Revista de Ciências Farmacêuticas Básica e Aplicada
Journal of Basic and Applied Pharmaceutical Sciences

Rev Ciênc Farm Básica Apl., 2014;35(2):195-203
ISSN 1808-4532

Characterization of atomized extract of *Opuntia ficus-indica* (L.) Mill. and assessment of its pharmaceutical potential

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ABSTRACT

Given the many traditional uses of *Opuntia ficus-indica* (L.) Mill. and the widespread employment of dry extracts in herbal medicine and phytocosmetics, the aim of this study is to characterize an atomized extract of *O. ficus-indica* cladodes, as well as to analyze its phytochemical composition and assay the total phenol content. In addition, the antioxidant, antimicrobial and photoprotective activities of the extract and its capacity to inhibit the enzyme tyrosinase were assessed, with a view to its pharmaceutical use. The physicochemical characterization was performed by pharmacopoeial tests, thermal analysis and infrared spectroscopy. Phytochemicals were analyzed by thin layer chromatography and total phenols by spectroscopy in the visible region. Antioxidant activity was detected by the method of free radical (DPPH●) scavenging and antimicrobial activity by the agar diffusion method, while inhibition of tyrosinase was estimated by the diphenolase activity assay and photoprotective activity by a spectrophotometric method. The pharmacopeial tests, IR spectroscopy and thermal analysis enabled the atomized extract to be characterized. Concerning the potential for pharmaceutical use, it was found that, under the study conditions, the extract did not show any antioxidant, antimicrobial or photoprotective activity. However, it did show a modest tyrosinase inhibitory capacity. The originality of the proposed research on *O. ficus-indica* in the pharmaceutical field should be emphasized, as it opens new prospects for the study of a species that is so abundant and adapted to Brazilian semi-arid regions.

Keywords: Phytochemical characterization. *Opuntia ficus-indica* (L.) Mill. Antimicrobial activity. Antioxidant activity. Inhibition of tyrosinase.

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INTRODUCTION

The selection of plants for research and development of herbal medicines and phytocosmetics, based on reports of specific therapeutic effects in humans and animals, is of great importance in the discovery of new assets, since the traditional use of a plant can be considered a pre-screening of its therapeutic usefulness. Species that grow in arid and semi-arid regions, where they are used daily in popular medicine, thus constitute a broad research object, possibly affording new and innovative applications in the pharmaceutical field (Elisabetsky & Souza, 2004; Barreiros et al., 2003).

Opuntia ficus-indica (L.) Mill., commonly known in Brazil as "palma forrageira" or fodder cactus (prickly pear or Indian fig in English), is a cactus native to arid Mexico, which has been disseminated in Central and South America as well as South Africa and throughout the Mediterranean region (Leo et al., 2010). Owing to its enormous productive potential and its multiple uses, the *O. ficus-indica* has been employed for human consumption, the production of medicines, cosmetics and dyes, in the conservation and recovery of soils, hedges, landscaping, and in many other ways, such as the manufacture of adhesives, glues, fiber craft, paper, mucilage and ornamentation (Barbera et al., 2001). It is known that this vegetable is a rich source of minerals, vitamins (A, C and E), pectins and phenolic compounds, especially flavonoids (Ramadan & Mörsel, 2003; Falcão & Oliveira, 2013).

In popular medicine, the fruit of *O. ficus-indica* is considered antidiarrhetic, antiasthmatic, diuretic, anti-inflammatory and cardiotonic (Chiacchio et al., 2006). It is also said popularly that crushed pads (cladodes) of *O. ficus-indica* are used to make anti-hair fall shampoo, while its sap and oil are used to prevent sunburn. However, scientific research is needed to verify the popular use of this plant for such purposes (Sáenz-Hernández, 2001; Hamou, 2008). Thus, Schmid et al. (2005) conducted a study which verified that *O. ficus-indica* has hydrating, softening and anti-inflammatory action and protects against exposure to UVA radiation.

