Research Article

IN VITRO SUN PROTECTING FACTOR, PHOTOSTABILITY, CHEMICAL STABILITY AND PHYTOCHEMICAL SCREENING OF *DELONIX REGIA* FLOWER EXTRACT GEL

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ABSTRACT

Delonix regia flowers Due to its antioxidant and photoprotective properties, a promising for use in cosmetic and pharmaceutical formulations. A high performance liquid chromatography method was used to evaluate the chemical stability using *Delonix regia* extract as marker at 5, 25 and 45 °C for 103 days. The sun protection factors were analyzed by ultraviolet (UV) spectrophotometry using samples irradiated with UVB lamp. The chemical stability of the *Delonix regia* flower extract gel was determined according to the concentration of *Delonix regia* flower extracts at different storage temperatures (5, 25 and 45 °C) for 103 days. It is screened for In vitro sun protection factor in the *Delonix regia* flower extract and of its gel formulation and determines Photostability of the isolated *Delonix regia* flower extract and SPF. This study has shown that the *Delonix regia* flower extract gel is stable for at least 2 to 3 months when stored at 5 and 25°C. This proved activity of plant showed its importance and prophylactic utility in anti- solar formulation.

Keywords: UV protective, SPF, Photostability, Chemical stability and *Delonix regia*.

INTRODUCTION

The various herbal formulation and chemicals are available to block various ranges of UV rays which prevent all types of skin from various damages. Our investigation is to study and find out such flowers that are widely used as sunscreen from ancient time. Therefore, we report here the promise of the *Delonix regia* flower extract in cosmetic formulations; there are no prior data available about several aspects of the cosmetic formulation.

Every year, more than one million people are diagnosed with skin cancer and about 10,000 die from malignant melanoma. Most skin cancers occur on the areas that are most frequently exposed to the sun, such as the face, neck, and the back of the hands.¹⁻⁴

The red color in *Delonix regia* flower is most probably due to co-pigmentation between anthocyanins and other flavonoid, the color of the reddish-yellow flowers is mostly attributed to an increase in the isosalipurposide concentration, along with an increase in the background of the yellowish cytoplasmic carotenoids. The red color in *Delonix regia* flower is most probably due to co-pigmentation between anthocyanins and other flavonoid.⁵⁻⁷

MATERIALS AND METHOD

Chemicals and Reagents

Analytically pure sample of Carbapol 940, Propylene glycol, Methyl paraben were obtained from Sahyadri Scientific and Research Lab, Islampur, Maharashtra, India. Trietanalamine, Methanol, Ethanol (AR Grade) were obtained from Rajesh Chemicals Co., Mumbai, Maharashtra, India.

Methods

Delonix regia were obtained from trees available in our college campus in Miraj Maharashtra, India. Flower were selected for uniform size, color, level of petals, and divided into equal parts. The petals of gulmohor flower were percolated methanol: water (1:1) (100 ml/g of petals of flower) and the extract was freeze-dried. The final concentration of the *Delonix regia* in the crude extract was 7.1% (w/w), as evaluated by HPLC with electrochemical detection.^{8,9}

Formulations

For the chemical stability study, gel formulation containing *Delonix regia* flower extract with final concentration of 0.1% (w/w) and 1.5% (w/w) of carbapol 940 was prepared. All formulations were stored in well-closed dark glass flasks and were compounded fresh for all studies. The concentration was the minimal active antioxidant concentration. A formulation was prepared with the addition of active ingredient % (w/w) which is shown in Table 1.

Table 1: Composition (% w/w) of gel formulations used for the chemical stability study and for the determination of SPF

Active Ingredients	Quantity
Carbapol 940	1.5 mg
Propylene glycol	10 ml
Trietanalamine	0.5 ml
Methyl paraben	0.25 mg
<i>Delonix regia</i> flower extract	1.42 mg
Distilled water qs	100 ml

Physicochemical parameters of the extract gel

Physicochemical parameters of the extract gel were determined according to the standard method which is shown in Table 2

Table 2: Physicochemical parameters of the extract gel

Parameters	%w/w (±) S.D.
Foreign organic matter	0.047 % ± 0.211
Methanol soluble extractive	13.21 % ± 0.3977
Water soluble extractive	39.25 % ± 1.524
Total ash	5.57 % ± 0.258
Acid-soluble ash	1.68 % ± 0.233
Acid-insoluble ash	9.1 % ± 0.977
Loss on drying	4.45 % ± 0.285
Moisture content	9.19 % ± 9.19 % ± 0.168

Chemical stability study

The stability of *Delonix regia* flower extract over time and the influence of temperature on the degradation of *Delonix regia* flower extract gel without and in the presence of antioxidant were investigated. Gel formulations were stored in well-closed 10 g dark glass flasks under different conditions: 5, 25 and 45°C (±1°C). The amount of crude extract in samples was quantitatively determined at 2 to 3 months stability studies. Briefly, 1.0 ml of distilled water and 10 ml of hexane were added to 50 mg of the samples. A fraction of

the hexane layer was evaporated under nitrogen, dissolved in methanol and analyzed by HPLC with electrochemical detection.

