regulatory authorities, such as USFDA and COLIPA (The European Cosmetic Tolley and Perfumery Association), mandate in-vivo testing on human subjects, using an erythematous endpoint to determine the SPF of a topical sunscreen. Being costly and time-consuming in-vivo tests may not be practically feasible for routine product evaluation (Sayre et al., 1979). Therefore in vitro methods are used for screening purposes and also to reduce the risk related to UV exposure to human subjects. One of them involves the measurement of absorption or transmittance of ultraviolet radiation by placing the sunscreen product film in the quartz plates, and the method includes spectrophotometric measurement of absorbance (Mansur et al., 1986). Varieties of chemicals are incorporated in sunscreen formulations, which show specific absorbance in the Ultraviolet spectrum. Plant extracts usually covers full range on UV spectrum as they contain a wide range of phytoconstituents such as polyphenols, flavonoids, carotenoids, vitamin A, Ascorbic acid, essential oil, etc. Herbs commonly employed in sunscreen preparations includes Aloe vera, almond, jojoba, cucumber, etc. (Ashawat et al., 2006). They are efficacious over synthetic chemicals because of their free radical scavenging action. Hence plants can be an ideal approaches and should be explored as sunscreen agents (Saraf et al., 2009; Saewan and Jimtaisong, 2015).

Viola odorata Linn, commonly known as sweet violet or Banafsha family Violaceae, is an evergreen perennial herb and is widely distributed at an altitude of 1500 to 1800 m in Kashmir and western Himalayan regions. It contains saponins, salicylates, alkaloids, flavonoids, tannins, phenolics, and coumarins (Mittal et al., 2015). Because of the presence of high content of flavonoids and phenolic compounds, this plant may be utilized for the prevention of the formation of UV-induced oxygen free radicals (Ebrahimzadeh et al., 2010). The present work strives to determine the sun protection factor (SPF) of Viola odorata ethanolic extract and its formulated gels by the Ultraviolet spectroscopy method.

MATERIALS AND METHODS

**Instruments**

pH meter (101 EI), Brookfield viscometer (LV model), PC based Double Beam Spectrophotometer (Systronics – 2202 ), and Stability chambers (Remi) were used in the study.

**Reagents**

Methyl paraben (Loba Chemie), Propyl paraben (Loba Chemie), Carbopol 940 (Loba Chemie), Propylene glycol 400 (Loba Chemie) and Triethanolamine (Fischer Scientific) were purchased for the study. Ethanol (Merck) analytical grade was used.

**Plant material and Preparation of extract**

Viola odorata aerial parts were purchased from the local market of Patiala and authenticated in the Department of Pharmacognosy, Chitkara University Punjab, India, and its voucher specimen was deposited in Chitkara University Punjab for future reference. The plant was powdered and extracted in Soxhlet assembly with ethanol (60-80°C) for 6 h and filtered through whatman filter paper and concentrated by hot plate and dried at a temperature not exceeding 40±2°C to obtain a solid residue.
Table 1: Composition (% w/w) of gel containing Viola odorata ethanol extract.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F0 gel</th>
<th>F1 gel</th>
<th>F2 gel</th>
<th>F3 gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbopol 940</td>
<td>1gm</td>
<td>1gm</td>
<td>1gm</td>
<td>1gm</td>
</tr>
<tr>
<td>Methyl Paraben (0.5%)</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Propyl Paraben (0.2%)</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Propylene glycol 400</td>
<td>2ml</td>
<td>2ml</td>
<td>2ml</td>
<td>2ml</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>1ml</td>
<td>1ml</td>
<td>1ml</td>
<td>1ml</td>
</tr>
<tr>
<td>Viola odorata extract (200 (\mu g/ml))</td>
<td>1ml</td>
<td>2ml</td>
<td>4ml</td>
<td></td>
</tr>
<tr>
<td>Distilled water (q.s)</td>
<td>100 ml</td>
<td>100 ml</td>
<td>100 ml</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

*Amaranth was added as a colouring agent

Table 2: Evaluation of Physical parameters of gels.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>F0 gel</th>
<th>F1gel</th>
<th>F2 gel</th>
<th>F3 gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>Pinkish</td>
<td>Pinkish brown</td>
<td>Brownish pink</td>
<td>Brownish pink</td>
</tr>
<tr>
<td>2.</td>
<td>Appearance</td>
<td>Clear transparent</td>
<td>Clear transparent</td>
<td>Clear translucent</td>
<td>Clear translucent</td>
</tr>
<tr>
<td>3.</td>
<td>pH</td>
<td>6.5</td>
<td>6.7</td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td>4.</td>
<td>Homogeneity</td>
<td>No aggregates</td>
<td>No aggregates</td>
<td>No aggregates</td>
<td>No aggregates</td>
</tr>
<tr>
<td>5.</td>
<td>Viscosity(cps)</td>
<td>1680</td>
<td>1630</td>
<td>1605</td>
<td>1600</td>
</tr>
</tbody>
</table>

The ethanol extract was subjected to a qualitative test for the identification of various phytoconstituents (Kokate, 2014).