Among the numerous ways of using plant raw materials, the atomized extract stands out as a final or intermediate product used to prepare several pharmaceutical forms, since these are, in general, two

to six times more potent than the fluid extracts that gave rise to them (Vasconcelos et al., 2005). The function of an atomized extract is to provide the action and characteristics of medicinal plants in small amounts and in a stable and convenient physical form (Ansel et al., 2000).

However, to control the quality of a product based on dried plant extract, it is necessary to check the chemical constitution and physicochemical properties of this extract, to ensure the desired final action. Thus, there are several analytical techniques used to standardize plant materials, among which we can list: thermal analysis, chromatography, infrared and ultraviolet spectroscopy (Souza, 2012).

The aims of this study were firstly to characterize the atomized extract of *O. ficus-indica* cladodes by pharmacopeial methods and analytical techniques, as well as to analyze the phytochemical composition and estimate the total phenol content. Secondly, it was proposed to assess the antioxidant, antimicrobial, tyrosinase inhibitory capacity and photoprotective activity of this extract, with a view to its pharmaceutical use.

MATERIALS AND METHODS

Plant material

The cladodes of *O. ficus-indica* were collected in March, 2011, at the city of Juazeirinho, PB (Brazil), which has a semi-arid climate. The plant material was identified by a botanist, Dr. José Iranildo Miranda de Melo, and dried plant specimens are deposited at the Manoel de Arruda Câmara Herbarium (ACAM) of the State University of Paraíba (UEPB), under voucher number 907. The collected pads were sanitized, their spines were taken out with tweezers and the stems sliced into small cubes. These were dried at 40°C in an oven with forced air circulation, until reaching a constant weight, and later ground in a knife mill (Whiley®).

Preparation of spray-dried extract

The powdered cladodes were extracted by percolation for 10 days with 70% aqueous ethanol, in a plant : solvent ratio of 1:5. The hydroalcoholic extract was transferred to a rotary evaporator (Tecnal TE-211) to decrease the concentration of ethanol to a safe level (45-50%) for the drying process. The extract was dried by atomization in a spray-drying tower in the SD-05 laboratory-scale spray dryer (Lab Plant, UK). The drying temperature was 160 ± 1°C, the pump flow rate was 8 $mL.min^{-1}$, while the exhaust temperature and air flow were not controlled. To aid the drying process, 20% colloidal silicon dioxide (Aerosil 200®) was added, relative to the content of solid (3.51%). The dried extract was collected at the base of a cyclone, stored in closed containers and kept in the dark in a silica gel desiccator.

Phytochemical characterization

Phytochemical screening of the dried extract from *O. ficus-indica* was performed by thin layer chromatography (TLC) on silica gel plates (Merck), in accordance with the general method described in the Brazilian Pharmacopeia (Brasil, 2010). The extract was tested for: flavonoids, terpenes, alkaloids, tannins and proanthocyanidins. To detect each of these classes of secondary metabolite, specific test reagents were used. The presence of saponins of was assessed by the formation of persistent foam after shaking with water (Harbone, 1998).

Determination of total phenolic contents

The total phenolic (TP) content of the dried extract was determined by UV-Vis spectroscopy, using the Folin-Ciocalteu method, with modifications (Velioglu et al., 1998). A methanolic solution of the extract ($1mg.mL^{-1}$) was prepared and 0.2 mL was mixed with 1.5 mL of 10% Folin-Ciocalteu solution in an amber vial, with vigorous stirring. After 5 minutes, 1.5 mL of 6% $NaHCO_3$ buffer was added. After 90 minutes, the absorbance of the mixture was measured at 725nm. The same procedure was performed for gallic acid standard solutions at the concentrations 0.5, 0.25, 0.125, 0.062, 0.031 and 0.015 $mg.mL^{-1}$, in methanol. From the gallic acid data, a standard curve was constructed and its linearity estimated by least squares linear regression analysis. The curve was represented by the equation $y = ax + b$, where x is the concentration of gallic acid and y the absorbance at 725nm. The TP content was determined by reading the absorbance of the sample against the calibration curve.