Flavonoid identification test

The general flavonoid identification test was performed on the extract as previously described.¹⁰

Determination of the in vitro sun protection factor

The crude *Delonix regia* flower extract, the gel formulation (1.5% carbapol 940) containing *Delonix regia* flower extract were analyzed for the in vitro SPF. The crude *Delonix regia* flower extract gel formulation was dissolved in methanol solvent: water (6:4). Scans of the samples in solution were run from 320 to 290 nm using 1 cm quartz cuvettes in a Jasco UV-1700 spectrophotometer. The commercial sunscreens, Himalaya® SPF 15, were used for the calculation of the correction factor and a solution of 8% homosalate (v/v) diluted to 0.2 mg/ml was used as standard. The SPF model used in this study was based on the following equation proposed by Mansur et al.¹¹

The SPF model used in this study was based on the following equation proposed by Mansur et al.

$$SPF = CF \times \sum_{290}^{320} EE\lambda \times I(\lambda) \times abs(\lambda) \quad (1)$$

Where CF is correction factor, determined by sunscreens with known SPF, so that a solution containing 8% of homosalate gives SPF = 8; $EE(\lambda)$ the erythemal efficiency spectrum; $I(\lambda)$ the solar simulator spectrum as measured with a calibrated spectroradiometer.

$$\sum_{290}^{320} EE(\lambda) \times I(\lambda) = 290 - 320nm \quad (2)$$

Where, 290–320 nm in 5 nm increments; $abs(\lambda)$ is the spectroradiometer measure of sunscreen product absorbance. Table 2 shows the normalized values of the product function used in these studies and were calculated.^{12-14, 16}

RESULTS AND DISCUSSION

Chemical stability of the *Delonix regia* flower extracts gel formulation

The chemical stability of the *Delonix regia* flower extract gel was determined according to the concentration of *Delonix regia* flower extracts at different storage temperatures (5, 25 and 45 °C) for 103 days. The final concentration was expressed as micrograms extracts per gram of gel formulation. All samples stored at 5 and 25 °C were stable over the time of experiment (103 days). All of them showed an initial decrease (20%) between days 0 and 1 and then remain constant over time. The samples stored at 45°C were stable for 7 days but then a degradation of gel structure was observed.

Determination of the correction factor

The correction factor was calculated for commercial sunscreen (Himalaya® SPF 15) using Eq. (1) data given in Table 2 and the total SPF given in Table 3.

Table 3: The normalized product function used in the calculation of SPF data

λ (nm)	$EE \times I$ (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
	=1.000

EE: erythemal efficiency spectrum; I: solar simulator intensity spectrum

Determination of SPF in the *Delonix regia* extract and of its gel formulation

According to Table 4 summarizes the SPF values determined for each solution described. As expected. In vitro SPF value for the *Delonix regia* extract was 1.2±0.1. When 1.42% *Delonix regia* extract was added to the carbapol 940 gel formulations, the SPF value was 3.57±0.05.

Table 4: SPF calculated for commercial sunscreens (Himalaya® SPF 15) using Eq. (1) and data given in Table 2

λ (nm)	EE×I (normalized)	Himalaya® SPF 15	
		Absorbance	SPF
290	0.0150	0.7943	0.0198
295	0.0817	0.7723	0.0676
300	0.2874	0.7625	0.2145
305	0.3278	0.7443	0.2434
310	0.1864	0.7167	0.1356
315	0.0839	0.6906	0.0578
320	0.0180	0.6688	0.0199
Total			0.7586

EE: erythral efficiency spectrum; I: solar simulator intensity spectrum.

Table 5: Results expressed as the average and S.D. of three determinations replicated of the SPF values

Sample	SPF
<i>Delonix regia</i> flower extract	1.2±0.1.
<i>Delonix regia</i> flower extract gel	3.57±0.05.

Photostability of the isolated *Delonix regia* extract

A methanol solution of 10 μ g/ml *Delonix regia* extract was irradiated with a UVB lamp. Absorbance spectra of the *Delonix regia* extract solution were stable over time of irradiation (Figure. 1). Here Absorbance spectra of mm ethanol solution of 10 μ g/ml *Delonix regia* extract: (A) just after preparation and (B) after 3 weeks of UVB irradiation. All values are means of three replicated experiments. The concentration difference between times was considered not significant in the statistical analysis.

Phytochemical Screening

The preliminary phytochemical investigation was carried out for petroleum ether (80-40) chloroform, 80% alcoholic & aqueous extract of flowers of *Delonix regia* for the detection of various phytoconstituents by following standard methods. The results was shown in Table 6.¹⁵

Table 6: Qualitative Phytochemical Tests

Tests for Phytoconstituents	Result
Test for reducing sugar	
1) Fehling's test	+++
2) Benedict's test	++
Test for proteins	
1) Biuret test	++
2) Million's test	+++
Test for amino acid	
1) Test for tyrosine	++
Test for cardiac glycoside	
1) Baljet's test	+++
Test for flavonoid	
1) Shinoda test	+++
2) Sodium hydroxide test	++
Test for alkaloids	
1) Dragendroff's test	++
2) Mayer's test	+++
3) Hager's test	++
4) Wagner's test	+++

Note: '+++' indicates highly significant, '++' indicates significant, '+' indicates presence.

Figure 1: Absorbance spectra of a methanol solution of 10 μ g/ml *Delonix regia* flower extract just after preparation **Figure 2: Absorbance spectra of a methanol solution of 10 μ g/ml *Delonix regia* flower extract after 3 weeks of UVB irradiation**

CONCLUSION

The result obtained were showed that ability of extract to absorb UV radiation and hence proved UV protection ability. This study has shown that the *Delonix regia* flower extract gel is stable for at least 2 to 3 months when stored at 5 and 25°C or Sometime heat is a possible factor responsible for the gel degradation over time. Further, isolated extract have, the major antioxidant of *Delonix regia*, is also stable when exposed to UVB irradiation. It is essential for collection of similar data for different plant and there flowers, as well as other parts. This proved activity of plant showed its importance and prophylactic utility in anti-solar formulation. This will be a better, cheaper and safe alternative to harmful chemical sunscreens that used now a day in the industry.

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