Physicochemical characterization and *in vitro* evaluation of the photoprotective activity of the oil from *Opuntia ficus-indica* (L.) Mill. seeds

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*Opuntia ficus-indica* (L.) Mill. is a cactus widely cultivated in northeastern Brazil due to its enormous growth potential and multiple uses. This study aimed to perform physicochemical characterization and *in vitro* evaluation of the photoprotective potential in the region of ultraviolet B (UVB) radiation of the oil from *O. ficus-indica* (L.) Mill. seeds. For physicochemical characterization of the oil, the following techniques were used: thermal analysis (TA), infrared spectrometry (IR) and gas chromatography-mass spectrometry (GC-MS). The photoprotective potential was determined by UV spectrophotometry. It was observed that *O. ficus-indica* (L.) Mill. oil is rich in saturated and unsaturated fatty acids, primarily linoleic acid (~65%), which may be related to the protection against ultraviolet rays (UVB). However, the results of evaluation of *in vitro* sun protection factor (SPF) indicated that under the conditions studied the oil from the *O. ficus indica* (L.) Mill. seeds does not have photoprotection activity.

**Key words:** *Opuntia ficus-indica* (L.) Mill., phytochemistry, physicochemical characterization, fatty acids composition, photoprotection.

**INTRODUCTION**

*Opuntia ficus-indica* (L.) Mill. is a cactus native to Mexico’s arid regions and is widespread through South America, Australia, South Africa and throughout the Mediterranean region (Leo et al., 2010). Members of the cactus family are biologically adapted to resist intense sunlight, drought and extreme temperature variations...
through day and night. In the Northeast of Brazil, a common species is *O. ficus-indica* that has been used as animal feed (Barbera et al., 2001). In many countries *O. ficus-indica* fruits have been used in human food due to the sweet taste and high content of nutritional compounds, such as the ascorbic acid, polyphenols, amino acids, minerals, vitamins and others; besides these products are juicy (Leo et al., 2010; Ozcan and Al Juhaimi, 2011).

Current researches with this species revealed their antiviral, anticancer, anti-inflammatory, hypoglycemic, antioxidant and diuretic properties (Guevara-Arauza, 2009; Galati et al., 2003; Zou et al., 2005; Chavez-Santoscoy et al., 2009). Furthermore, studies on nutrition indicated that this seeds are sources of natural fibers and due to the high concentration of essential fatty acids, sterols, carotenoids and fat soluble vitamins, the oil seeds can be used as nutraceutical agent (Ramadan and Mörsel, 2003; Ozcan and Al Juhaimi, 2011).

It is remarkable that current studies with *O. ficus-indica* have been performed in the researches for development of new drugs and functional foods. However, there is a need of studies for physicochemical characterization of the oil from *O. ficus-indica* seeds (Ennouri et al., 2005) and demonstrate potential activities and employability of this raw material in the area of new cosmetic development, such as the study performed by Schmid et al. (2005) which created a moisturizing, soothing and protective from ultraviolet A radiation –UVA- product.

Species such as *O. ficus-indica*, which have phenolic constituents and fatty acid composition of its oils may be capable to absorb the ultraviolet light and when associated with the possible antioxidant activity can express photoprotective activity (Violante, 2009; Wagemaker et al., 2011). The photoprotective preparations for topical use are designed to protect the skin from the deleterious effects of solar radiation (Rangel and Corrêa, 2002). A promising tendency is the association of natural products in sunscreens to improve their photoprotective activity by intensifying the sun protection factor (SPF) into the final product (Ferrari et al., 2007). The SPF determination of sunscreens can be performed by *in vivo* or *in vitro* methods. In Brazil, *in vivo* method is highly recommended, according to the Resolution RDC n. 30/12, that presents the Mercosul technical regulation of sunscreens in the cosmetics area (Brasil, 2012). However, there are other *in vitro* methodologies developed which are based on absorbing or reflecting filter properties that can be used in a preliminary evaluation of the SPF during the development of formulations and for a routine quality control, bath to bath. These methods present the advantage of not requiring the use of human volunteers to determinate the SPF (Nascimento et al., 2009). The spectrophotometric method developed by Mansur et al. (1986), proved to be effective and fast, besides showing a good correlation with *in vivo* results that are phototests (Mansur et al., 1986; Ferrari et al., 2008). The present study aimed to perform physicochemical characterization and *in vitro* evaluation of the photoprotective potential in the region of UVB radiation of the oil from *O. ficus-indica* (L.) Mill. seeds cultivated in the Northeast of Brazil.

