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Chemical stability and SPF determination of *Pothomorphe umbellata* extract gel and photostability of 4-nerolidylcathecol

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Abstract

Due to its antioxidant and photoprotective properties, *Pothomorphe umbellata* is a promising candidate for use in cosmetic and pharmaceutical formulations. These properties arise from the presence of 4-nerolidylcathecol (4-NC), a polyphenolic compound isolated from *P. umbellata* roots. This study investigates its photostability properties, as well as the chemical and the in vitro sun protection factor (SPF) of *P. umbellata* root extract in a gel formulation. A high performance liquid chromatography method was used to evaluate the chemical stability using 4-NC as marker at 5, 25 and 45 °C for 103 days. The photostability and the sun protection factor were analyzed by ultraviolet (UV) spectophotometry using samples irradiated with UVB lamp. No significant difference of the 4-NC concentration was found in formulations stored at 5 and 25 °C. All samples stored at 45 °C, however, showed degradation of gel structure. After 2 h of UVB exposure, there was no change in the absorption profile of 4-NC. The sun protection factor of *P. umbellata* root extract gel to final concentration of 0.1% 4-NC was not expressive (SPF = 3.35 ± 0.02), suggesting the predominance of its antioxidant activity.

Keywords: *Pothomorphe umbellata*; 4-Nerolidylcathecol; Stability; Photostability; Sun protection factor

1. Introduction

Chronic exposure of human skin to solar ultraviolet (UV) radiation may cause several skin damages. These damages include sunburn, skin cancer, oxidative stress as well as photoaging depending on the amount and form of the UV radiation and on the type of the individual exposed (Ichihashi et al., 2003; Melnikova and Ananthaswamy, 2005).

There has been an increasing interest in the use of antioxidants in sunscreens to provide supplemental photoprotective action activity. Antioxidants from

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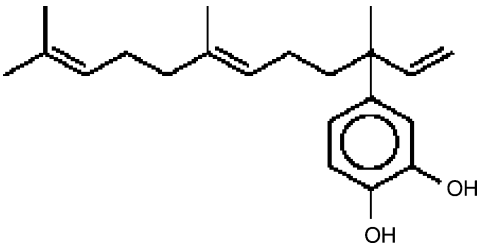

Fig. 1. 4-Nerolidylcathecol (Kijjoa et al., 1980).

natural sources may provide new possibilities for the treatment and prevention of UV-mediated diseases (Bonina et al., 1996; Saija et al., 1998; F'guyer et al., 2003). The Brazilian flora is rich in medicinal plants with a high potential for providing these antioxidants. Among them, the Piperaceae family, and in particular, the "pariparoba", is widely used in Brazilian folk medicine for the treatment of liver diseases. The root of *Pothomorphe umbellata* (L.) Miq was included in the first edition of the *Brazilian Pharmacopeia* (Silva, 1926).

Different experimental models displayed a significant activity of crude root ethanolic extracts of *P. umbellata* as antioxidant and photoprotective agent (Barros et al., 1996; Desmarchelier et al., 1997; Ropke et al., 2000). This activity was attributed to 4-nerolidylcathecol (4-NC) (Fig. 1), a compound isolated from the roots and leaves of *P. umbellata* (Kijjoa et al., 1980). In our previous study, skin alpha-tocopherol concentrations diminished (≈40%) in irradiated hairless mice control groups, but were totally preserved in the *P. umbellata* treated group (≈100%) (Ropke et al., 2003). Further, a cutaneous absorption study using four topical formulations (4-NC gel, 4-NC gel emulsion, 4-NC emulsion and crude root extract *P. umbellata* gel) showed that a higher concentration of 4-NC in the skin was found in the 4-NC gel formulation (Ropke et al., 2002). Even at a lower rate of percutaneous absorption of crude root extract *P. umbellata* gel, a total reactive antioxidant potential study showed that it has a higher antioxidant potential than the isolated 4-NC, suggesting the presence of additional compounds with antioxidant activity (Desmarchelier et al., 1997). An in vivo study showed that *P. umbellata* root extract gel was able to effectively reduce the incidence of visible and histological skin alterations in chronically UVB irradiated mice (Ropke et al., 2005).

Despite the promise of the *P. umbellata* root extract in cosmetic formulations, there are no data available about several aspects of the formulation. The goals of this research are to evaluate: (1) its stability at 0, 1, 3, 7, 37, 44, 81 and 103 days stored at 5, 25 and 45 °C; (2) the in vitro sun protection factor; (3) the photostability of isolated 4-NC over UVB exposure for 30, 60, 90 and 120 min. These experiments were carried out using a carbomer 940 (1.5%, w/w) gel of the *P. umbellata* root extract to final concentration of 0.1% 4-NC (w/w).