The extract (100 mg) was accurately weighed and dissolved in 100 ml of ethanol in a volumetric flask to form 1mg/ml concentration. From this 1 ml and 2 ml of solution was withdrawn and diluted to 10 ml further to produce 100 \(\mu g/ml\) and 200 \(\mu g/ml\), respectively.

**Preparation of Gel**

F0-F3 gel formulations were prepared, as shown in Table 1.

**Physicochemical evaluation of gels**

**Organoleptic parameters**

In organoleptic parameters, appearance, color, transparency, and smoothness of gel were studied (Baird, 1997).

**pH**

1gm of the gel was dispersed in 100 ml of distilled water and dissolved. pH of the solution was evaluated by digital pH meter (Kumar and Verma, 2010). All measurements were taken in triplicate and calculated as mean.

**Spreadability**

Spreadability was observed by spreading 1 g of formulation on a clean even glass surface (Kumar and Verma, 2010).

**Homogeneity**

The formulated gels were investigated for homogeneity by visual inspection for their appearance and presence of any aggregates (Kumar and Verma, 2010).

**Viscosity**

Viscosity profile (cps) of each formulation was determined using a Brookfield viscometer at 10 to100 rpm, at a temperature of 25°C using LV spindle 64 (Patel et al., 2009). All measurements were taken in triplicate and calculated as mean.

**Determination of the in vitro sun protection factor**

The ethanolic extract of Viola odorata (100 \(\mu g/ml\), 200\(\mu g/ml\)) and formulated gels (F0-F3) containing Viola odorata extract were estimated for the in vitro SPF. For comparative evaluation, one marketed formulations having known SPF 50 were purchased from the market.1 g of the gel was accurately weighed and transferred to a volumetric flask of 100ml capacity, diluted to volume with ethanol followed by ultrasonication for 5 min. The resulting mixture is filtered through cotton; the first, 10 ml of filtrate was rejected. A 5.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol. Then 5.0 ml of aliquot was
Table 3: Determination of SPF value of *V. odorata* extract and its gel formulations (F1-F3)

<table>
<thead>
<tr>
<th>S.no</th>
<th>Wave length</th>
<th>EE*I (Normalized)</th>
<th>Viola odorata ethanol extract (100µg/ml)</th>
<th>Viola odorata ethanol extract [200µg/ml]</th>
<th>F0 gel</th>
<th>F1 gel</th>
<th>F2 gel</th>
<th>F3 gel</th>
<th>Marketed sunscreen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>290</td>
<td>0.0150</td>
<td>0.364±0.002</td>
<td>1.114±0.002</td>
<td>0.028±0.003</td>
<td>0.249±0.002</td>
<td>0.312±0.037</td>
<td>0.525±0.002</td>
<td>2.62±0.004</td>
</tr>
<tr>
<td>2.</td>
<td>295</td>
<td>0.0817</td>
<td>0.388±0.004</td>
<td>1.142±0.023</td>
<td>0.051±0.001</td>
<td>0.268±0.004</td>
<td>0.388±0.027</td>
<td>0.531±0.003</td>
<td>2.75±0.002</td>
</tr>
<tr>
<td>3.</td>
<td>300</td>
<td>0.2874</td>
<td>0.401±0.011</td>
<td>1.158±0.033</td>
<td>0.083±0.011</td>
<td>0.279±0.001</td>
<td>0.401±0.051</td>
<td>0.548±0.001</td>
<td>0.021±0.001</td>
</tr>
<tr>
<td>4.</td>
<td>305</td>
<td>0.3278</td>
<td>0.410±0.004</td>
<td>1.169±0.043</td>
<td>0.095±0.002</td>
<td>0.285±0.004</td>
<td>0.432±0.041</td>
<td>0.565±0.012</td>
<td>0.001±0.003</td>
</tr>
<tr>
<td>5.</td>
<td>310</td>
<td>0.1864</td>
<td>0.422±0.003</td>
<td>1.172±0.035</td>
<td>0.120±0.001</td>
<td>0.292±0.023</td>
<td>0.450±0.062</td>
<td>0.585±0.023</td>
<td>0.004±0.003</td>
</tr>
<tr>
<td>6.</td>
<td>315</td>
<td>0.0837</td>
<td>0.423±0.023</td>
<td>1.186±0.043</td>
<td>0.132±0.022</td>
<td>0.305±0.012</td>
<td>0.487±0.013</td>
<td>0.605±0.003</td>
<td>0.001±0.003</td>
</tr>
<tr>
<td>7.</td>
<td>320</td>
<td>0.0180</td>
<td>0.467±0.003</td>
<td>1.195±0.023</td>
<td>0.135±0.002</td>
<td>0.307±0.003</td>
<td>0.521±0.043</td>
<td>0.636±0.013</td>
<td>0.002±0.002</td>
</tr>
<tr>
<td>SPF</td>
<td></td>
<td>4.05±0.07</td>
<td>11.66±0.04</td>
<td>0.98±0.06</td>
<td>2.89±0.11</td>
<td>4.20±0.08</td>
<td>5.63±0.07</td>
<td>5.98±0.12</td>
<td>29.91±0.12</td>
</tr>
</tbody>
</table>

