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In vitro evaluation of SPF, photostability and chemical stability of *Carica papaya L.* fruit extract gel

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ABSTRACT

In the present study, to investigates its chemical stability and the in vitro sun protection factor (SPF) of Carica papaya L fruit extract in a gel formulation. Due to its antioxidant and photo protective properties, Carica papaya L is a promising candidate for use in cosmetic and pharmaceutical formulations. A high performance liquid chromatography method was used to evaluate the chemical stability using Carica papaya L 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 Carica papaya L extract gel was determined according to the concentration of Carica papaya L extracts at different storage temperatures (5, 25 and 45 ◦C) for 103 days. It is screened for In vitro sun protection factor in the Carica papaya L extract and of its gel formulation and determinesPhoto stability of the isolated Carica papaya L extract and SPF. This study has shown that the Carica papaya L fruit extract gel is stable for at least 2 to 3 months when stored at 5 and 25oC. 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.

Key words: UV protective, SPF, Photostability, Chemical stability and *Carica papaya L.*

INTRODUCTION

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. [1,2]

Exposed sun UV light is classified into three types, by its wavelengths as UV-A, UV-B and UV-C. The dimensions of their wavelength are roughly 400-320nm for UV-A, 320-290nm for UV-B and 209-200nm for UV-C. Although it may be observed that the shorter wavelength and the lower the number, the greater the energy level of the light and the more damage it can do. Direct exposure to UV-C for a length of time would destroy the skin. Fortunately, UV-C is completely absorbed by gases in the atmospheres before it reaches the ground. In any time the longer wavelength of UV-B and UV-A pass right through the atmosphere. The molecules in sunscreen absorb most of UV-Band prevent it from reaching the skin just as the molecules of the atmospheres absorbs UV-C and prevent it from reaching the ground [3-6]

The *CaricaPapaya L* (Caricaceae) is a powerhouse of nutrients and is available throughout the year. It is a rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber. In addition to all this, it contains a digestive enzyme-papaintha effectively treats causes of trauma, allergies and sports injuries. All the nutrients of papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer. The fruit is an excellent source of beta carotene that prevents damage caused by free radicals that may cause some forms of cancer. Ripe fruit consumed regularly helps in habitual constipation. It is also reported that papaya prevents premature aging. It may be that it works because a poor digestion does not provide enough nutrients to our body. The fruit is regarded as a remedy for abdominal disorders. The skin of papaya works as a best medicine for wounds. Even you can use the pulp left after extracting the juice from papaya as poultice on the wounds. The enzymes papain and chymopapain and antioxidant nutrients found in papaya have been found helpful in lowering inflammation and healing burns. The presence of vitamin A helps to restore and rebuild damaged skin. Applied papaya peel used as skin lightening agent. When peel mixed with honey and applied it can act as soothe and moisturizers the skin.[7]

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 fruit that are widely used as sunscreen from ancient time.

Therefore, we report here the promise of the *Carica Papaya L* fruit extract in cosmetic formulations; there are no prior data available about several aspects of the cosmetic formulation. The goals of this research are to evaluate, its stability at 2 to 3 months stored at 5, 25 and 45 ◦C; the in vitro sun protection factor; the Photostability of the isolated *Carica Papaya L* extract.

MATERIALS AND METHODS

Materials and extract preparation

Papaya fruit (*Carica Papaya L*) were obtained from a local market in Sangli, Maharashta, India, and they were botanical authenticated at Dept. of botany Y.C College, Karad (MS). Fruit were selected for uniform size, color, level of external ripeness, and divided into four ripeness stages: RS1 re- presents papaya with yellow area on 0% - 25% of the skin; RS2 with >25% - 50% color; RS3 with >50% - 75%, and RS4 with >75% - 100%. The papaya with yellow soft area of fruit flower were percolated ethanol: water(1:1) (100 ml/g of dried yellow soft area of fruit) and the extract was freeze-dried. The final concentration of the C. papaya L in the crude extract was 7.1% (w/w), as evaluated by HPLC with electrochemical detection.[8]

Formulations

For the chemical stability study, gel formulation containing *Carica Papaya L* fruit extract with final concentration of 0.1% (w/w)and 1.5% (w/w) of carbopol 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
Triethanolamine	0.5 ml
Methyl paraben	0.25 mg
C. papaya L fruit extract	1.42 mg
Distilled water qs	100 ml

Physiochemical 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	0.040 % ± 0.211
Ethanol soluble extractive	12.41 % ± 0.417
Water soluble extractive	38.11 % ± 1.425
Total ash	5.57 % ± 0.258
Acid-insoluble ash	1.89 % ± 0.133
Acid-insoluble ash	8.9 % ± 0.977
Loss on drying	4.15 % ± 0.285
Moisture content	9.55 % ± 0.267

Chemical stability study

The stability of *Carica Papaya L* fruit extract over time and the influence of temperature on the degradation of *Carica Papaya L* fruit 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^{0}C (±1^{0}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 ethanol and analyzed by HPLC with electrochemical detection.