2. Material and methods

2.1. Test materials

Methanol, ethanol, hexane licrosolv grade, methanol UVsolv, KCl p.a., homosalate (Eusolex® HMS) were from Merck (Darmstadt, Germany). $LiCLO_4$ was from Aldrich (Milwaukee, WI). 4-Nerolidylcathecol was isolated as described previously from dried powdered roots of *P. umbellata* (Gustafson et al., 1992). Carbomer 940 (Carbopol® 940), propylene glycol, trietanolamine and methyl paraben were from Galena (Campinas, Brazil). Butylated hydroxytoluene (BHT) was from Sigma–Aldrich (St. Louis, MO). Sundown® SPF 15 was from Johnson & Johnson (batch number: lot 04, Brazil) and Helioblock® SPF 30 was from (batch number: bB080, La Roche Posay, France).

2.2. Extract preparation

The plant material was identified and a sample was deposited in the Herbarium of the Institute of Biosciences, University of São Paulo, Brazil. Powdered roots were percolated according to the *Brazilian Pharmacopeia* (Silva, 1926), with ethanol–water (1:1) (100 ml/g of dried powdered root) and the extract was freeze-dried. The final concentration of the 4-NC in the crude extract was 7.1% (w/w), as evaluated by HPLC with electrochemical detection as previously described (Ropke et al., 2002).

2.3. Formulations

For the chemical stability study, gel formulation containing *P. umbellata* root extract with final concentration of 0.1% (w/w) for the 4-NC and 1.5% (w/w)

Table 1
Composition (%, w/w) of gel formulations used for the chemical stability study (F1 and F2) and for the determination of SPF (F1)

	F1	F2
Carbomer 940	1.5	1.5
Propilene glycol	10	10
Trietanolamine	0.5	0.5
Methyl paraben	0.25	0.25
Butylated hydroxytoluene	–	0.05
P. umbellata root extract	1.41	1.41
Distilled water qs	100	100

of carbomer 940 was prepared. This concentration of 4-NC was chosen because it was the minimal active antioxidant concentration found in our previous study (Ropke, 1999; Ropke et al., 2000). A second formulation was prepared with the addition of 0.05% (w/w) of BHT (Table 1).

Two series of 1.5% (w/w) carbomer 940 hydrogels, with and without 0.05% (w/w), were prepared by neutralizing an aqueous dispersion of the carbomer with trietanolamine under constant stirring. The hydrogels were kept at room temperature. Then, the *P. umbellata* root extract was weighted and small amounts of the gel formulation were added until total gel weight was added under constant stirring. The gel was once more neutralized with trietanolamine under constant stirring. The final pH of the formulation was 5.0. All formulations were stored in well-closed dark glass flasks and were compounded fresh for all studies.

Determination of the sun protection factor (SPF) was carried out using the first gel formulation described above (without BHT) as well as the crude *P. umbellata* root extract. The isolated 4-NC was used for the photostability study.

2.4. Chemical stability study

The stability of 4-NC over time and the influence of temperature on the degradation of *P. umbellata* root extract gel without and in the presence of antioxidant were investigated. Gel formulations (with and without BHT) were stored in well-closed 10 g dark glass flasks under different conditions: 5, 25 and 45 °C (±1 °C). The amount of 4-NC in samples was quantitatively determined at 0, 1, 3, 7, 37, 44, 81 and 103 days (Ribeiro et al., 1996; Carlotti et al., 2002). The 4-NC was extracted from the gel formulation using a modification of the procedure described by Burton et al. (1985). 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 as described previously (Ropke et al., 2002).

2.5. Determination of the in vitro sun protection factor

The crude *P. umbellata* root extract, the gel formulation (1.5% carbomer 940) containing *P. umbellata* root extract with final concentration of 0.1% (w/w) of 4-NC and the isolated 4-NC were analyzed for the in vitro SPF. The crude *P. umbellata* root extract and the gel formulation were dissolved in methanol UVsolv:water (6:4) and the isolated 4-NC was dissolved in methanol UVsolv to 0.2 μg/ml. Scans of the samples in solution were run from 320 to 290 nm using 1 cm quartz cuvettes in a Hitashi DU-3210 spectrophotometer (Sayre et al., 1979). Two commercial sunscreens, Sundown® SPF 15 and Helioblock® SPF 30, were used for the calculation of the correction factor and a solution of 8% homosalate (v/v) diluted to 0.2 μg/ml was used as standard.

The SPF model used in this study was based on the following equation proposed by Mansur et al. (1986):

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

where CF is correction factor, determined by two sunscreens with known SPF, so that a solution containing 8% of homosalate gives SPF = 8; EE(λ) the erythemal efficiency spectrum; $I(\lambda)$ the solar simulator spectrum as measured with a calibrated spectroradiometer; $\sum_{290}^{320}\mathrm{EE}(\lambda) \times I(\lambda) = 290$–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 by Sayre et al. (1979).