**MATERIALS AND METHODS**

**Extraction of vegetable oil**

Ripe fruits of *O. ficus-indica* (L.) Mill. were collected in Juazeirinho - PB (Brazil), on March, 2011 and identified by Ivan Coelho Dantas. The voucher specimen was stored at the plant Manoel de Arruda Camara Herbarium (ACAM) of the State University of Paraíba, under registration number 907. The seeds were dried in a forced air circulation at 40°C and subsequently crushed in a Whiley® knife mill. The oil from *O. ficus-indica* seeds was extracted with hexane in a Soxhlet apparatus for nine hours. The organic phase was removed using a rotary evaporator under reduced pressure.

**Physicochemical characterization**

**Analysis of fatty acids**

The fatty acid content of oil from *O. ficus-indica* seeds was determined by gas chromatography (GC) after the esterification process, according to the methodology of Maia (1992). This process consists of replacing the reactive hydrogen of the carboxylic acid by a methyl, in order to get a derived product, which could be measured with more sensitivity and exactly and easily separated from the interferents (Lança, 1993). Thus, a solution of acid (H2SO4) with methanol (MeOH) was used as esterifying reagent. The esterified oil was used only for the GC analysis. Qualitative analysis of the substances was carried out in gas chromatograph couple to a mass spectrometer (GC-MS, Shimadzu, QP-5000), equipped with a fused silica capillary column OV-5 (30 m × 0.25 mm × 0.25 μm Ohio Valley Specialty Chemical, Inc.), operating by electrons impact (70 eV). The analysis conditions were: injector: 240°C; detector: 230°C; carrier gas: He; flow rate: 1.0 ml min⁻¹; dilution: 1 μl fixed oil/1.0 ml ethyl acetate, volume injection: 1 μl; Split: 1/20. The oven temperature program was: 110°C (1 min), 110 to 170°C, 10°C/min; 170°C (2 min); 170 to 173°C, 1.5°C/min; 173 to 180°C, 1.0°C/min; 180°C (7 min); 180 to 230°C, 6°C/min; 230°C (20 min). Quantitative analysis was conducted in gas chromatography with flame ionization detector (GC-FID), using the same condition as stated. The identification of substances was performed by comparing their mass spectra with database system GC-MS (Nist. 62 lib.) and retention index (Adams, 2007) and comparison with commercial standards of methyl esters fatty acids.

**Thermal analysis (TA)**

The thermal stability was determined through the differential scanning calorimetry (DSC) using a DSC Q20 of the TA Instruments®. The heating rate was 10°C/min, under nitrogen atmosphere of 50 ml/min. A sample of 2 mg was weighed and subjected to a temperature range from 25 to 500°C. For the thermogravimetry (TG) an SDT Q600 was used, TA instruments® brand, with a heating rate of 10°C/min under nitrogen atmosphere (50 ml/min). There were weighed 8.5 mg of oil sample in alumina crucibles and subjected to a temperature range from 25 to 900°C. For the analysis of DSC and TG curves we used the TA Universal Analysis
Table 1. Composition of formulations for SPF evaluation.

<table>
<thead>
<tr>
<th>Composition</th>
<th>F1 (%)*</th>
<th>F2 (%)*</th>
<th>F3 (%)*</th>
<th>F4 (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aristoflex AVL®</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Methylparaben</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Oil of O. ficus-indica</td>
<td>-</td>
<td>-</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Octyl methoxycinnamate</td>
<td>-</td>
<td>7.5</td>
<td>-</td>
<td>7.5</td>
</tr>
<tr>
<td>Water</td>
<td>qsp</td>
<td>qsp</td>
<td>qsp</td>
<td>qsp</td>
</tr>
</tbody>
</table>

*Percent by weight (w/w). F1 = emulsion base; F2 = base + sunscreen, F3 = base + oil; F4 = base + oil + sunscreen.

Analysis software.

Infrared absorption spectroscopy (IR)

The spectrum of absorption in the mid-infrared region was obtained using the equipment PerkinElmer® (Spectrum 400) with attenuated total reflectance device with crystal of selenium (ATR). The analysis was performed with 16 scans and resolution 4 cm⁻¹ in the region between 4000 and 650 cm⁻¹.