Physicochemical characterization

The spray-dried extract was analyzed, to determine the pH, density, total ash content, desiccation loss and particle size, by the official Brazilian Pharmacopeial methods (Brasil, 2010). All assays were performed in triplicate.

pH

The reading was performed with a digital pH meter (HI 221 Hanna instruments), by immersing the electrode in a 10% (w/v) solution of the spray-dried extract in distilled water at 25°C. To calibrate the pH meter, readings were taken with standard buffers at pH 4.0 and 7.0.

Density

To measure the density, a beaker of known weight and volume was filled with dry extract, while excess air was removed by tapping. The beaker was then weighed to obtain the mass of the extract. The density was calculated by dividing the weight by the volume occupied by the powder.

Total ash content

About 3g of extract was weighed in pre-weighed crucibles and then the samples (n = 3) were incinerated in a muffle furnace (Quimis®), by increasing the temperature gradually as follows: 30 minutes at 200°C, 60 minutes at 400° C and 90 minutes at 600°C. The result is expressed as the percent mass of ash in the extract (% w/w) and represents the mean of three determinations.

Loss on drying

The moisture in the sample was determined by gravimetry. Thus, 2g extract was weighed and transferred to a weighed, desiccated filter. The sample was then dried in an oven (Fanem®) at 105°C for 2 hours and the operation was repeated until constant weight.

Particle size distribution

To determine the particle size profile, 5g of the

sample was weighed and transferred to a graded series of sieves subjected to a mechanical sieve shaker (Bertel Ltda, Brazil), vibrating vigorously for 15 minutes. The percentage of extract retained on each sieve was calculated by Equation 1:

$$\% \text{ extract on sieve i} = \frac{M1}{M2} \times 100 \qquad \text{Eq. (1)}$$

where: M1=Mass of sample retained on sieve i; M2 = Sum of masses retained on all sieves and the collector.

Thermal Analysis

Differential Scanning Calorimetry (DSC)

The DSC curve of the extract was recorded with a TA Instruments calorimeter Q20. The sample (about 2 ± 0.1 mg) was placed in hermetically sealed aluminum cells in a nitrogen atmosphere flowing at 50 mL.min^{-1} and heated at 10°C.min^{-1} to 500°C. The system was calibrated with indium (T_f = 156.6 ± 0.2°C) and zinc (T_f = 419.5 ± 0.3°C) standards of purity 99.99%. TA Universal Analysis software was used to analyze the DSC curve.

Thermogravimetric Analysis (TGA)

The TGA curve of the extract was recorded with a TA Instruments SDT Q600 thermobalance. An alumina crucible holding about 5±0.1 mg sample was heated at 10°C.min^{-1} to 1200°C under synthetic air flowing at 20 mL.min^{-1}. Before the test, the equipment was calibrated with a standard sample of calcium oxalate monohydrate, under the same experimental conditions. TA Universal Analysis software was used to analyze the TGA curve.

Infrared absorption spectroscopy (IR)

Mid-infrared absorption spectra were collected in a Perkin Elmer® Spectrum 400 spectrometer, with an attenuated total reflectance (ATR) accessory, with a zinc selenide crystal. The spectra were acquired in 16 scans at a resolution of 4 cm^{-1}, in the region between 4000 and 650 cm^{-1}.

Antioxidant activity

The extract was tested for antioxidant activity by its ability to capture the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH●). Qualitative analysis was performed by TLC on silica gel plates (Merck®), with rutin, ascorbic acid and tannic acid as positive standards for comparison. The plates were eluted with CHCl3/MeOH/H2O (65:30:5) (v/v) and, after drying, were sprayed with a solution of 0.4 mmol.L^{-1} DPPH● in MeOH. The plates were observed for the appearance of yellow spots on a purple background, which would indicate possible antioxidant activity (Sousa et al., 2007; Soler-Rivas et al., 2000).