2.6. Photostability of 4-nerolidylcathecol

A methanol solution of 30 μg/ml 4-NC, in a 1 cm quartz cuvette perfectly stoppered, was irradiated with a Philips TL12rs 40W UVB lamp at a fixed distance (26 cm). UV irradiation was performed just after the

Table 2
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 (Sayre et al., 1979).

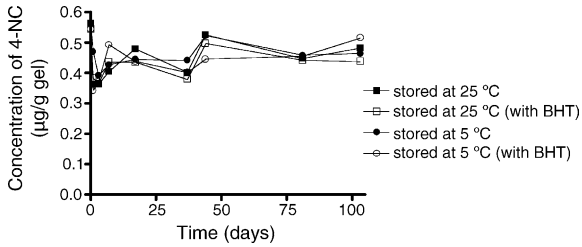

Fig. 2. Concentration of 4-NC (μg/g gel) in both formulations: without BHT and with BHT stored at 5 and 25 °C for 103 days. Data are the mean of three samples. Error bars were omitted to improve clarity. The difference between times was considered not significant.

preparation ($t = 0$) and at the following times: 30, 60, 90 and 120 min, with doses of 206, 412, 618 and 824 mJ/cm^2, respectively (Ioele et al., 2005). The total energy output of the lamp was measured using an International Light radiometer 1700 IL equipped with a UVB SED sensor. The content of 4-NC in each solution was determined quantitatively by HPLC with UV detection. The HPLC equipment consisted of a Consta Metric 3200 pump and a Linear Instruments model 525 spectrophotometric detector (λ = 282 nm) with a 7161 Rheodyne injector equipped with a 20 μl loop. Integration of the chromatographic peaks was achieved with an SP 4600 Thermoseparation Products integrator. Chromatography was performed on a Phenomenex C18 (3.9 mm × 300 mm) 10 μm column with a mobile phase of methanol–water (9:1). The flow rate was set at 1.0 ml/min. Each sample was filtered through 0.22 μm cellulose acetate filters (Costar®) and an aliquot of 20 μl was injected into the HPLC. UV spectra were performed in a Hitashi DU-3210 spectrophotometer.

2.7. Statistical analysis

Data were analyzed statistically by factorial analysis of variance (ANOVA). The Tukey–Kramer test was then used to determine significant differences between groups.

3. Results and discussion

3.1. Chemical stability of the P. umbellata extract gel formulation

The chemical stability of the *P. umbellata* root extract gel was determined according to the concentration of 4-NC at different storage temperatures (5, 25 and 45 °C) for 103 days. The final concentration was expressed as micrograms of 4-NC per gram of gel formulation. The results, plotted in Fig. 2, show the mean values of three samples for each time and temperature of storage.

Carbomer frequently interacts with cationic drugs and excipients due to its numerous carboxylic acid groups (Blanco-Fuente et al., 2002). In vitro studies using carbomers 934 and 940 showed that its interaction with substances commonly used in the pharmaceutical industry, such as lidocaine and mebeverine hydrochloride, was a function of pH, drug, polymer concentration and electrolytes (Al-Gohary and Foda, 1993; Jimenez-Kairuz et al., 2002; Glavas-Dodov et al., 2002). The 4-NC recovery, using water to break the carbomer 940 gel structures, was 60%. In order to improve the recovery, we used other solutions with different pH and amount of electrolytes, and higher volumes of hexane on the 4-NC extraction but none of these conditions showed better results.

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. Addition of 0.05% (w/w) of BHT did not influence the stability. The samples stored at 45 °C were stable for 7 days (data not shown) but then a degradation of gel structure was observed. Data are the mean of three samples for each day and each formulation. The difference between concentrations of 4-NC over time was considered not significant in the statistical analysis.

Table 3
SPF calculated for two commercial sunscreens (Sundown® SPF 15 and Helioblock® SPF 30) using Eq. (1) (Section 2.5) and data given in Table 2

λ (nm)	EE × *I* (normalized)	Sundown® SPF 15		Helioblock® SPF 30	
		Absorbance	SPF	Absorbance	SPF
290	0.0150	0.4413	0.0066	0.7940	0.0119
295	0.0817	0.4197	0.0343	0.7758	0.0634
300	0.2874	0.4068	0.1169	0.7648	0.2198
305	0.3278	0.3816	0.1251	0.7468	0.2448
310	0.1864	0.3672	0.0684	0.7190	0.1340
315	0.0839	0.3429	0.0288	0.6934	0.0582
320	0.0180	0.3130	0.0056	0.6690	0.0120
Total			0.3858		0.7441

EE: erythemal efficiency spectrum; *I*: solar simulator intensity spectrum (Sayre et al., 1979).