Evaluation of the potential photoprotective

SPF evaluation of vegetable oil

The SPF evaluation followed the methodology proposed by Mansur et al. (1986). The SPF was measured at different oil concentrations (screening). For this, the oil from the O. ficus-indica seeds was dissolved in hexane (Merck®) analytical grade, at concentrations of 50, 40, 30, 20, 10, 5, 2, 1, 0.5 and 0.2 µl/ml. It a spectrophotometer UV/VIS (Shimadzu, model 1240) was used in quartz cuvette of 1.0 cm optical path in the range of 290 to 320 nm, at intervals of 5 nm. The absorbances obtained were added to the equation proposed by Mansur (Equation 1).

\[
\text{SPF} = \text{CF} \sum_{290}^{320} \text{EE}(\lambda) \cdot i(\lambda) \cdot \text{Abs}(\lambda)
\]

Equation 1. Calculation of SPF according to Mansur et al. (1986).

Where CF = correction factor (equal 10); EE(\lambda) erythemal effect of solar radiation at each wavelength \(\lambda\); i(\lambda) = sunlight intensity at wavelength \(\lambda\); Abs(\lambda) = spectrophotometric reading of sample’s absorbance at each wavelength.

The correction factor (CF) was determined so that a standard sunscreen formulation containing 8% homosalate presented a SPF value of 4, determined by UV spectrophotometry (Mansur et al., 1986). The relation between the erythemal effect and the sunlight intensity at wavelength (EE x i) is constant and was determined by Sayre et al. (1979). The experiment was performed in triplicate. The results of the SPF were expressed by the arithmetic mean of three determinations.

Evaluation of SPF of emulsions with oil of Opuntia ficus-indica (L.) Mill.

The SPF emulsion base was evaluated with 10% oil as well as the SPF photoprotective emulsion with 10% oil addition in order to verify the occurrence of SPF potentialization. As a vehicle for incorporating the derivative of O. ficus-indica self-emulsifying base, Aristoflex AVL® (Clariant Brazil), was used which is a mixture of emulsifiers, emollients and thickening polymer. The emulsions were prepared by the method of reverse phase cold and the preservative methylparaben and the surfactant polysorbate 80 were used as adjuvants. Octyl methoxycinnamate was used as synthetic sunscreen (Table 1). To evaluate the SPF of these formulations, 1.0 g of all samples was weighed, transferred to a 100 ml volumetric flask, diluted to volume with ethanol (Merck®) analytical grade, followed by ultrasonication for 5 min and then filtered through cotton, rejecting the first 10 ml. A 5.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol. Then a 5.0 ml aliquot was transferred to a 25 ml volumetric flask and the volume completed with ethanol. The absorption spectra of samples in solution were obtained in the range of 290 to 450 nm using 1 cm quartz cell, and ethanol as a blank. The absorption data were obtained in the range of 290 to 320, every 5 nm, and 3 determinations were made at each point, followed by the application of Mansur equation. The results of the SPF were expressed by the arithmetic mean of three determinations.

RESULTS AND DISCUSSION

Composition of fatty acids

The saturated and unsaturated fatty acids content of the oil from the seeds of O. ficus-indica can be viewed in Table 2. It was observed that the oil in this study has predominantly unsaturated fatty acids 79.61%. This result is in agreement with those of Ramadan and Mörsel (2003), who previously reported the major saturated and unsaturated fatty acids present in the oil of O. ficus-indica cultivated in Germany. The pattern of lipids in the cactus is comparable to that of sunflower (Helianthus annuus) and grape (Vitis vinifera) oils seeds (Tan and Che Man, 2000). Recently, Ennouri et al. (2005) reported levels of linoleic and palmitic acid superior to the present study: (74.00% vs. 64.78%) and (7.20% vs. 4.84%), respectively. However, to oleic acid, the concentration detected by this study was lower (12.80% vs. 14.83%). According to Faria et al. (2002), the proportion of different unsaturated and saturated fatty acids found in vegetable oil from the same species may vary according to climatic conditions and soil types in which these species are cultured. Epidemiologically, the linoleic acid (omega-6)
Table 2. Chemical composition of esterified oil from the seeds of *Opuntia ficus-indica*.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Substance</th>
<th>Saturation</th>
<th>Retention Time*</th>
<th>Relative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Undecane</td>
<td>-</td>
<td>3.2</td>
<td>5.52</td>
</tr>
<tr>
<td>2</td>
<td>n-Dodecane</td>
<td>-</td>
<td>3.8</td>
<td>1.95</td>
</tr>
<tr>
<td>3</td>
<td>n-Tridecane</td>
<td>-</td>
<td>4.1</td>
<td>2.60</td>
</tr>
<tr>
<td>4</td>
<td>Palmitic acid</td>
<td>C16:0</td>
<td>18.4</td>
<td>4.84</td>
</tr>
<tr>
<td>5</td>
<td>Linoleic acid</td>
<td>C18:2 (9c,12c)</td>
<td>27.3</td>
<td>64.78</td>
</tr>
<tr>
<td>6</td>
<td>Oleic acid</td>
<td>C18:1 (9c)</td>
<td>27.5</td>
<td>14.83</td>
</tr>
<tr>
<td>7</td>
<td>Us**</td>
<td>-</td>
<td>27.8</td>
<td>4.95</td>
</tr>
<tr>
<td>8</td>
<td>Stearic acid</td>
<td>C18:0</td>
<td>28.6</td>
<td>0.52</td>
</tr>
</tbody>
</table>