Tyrosinase inhibitory activity

Inhibition of the enzyme tyrosinase was assayed as described by Hearing (1987). In a 96-well microplate, the control well contained 80µL of the tyrosinase solution, 20µL of diluent (DMSO or phosphate buffer, pH 6.5) and 100µL of DOPA (3,4-dihydroxy-L-phenylalanine). After 20 min incubation at 37°C, the final absorbance should be ~ 0.800 with DMSO and ~1.000 with buffer as diluent. In the other wells, the assay mixture was: 20 µL of inhibitor [extract or standard (kojic acid)] + 80µL of the tyrosinase solution. The plate was incubated for 5 min at 37°C. Thereafter, 100 µL of the color reagent DOPA was added and the plate incubated at 37°C for 20 min. The absorbance at 492 nm was read before the addition of DOPA and at 5, 10, 15 and 20 minutes. Inhibition was calculated by equation 2.

% Inhibition = 100 – [At_{20}samp – At_0samp)/ (At_{20}cont–At_0cont)]*100 Eq. (2)

where: At_{20}= absorbance after 20 minute of reaction; At_0= initial absorbance; samp= sample; cont= control.

Antimicrobial activity

The microbiological assay was performed by the agar diffusion cylinder plate method, following the Brazilian Pharmacopoeia (Brasil, 2010) recommendations. The standard strains of bacteria, from the American Type Culture Collection, were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853), which were provided by the Oswaldo Cruz Foundation (FIOCRUZ – RJ, Brazil).

Mueller Hinton agar plates were used, with a bacterial inoculum of 85% transmittance at a wavelength of 625 nm (spectrophotometer Biospectro SP-22), which corresponds to a final density of 10^6 CFU.mL^{-1}. The inoculum was added to a top layer of agar at a dilution of 1:100 (v/v). After this layer had set, 4 stainless steel cylinders were placed on the surface, to each of which was added 100 µL of sample (1 g.mL^{-1}). The assay was performed in triplicate. After an incubation period of 48 hours, the diameters (in mm) of inhibition zones were measured around the cylinders, with the aid of a digital caliper. As a negative control, 70% alcohol was used and, as positive controls, the injectable antibiotic cephalothin (1g.mL^{-1}) for *Staphylococcus aureus* (Gram-positive bacteria) and injectable gentamycin sulfate (40 mg.mL^{-1}) for the other two bacteria (Gram-negative).

Photoprotective activity

The Sun Protection Factor (SPF) of a protective cream formulation, to which the atomized extract of *O. ficus-indica* was added, was determined, to test for potentiation of SPF formulations containing a chemical UV filter and the extract. The auto-emulsifier base, Aristoflex® AVL (Clariant Brazil), which is a mixture of emulsifiers, emollients and thickening polymer, was used as a vehicle for incorporation of the extract. The emulsion was prepared by the phase inversion method for cold emulsification and methylparaben (preservative) and polysorbate 80 (surfactant) were used as adjuvants. The chemical filter was octyl methoxycinnamate (Table 1).

Table 1. Composition of cream formulations for SPF assessment.

COMPOSITION	F1 (%)*	F2 (%)*	F3 (%)*	F4 (%)*
Aristoflex AVL®	5.0	5.0	5.0	5.0
Methylparaben	0.15	0.15	0.15	0.15
Polysorbate 80	2.0	2.0	2.0	2.0
Atomized extract of O. ficus-indica	-	-	10.0	10.0
Octyl methoxycinnamate	-	7.5	-	7.5
Water	qsp	qsp	qsp	qsp

*Percent by weight (w/w).
Legend: F1 = base; F2 = base + sunscreen 7.5%; F3 = base + extract 10%, F4 = base + extract 10% + filter 7.5%.