Flavonoid identification test

The general flavonoid identification test was performed on the extract as previously described. [9]

Determination of the in vitro sun protection factor

The crude *Carica Papaya L* fruit extract, the gel formulation (1.5% carbopol 940) containing *Carica Papaya L* fruit extract were analyzed for the in vitro SPF. The crude *Carica Papaya L* fruit extract gel formulation was dissolved in methanol UV solv:water (6:4). Scans of the samples in solution were run from 320 to 290 nm using 1 cm quartz cuvettes in a Jascco 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. [10]

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

$$SPF = CFX \sum_{290}^{320} EE\lambda\ X\ I(\lambda) X\ abs\ (\lambda) \tag{1}$$

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

$$\sum_{290}^{320} EE(\lambda) X I(\lambda) = 290–320\ \mathrm{nm} \tag{2}$$

Where, 290–320 nm in 5 nm increments; abs(λ) 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. [10, 11]

RESULTS AND DISCUSSION

Chemical stability of the Carica Papaya L fruit extracts gel formulation

The chemical stability of the *Carica Papaya L* fruit extract gel was determined according to the concentration of *Carica Papaya L* fruit 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.

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

λ (nm)	EE×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 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.

Determination of SPF in the Carica Papaya L 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 *Carica Papaya L* extract was 1.88±0.01. When 1.42% *Carica Papaya L* extract was added to the cabapol 940 gel formulation, the SPF value was 16.047±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: erythemal efficiency spectrum; I: solar simulator intensity spectrum.

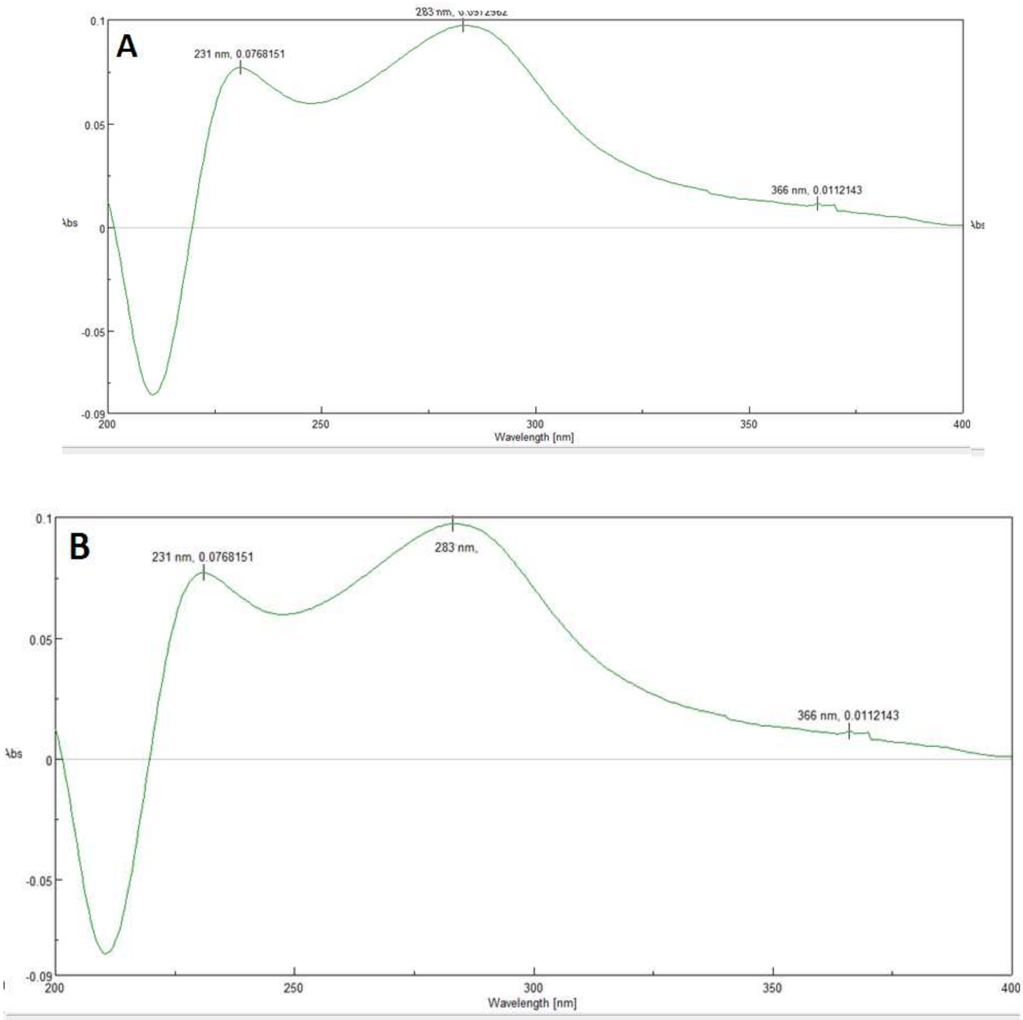

Figure 1. Absorbance spectra of a ethanol solution of10µg/ml Carica Papaya L extract: (A) just after preparation and (B) after 3 weeks of UVB irradiation

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

Sample	SPF
*Carica Papaya L*fruitextract gel	1.88±0.01.
Crude *Carica Papaya L*extract	16.047±0.05

Photostability of the isolated Carica Papaya L extract
An ethanol solution of 10µg/ml *Carica Papaya L* extract was irradiated with a UVB lamp. Absorbance spectra of the *Carica Papaya L* extract solution were stable over time of irradiation (Figure. 1). Here Absorbance spectra of aethanol solution of10µg/ml *Carica Papaya L* 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.

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 *Carica Papaya L* fruit 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 *Carica Papaya L,* 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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