*Retention time in minute. **Unidentifiable substances.

Figure 1. DSC curve of the oil from the seeds of *Opuntia ficus-indica*.

plays an important physiological role as a potent mediator of the inflammation and the beneficial effect upon the immune system (Luzia et al., 2010). Furthermore, the presence of oleic and linoleic acids on skin is essential for maintenance of skin hydration, skin barrier and hydrolipidic mantle (Pianovski et al., 2008). And for presenting absorption band at UV region, these essential fatty acids are strong candidates for raw material in sunscreen industry (Remédios et al., 2006). Then, that several layers of fat-cells formed the skin, as the more lipophilic the sunscreen, the greater its substantivity. In other words, the product has the ability to retain its effectiveness for prolonged periods of time, especially when exposed to water (Wagemaker et al., 2011).

**Thermal behavior**

In the DSC curve (Figure 1) of the oil from *O. ficus-indica* seeds two events were observed: one exothermic with peak at 159.56°C and another endothermic with peak at 432.57°C. The first event may be associated with a crystallization peak favored by the power supply to the sample in sufficient quantity to promote a structural reorganization of the oil molecules (Herrera, 2005). According to Prado et al. (2007), the main factors that influence on the crystallization temperature of lipids are
the size of their molecules and the presence of double bonds in carbon chains. Considering the palm oil is rich in saturated and unsaturated fatty acids, the endothermic event can match the thermal decomposition of these compounds, since according to Reda and Carneiro (2007) at temperatures above 200°C maximum decomposition of the oils occurs. Faria et al. (2002) analyzed the thermal stability of some seed oils typical of the cerrado and observed similar results to the present study about the final temperature of decomposition of guaíiroba (Syagrus oleracea) (433°C), babaçu (Attalea ssp) (440°C) murici (Pterogyne nitens) (477°C), araticum (Annona glabra L.) (478°C) and buriti (Mauritia flexuosa L.) (483°C) oils.

According to Kasprzycha-Guttman and Cozeniak (2010), the thermal decomposition of saturated fatty acids requires more energy than the unsaturated acids. Then, as shown in the results of this study, the oil from *O. ficus-indica* seeds contains mainly unsaturated fatty acids (~80%), then we can try to justify that the enthalpy (ΔH) required for decomposition of this oil was relatively low (27.11 cal/g). The ΔH required for transition from decomposition of palm oil was higher than that of soybean (Glycine Max (L.) Merrill) oil (19.00 cal/g), corn (Zea mays) (11.60 cal/g), sunflower (H. annuus) (21.61 cal/g) and less than the canola oil (Brassica napus L.) (35.30 cal/g), rice (Oryza sativa) (51.13 cal/g) and olive (Olea europaea) (46.37 cal/g), which has significant levels of antioxidants (Santos et al., 2002). The thermal decomposition of oil from *O. ficus-indica* seeds (Figure 2) occurs in two main steps. The first stage begins at 219.39°C and has a weight loss of 17.74%. The second phase begins at 336.25°C and has a mass loss of 76.15%.