The SPF of the emulsions was estimated by the spectrophotometric method proposed by Mansur et al. (1986). In this, the formulations were diluted in ethanol A.R. at a concentration of 0.2 µg.mL^{-1} and the absorbance was read in a UV/Vis spectrophotometer (Schimadzu 1240), in a quartz cuvette of light path 1 cm, in the range 290-320 nm, at intervals of 5 nm. The absorbance values were substituted in equation 3 to give the in vitro SPF.

$$\mathbf{SPF = CF.} \sum_{290}^{320} \mathbf{EE(\lambda) .I(\lambda) .Abs\ (\lambda)} \qquad \text{Eq. (3)}$$

where CF= Correction Factor (= 10); EE (λ) = Erythemal effect of solar radiation at wavelength λ; I(λ) = Solar intensity at wavelength λ; Abs(λ)= absorbance of sample at each wavelength.

The product of the erythemal effect and the sunlight intensity at a given wavelength (EE x I) is constant and was determined by Sayre et al. (1979). The experiment was performed in triplicate and the SPF was expressed as the arithmetic mean of three determinations.

Besides the SPF, the organoleptic characteristics and the pH (pHmetro HI 221- Hanna Instruments) of each formulation presented in Table 1 were noted.

RESULTS

To obtain an intermediate product with the best technological characteristics, a dry extract of cladodes of *O. ficus-indica* was prepared by the spray-drying method, with addition of 20% Aerosil 200®, giving a final yield of 41.28 ± 2.71%.

The phytochemical analysis of this atomized extract of *O. ficus-indica* detected the presence of secondary metabolites such as flavonoids and terpenes.

In order to determine the total content of phenolic compounds (TP) in the sample, a linear calibration curve was constructed for the gallic acid standard, represented by the equation: y=5.651x + 0.032. The coefficient of determination was R^2=0.999. The result obtained for TP by the Folin-Ciocalteu method (Velioglu et al., 1998), expressed as gallic acid equivalents (GAE) per gram of spray-dried extract, was 42.36 ± 1.38 mg GAE.g^{-1}.

For the standardization of plant raw materials, it is very important to monitor the chemical constitution of the plant and, in order to ensure the quality of the final product, physicochemical tests were performed on the atomized extract of O. ficus-indica. The results of pharmacopeial tests on the extract can be seen in Table 2, except for particle size analysis, which showed that 53.60% of the extract was retained on the sieve of mesh 355µm.

Table 2. Pharmacopoeial tests applied to atomized extract of O. ficus-indica.

Pharmacopoeial Tests	Mean + SD *
pH	4.54 ± 0.05
Density (g.mL^{-1})	0.64 ± 0.02
Ash (%)	35.13 ± 0.01
Loss on Drying (%)	5.05 ± 0.06

*SD= standard deviation.

The thermal behavior of the spray-dried extract was assessed from DSC and TGA curves, presented respectively in Figures 1 and 2. The IR absorption spectrum can be seen in Figure 3.

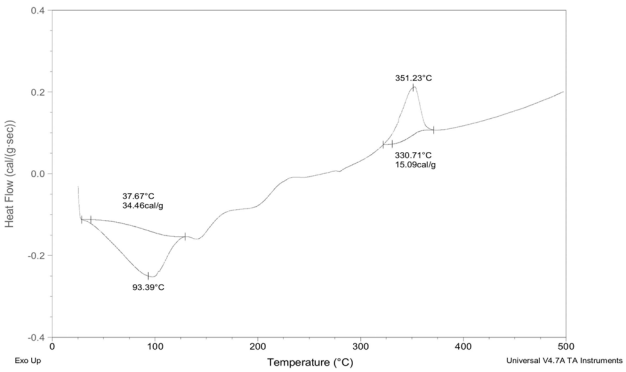

Figure 1- DSC curve of nebulized extract of Opuntia ficus-indica.