All values are represented as mean±SD (n=3)

Table 4: SPF determination during stability study

<table>
<thead>
<tr>
<th>Day</th>
<th>SPF value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.63±0.07</td>
</tr>
<tr>
<td>3 months (at 25°C and 60%±5% RH)</td>
<td>5.59±0.11</td>
</tr>
<tr>
<td>3 months (at 40°C and 75%±5% RH)</td>
<td>5.14±0.23</td>
</tr>
</tbody>
</table>

All values are represented as mean±SD (n=3)

transferred to a volumetric flask of capacity 25 ml and diluted with ethanol. Then spectrophotometer readings of each aliquot were taken in wavelength ranging from 290 to 320 at 5nm interval, and absorbance were measured. SPF was calculated from the formula given by Mansur et al. (1986) by utilizing values given by (Mansur et al., 1986; Sayre et al., 1979) SPF was calculated thrice and then means the value was taken in consideration. In vitro SPF can be calculated by the following equation:

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

Where CF is the correction factor and is equal to 10
EE(\lambda) is erythmogenic effect of radiation with wavelength \( \lambda \)
Abs(\lambda) is spectrophotometric absorbance value at a wavelength

The values of EE X I are constant and were determined by Sayre et al. (1979) and are given in Table 3.

**Stability study**

Based on the calculated SPF value, F3 formulation was chosen for the stability study. F3 gel was stored at room temperature (25°C and 60%±5% RH) for 3 months, and SPF value was determined.
The yield of ethanol extract of *Viola odorata* was observed to be 2.8%. Phytochemical screening revealed that alkaloids, carbohydrates, glycosides, saponins, flavonoids, and polyphenols are present in the plant. In the present study, ethanol extract of *viola, odorata* were subjected for SPF determination by the ultraviolet spectroscopic method. Ethanol extract of *Viola odorata* (100 μg/ml and 200 μg/ml) have SPF value 4.05±0.07 and 11.66±0.04, respectively. 1ml, 2ml, and 4ml of ethanol extract (200μg/ml) of the plant were used to formulate three gels (F1-F3) using Carabopol 940, 0.5% Methyl paraben, 0.2% Propyl paraben, Propylene glycol 400 and Triethanolamine (Figure 1). Prepared gel formulations (F0-F3) were evaluated for color, appearance, spreadability, pH, homogeneity, and viscosity, and results are summarised in Table 2.

The in vitro SPF of the formulated gels (F0-F3) was determined according to the UV spectrophotometric method of *Mansur et al.* (1986) and is shown in Table 3. For comparative evaluation, one marketed formulations having known SPF 50 was also done. Gel formulations (F1-F3) containing 1, 2, 3ml of 200μg/ml of the ethanolic extract have SPF val-

**RESULTS AND DISCUSSION**

Figure 1: Representative photographs of control gel (F0), and formulated gels (F1, F2, and F3) containing 1 ml, 2 ml and 4 ml of ethanolic extract (200 μg/ml) of *V. odorata*.
ues about 2.89±0.01, 4.20±0.08, and 5.63±0.07, respectively. The result revealed that gels have the ability to absorb UV radiation and hence confirmed its sun protection activity. SPF determination of marketed sunscreen and its comparison with claimed value confirmed the reliability and suitability of the method.

During stability studies of F3 gel, no significant changes in colour, appearance, spreadability, pH, homogeneity, and viscosity was observed. No phase separation was observed in F3 gel during centrifugation indicating the stability of gel under stressed conditions. No significant changes in SPF value were observed during stability evaluation (Table 4).

CONCLUSIONS

Result obtained in the study revealed that Viola odorata have significant UV absorbing property. Sunscreen property of the Viola odorata extract may be due to the presence of polyphenols, flavonoids, and carotenoids. Results proved that Viola odorata can be a safer and cheaper alternative which can be used over harmful chemicals in various sunscreen formulations and in a future plant can be explored further for the isolation of active component responsible for protection against sun rays.

ACKNOWLEDGEMENT

The authors are thankful to Chitkara University, Punjab India for giving necessary research facilities to carry out this project.

REFERENCES


Cleveland Clinic Journal of Medicine, 79(6):427–436.