The first step is probably the decomposition of compounds with chains from 8 to 16 carbon atoms, and the second step is probably the decomposition of other saturated fatty acids and higher unsaturated carbon chains such as stearic acid (C18:0), oleic (C18:1) and linoleic (C18:2) (Santos, 2008). It is observed that there was no formation of residue, which may correspond to the complete decomposition and carbonization of the sample. Similar results were found in the thermal stability study by Santos (2008) with babaçu (Attalea speciosa) oil, which also breaks down into two stages and has thermal stability up to 209.30°C. Comparing the DSC with TG curve it was observed that the first event registered in the DSC (159.56°C) was not accompanied by significant mass loss. Therefore, it was just a physical change, which contributes to the proposition of an event of crystallization.

**Infrared absorption spectroscopy**

The analysis of the IR spectrum of the *O. ficus-indica* oil (Figure 3) shows the presence of intense bands around 2900 cm⁻¹, which are characteristic of methyl groups (-CH₃); methylene (-CH₂) and methyl (-CH), similar to the spectral bands of sesame and buriti oils (Albuquerque et al., 2003; Barros et al., 2007). The greater intensity of these bands may be related to the accumulation of signal generated by the large amount of lead type C-H. The band of low intensity at 3009 cm⁻¹ refers to the asymmetric
Figure 3. IR spectrum of the oil of *(Opuntia ficus-indica)*.

Thus, the higher the concentration of oil, the higher the SPF. Given the data, it is important to point out that on the concentration of 50 µg/ml a 22 FPS was observed, which is quite useful on photoprotection, since vegetable products that have SPF > 4 compared to homossalato, could be applied for the same purpose, since its production follow current legislation marketing of sunscreens (Rosa et al., 2008).

Evaluation of SPF of emulsions with oil of *(Opuntia ficus-indica)* (L.) Mill.

The positive control (F2) performed with the incorporation of 7.5% (w/w) of octyl methoxycinnamate (synthetic sunscreen) showed *in vitro* SPF is equal to 12.53, which is in agreement with results of the literature 13.21 ± 1.07 (Violante, 2009). However, the formulation with oil from *(O. ficus-indica)* seeds incorporated at a concentration of 10% (w/w) showed no significant SPF (F3) and was not capable of increasing the SPF of the formulation with synthetic filter (F4) (Table 3). Similar results were found by Violante et al. (2009) while working with dry ethanol extracted from *Macrosiphonia velame*. This vegetable species was presented in UVB absorption, but when it was subjected to the *in vitro* determination of SPF developed by Mansur test, the result was 0.36 ± 0.01. Thus, at the concentration used and standardized to *Macrosiphonia velame*, it cannot be considered a plant...

stretch of C-H bond sp² carbon (Silverstain and Webster, 2000). It also marks the existence of a band at 1744 cm⁻¹ characteristic of the carbonyl group (C=O) of esters, often long-chain fatty acids are also found in oils such as cotton and buriti (Albuquerque et al., 2003; Salgado et al., 2007). The bands at 1464 and 1377 cm⁻¹ may be related, respectively, the angular deformation of the asymmetric and symmetric methylene group (-CH₂). And the band in the range of 1161 cm⁻¹ is characteristic of stretching C-O (Silverstain and Webster, 2000). The band observed around 722 cm⁻¹ can be related to the synchronous vibration of the sequence of aliphatic chains of fatty acids (Albuquerque et al., 2003; Silverstain and Webster, 2000). Therefore, the bands observed in this IR spectrum are characteristic of the fatty acids detected in the GC-MS of the *(O. ficus-indica)* oil.

**Evaluation of SPF of vegetable oil**

According to the literature, polyunsaturated fatty acids present in the plants might absorb ultraviolet light. This shows the possibility to use the vegetable oils as sunscreens in photoprotection preparations (UVA/UVB) (Wagemaker et al., 2011). Corroborating with this assertion, it was detected that *(O. ficus-indica)* seed oil showed significant SFP on different oil concentrations. According to Figure 4, the SPF is directly related to the amount of oil in the solution spectrophotometric reading.
Table 3. Results of the SPF of the formulations studied.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>SPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>F2</td>
<td>12.53 ± 0.03</td>
</tr>
<tr>
<td>F3</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>F4</td>
<td>12.72 ± 0.06</td>
</tr>
</tbody>
</table>

F1 = Emulsion base; F2 = base + 7.5% sunscreen, F3 = base + 10% oil, F4 = base + 10% oil + 7.5% sunscreen.

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Conflict of interest

Authors declare that they have no conflicts of interest.
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Pereira de Souza et al. 831