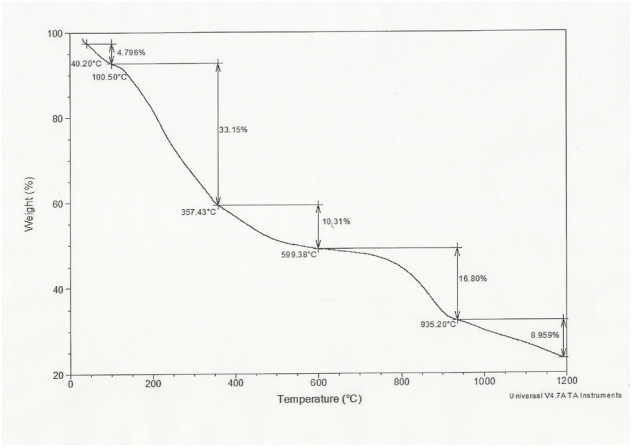

Figure 2- TGA curve of nebulized extract from Opuntia ficus-indica.

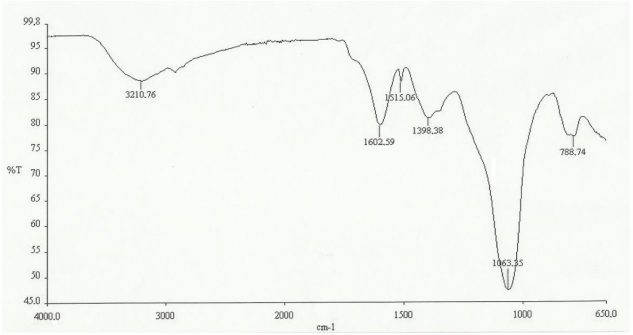

Figure 3- IR spectrum of nebulized extract of Opuntia ficus-indica.

With regard to the assessment of intermediate plant product cosmetic uses, it was found, in relation to antioxidant activity, that the extract, tested by the qualitative DPPH● method on TLC plates, did not produce yellow stains on a purple background, which would have indicated possible antioxidants.

However, in the assay to determine inhibition of tyrosinase, the extract showed an inhibitory activity of 11.39 ± 0.21%, while the standard inhibitor, kojic acid, tested at the same concentration, showed an inhibition of 38.17 ± 2.69%.

In the test for antibacterial activity in the extract of O. ficus-indica, inhibition zones against the strains tested were not found under the study conditions. However, with regard to the positive controls, gentamicin exhibited zones of 28.80 ± 0.30mm against E. coli and 25.80 ± 0.20mm against *P. aeruginosa*, while cephalothin produced a growth inhibition zone against S. aureus of 23.50 ± 0.30mm.

According to the method of assessing photoprotective action proposed by Mansur *et al* (1986), the atomized extract of *O. ficus-indica* showed no significant SPF and was not able to increase the SPF of a formulation based on chemical sunscreen, as can be seen in Table 3.

Table 3. In vitro SPF of cosmetic formulations tested.

Formulations	SPF	pH
F1	0.18 ± 0.01	6.29 ± 0.01
F2	12.53 ± 0.03	6.24 ± 0.02
F3	0.21 ± 0.02	5.07 ± 0.03
F4	12.54 ± 0.05	5.13 ± 0.02

Legend: F1 = base; F2 = base + 7.5% chemical sunscreen; F3 = base + 10% extract; F4 = base + 10% extract + 7.5% sunscreen.

Concerning the organoleptic properties of the emulsions made for this photoprotective activity, it was observed that after incorporation of the dried extract into the cosmetic base, the formulations had the macroscopic appearance of cream and there was no separation of phases 24 hours after the preparation. Formulations without the plant product were white (F1 and F2), while those with extract exhibited a green color (F3 and F4) and characteristic odor. Given the nature of the hydrophilic surfactants used, the formulations were classified as O/W and those containing extract exhibited a slightly lower pH (Table 3).