3.2. *In vitro sun protection factor in the P. umbellata extract and of its gel formulation*

3.2.1. *Determination of the correction factor*

The correction factor was calculated for each commercial sunscreen (Sundown® SPF 15 and Helioblock® SPF 30) using Eq. (1) (Section 2.5), data given in Table 2 and the total SPF given in Table 3. The final average correction factor is 39.60.

3.2.2. *Determination of SPF in the P. umbellata extract and of its gel formulation*

Table 4 summarizes the SPF values determined for each solution described. All values are means of three replicated determinations. As expected, the SPF observed for the 8% homosalate solution was approximately 8 (7.86 ± 0.12). Thus, in vitro SPF value for the crude *P. umbellata* root extract (7.1% of 4-NC) was 21.53 ± 0.04. When 1.41% *P. umbellata* root extract was added to the carbomer gel formulation, the SPF value was 3.35 ± 0.02. The SFP of the isolated 4-NC was 4.00 ± 0.59, suggesting the presence of additional compounds with sunscreen activity in the extract.

Table 4
Results expressed as the average and S.D. of three determinations replicated of the SPF values

Sample	SPF
Homosalate 8%	7.86 ± 0.12
P. umbellate extract gel 1.41%	3.35 ± 0.02
Isolated 4-nerolidylcathecol	4.00 ± 0.59
Crude *P. umbellate* root extract	21.53 ± 0.04

3.3. *Photostability of the isolated 4-nerolidylcathecol*

A methanol solution of 30 μg/ml 4-NC was irradiated with a UVB lamp. Absorbance spectra of the 4-NC

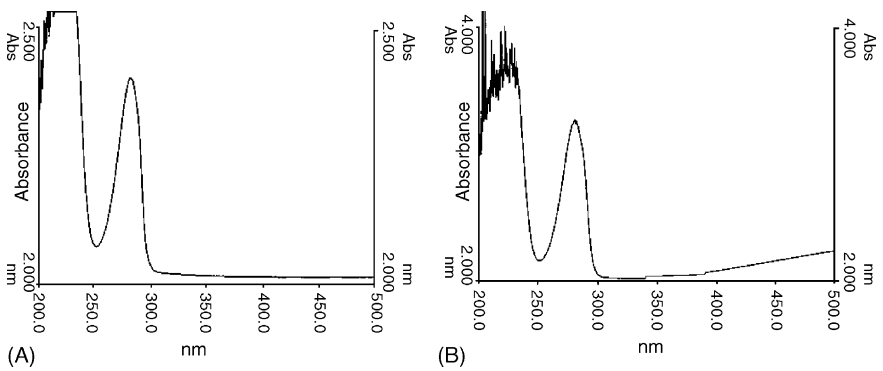

Fig. 3. Absorbance spectra of a methanol solution of 30 μg/ml 4-NC: (A) just after preparation and (B) after 60 min of UVB irradiation.

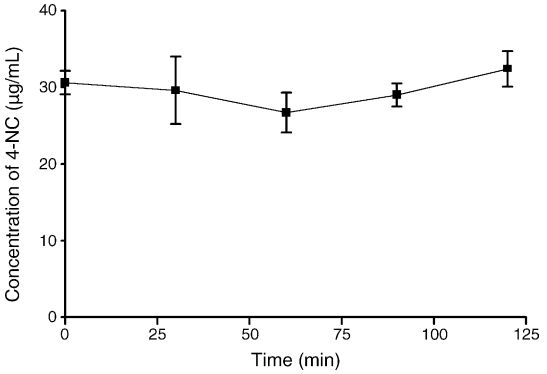

Fig. 4. Concentration of 4-NC in an irradiated methanol solution as a function of irradiation time. All values are means of three replicated experiments. The concentration difference between times was considered not significant.

solution were stable over time of irradiation (Fig. 3) as well as its concentration determined by HPLC with UV detection (Fig. 4). All values are means of three replicated experiments. The concentration difference between times was considered not significant in the statistical analysis.

4. Conclusions

This study has shown that the *P. umbellata* root extract gel is stable for at least 3 months when stored at 5 and 25 °C and that addition of BHT does not affect the stability. Heat is a possible factor responsible for the gel degradation over time. Further, 4-NC, the major antioxidant of *P. umbellata*, is also stable when exposed to UVB irradiation. Moreover, the low sun protection factor value observed for the *P. umbellata* root extract gel used in these experiments supports that the alpha-tocopherol preservation and photoprotective effects of the *P. umbellata* observed in our previous studies (Ropke et al., 2003, 2005) are due to its antioxidant activity.

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