DISCUSSION

The yield of the atomized extract of *O. ficus-indica* achieved by adding silica (41%) was much higher than that of the extract of *Symphytum officinale* L. dried free of adjuvants, which was 20% (Silva Júnior et al., 2006). Therefore, the incorporation of technological adjuvants has a decisive influence in increasing the drying process efficiency and contributes positively to the recomposition of the product in water (Soares, 2002).

In the phytochemical screening, there was a positive response for flavonoid compounds, which are reported to have antioxidant, anti-inflammatory, antimicrobial, photoprotective and enzymatic inhibitory activities. Thus, plants that contain these metabolites may have many applications in the pharmaceutical field. The negative response for other secondary metabolites does not necessarily imply their absence, as they could be in small amounts, insufficient for positive identification; indeed, the time of harvest may have influenced their production by the plant (Perruchon, 2002; Souza, 2011).

The determination of the phenolic compound content in the dried *O. ficus-indica* extract afforded similar results to those reported by Sousa et al. (2007), who studied plants in the semi-arid area of the state of Piauí (NE Brazil), where the lyophilized extract from the bark and leaves of *Terminalia brasiliensis* Camb. demonstrated total phenolic contents, respectively, of 45.82 ± 0.78 mg and 38.53 ± 0.63 mg GAE.g^{-1}. According to those authors, there is a positive correlation between the content of total phenolics and antioxidant capacity. Thus, the ethanol extract of *T. brasiliensis* bark displayed an antioxidant activity of about 90% at a concentration of 100 μg.mL^{-1}.

According to Luzia et al. (2010), the extraction of phenolic compounds from natural products is strongly affected by the solvent used, in the sense that the greater the polarity of the extracting solvent, the greater the amount of phenolic compounds extracted. They used ethanol (dielectric constant = 24.30) to obtain an extract of *Eugenia uniflora* L. seeds and obtained a phenolic compounds content of 75.64 mg GAE.g^{-1}. We employed a hydroalcoholic solution of 70% ethanol, since, as Medeiros & Kanis (2010) point out, in most processes to prepare liquid extracts of plants in the pharmaceutical industry, the solvents used are various mixtures of water and ethanol. The presence of water (dielectric constant = 78.36) increases the dielectric constant and may favor the extraction of substances of intermediate polarity. Thus, Spagolla et al. (2009) observed that the mixtures ethanol/water 60:40 (v/v) and methanol/water 80:20 (v/v) yielded the highest contents of phenolics and flavonoids of all the hydroalcoholic extracts of *Vaccinium ashei* tested.

Regarding the physicochemical characteristics of the atomized extract of O. ficus-indica, it can be seen that the solution of 10% extract (w/v) showed an acid pH (4.54). However, according to Coskuner et al. (2006), the natural pH of *O. ficus-indica* cladodes is between 5.40 and 5.75. This fall in pH of the dried extract may be attributed to the mixture with silica, which has a pH around 3.70 to 4.70 (Balköse, 1990).

The determination of residual water content in herbal drugs is a test of the quality of their preparation, as well as a warranty of their conservation. The result for the atomized extract indicated good conservation and efficient drying of the extraction solvent. The importance of determining the loss of mass on drying is linked to the microbiological stability of raw plant material, as an expression of its susceptibility to the growth of fungi and bacteria and its chemical stability, especially against hydrolytic processes (Coskuner et al., 2006).

The main objective of measuring total ash is to estimate the proportion of inorganic compounds or impurities contained in organic substances (Brasil, 2010). A high result was obtained because 20% of the inorganic compound silica was used in the extract drying process; in addition, according to the literature, the fodder *Opuntia* is rich in minerals, especially calcium and iron (Chiacchio et al., 2006).

The density of the atomized extract of *O. ficus-indica* was 0.64 ± 0.02 g.mL^{-1}. This is an important quality control parameter from the technological point of view, since it influences the processing of phytotherapeutic and phytocosmetic forms.

In granulometric analyses, spray-dried extracts of *O. ficus-indica* are found to be a moderately dense powder, established in the Brazilian Pharmacopeia (Brasil, 2010) as composed of particles that pass entirely through a silk sieve of 710μm mesh, while up to 40% pass through one of nominal mesh 250μm. The same result was found by Costa et al. (2009) for the granulometric characterization of a spray-dried extract of *Cynara scolymus* L.

In the DSC curve of the dried extract (Figure 1), analyzed by the tangent method, two main events could be identified – one endothermic and one exothermic. The first peak probably indicates the water vaporization process, in other words, the loss of moisture from the extract. The second possibly corresponds to the decomposition of plant organic matter.

However, between these two thermal events, there is a steadily rising baseline, indicating that poorly defined thermal events may be occurring in the DSC curve in this range (from 129.40 °C to 330.71 °C). Comparison with the TG curve of the extract (Figure 2) suggests that these events also refer to thermal decomposition, since significant mass loss occurred in this temperature range.

The small quantity of sample, rapid results and the cleanliness of the technique make thermogravimetry a powerful tool for technological research in the standardization of raw materials used in herbal medicine and phytocosmetics (Aragão et al., 2002). According to the thermogravimetric curve of the dried extract (Figure 2), the weight loss occurred in several stages, probably due to the complexity of the sample, which is a plant extract and contains many different chemical compounds.

We also observe in Figure 2 that up to 100.50 °C there was a weight loss of 4.79%, possibly associated with loss of water. This result was consistent with the value obtained in the gravimetric loss on drying test (5.05%), showing that thermogravimetry can also provide an estimate of the residual water content present in the material after processing. The weight loss steps occurring at higher temperatures are related to the thermal decomposition of organic compounds in the extract. The step with the highest mass loss (33.15%) is seen between the temperatures 100.50 °C and 357.43ºC, similar to the findings of Silva Júnior et al. (2006) on the dry extract of *Symphytum officinale* L. The mean cumulative loss until the end of the test (at 1200 °C) was 74.02%. Thus, in this analysis, the mineral residue averaged 25.98%.

In their study of the thermal behavior of *Origanum mangerona* L. dried leaves, Medeiros et al. (2007) noted that the sample went through four processes of thermal decomposition, up to 900°C, showing a thermogravimetric profile similar to that in this research. However, those authors reported a mass loss of moisture of 10.19%. Despite thermoanalytical techniques being good tools for the characterization of materials, studies in which thermal techniques are applied to plant products are rare in the literature (Aragão et al., 2002).

According to Luz (2005), the infrared ATR spectrum shows how the compounds at the sample surface absorb in the infrared region. The analysis of the IR spectrum of the *O. ficus-indica* atomized extract (Figure 3) shows a broad band at 3210 cm^{-1}, which may represent the symmetric axial stretching of hydroxyl (O-H), a chemical grouping present in phenolic compounds. The absorption in the region of 1602 cm^{-1} suggests the axial vibration of C=C double bonds in the aromatic groups and an intense band at 1063cm^{-1} suggests the presence of C-O bonds in phenols. The band of low intensity at 788cm^{-1} may be due to the angular deformation of adjacent hydrogens of the aromatic ring (Silverstain & Webster, 2000).

Antioxidants are substances that characteristically decrease or block the oxidation reactions induced by free radicals (Costa et al., 2012), and it is a constant concern of cosmetology to prevent or attenuate the aging process by searching for and studying effective antioxidants, which can be offered to consumers in cosmetic products. These compounds are increasingly sought in plants, as they hold a vast amount of natural antioxidant substances (Magalhães, 2000).

In the qualitative analysis of DPPH● radical scavenging activity, the dried extract did not show antioxidant activity. This finding contradicts claims in the literature that *O. ficus-indica* exhibits antioxidant activity, consistent with the presence of phenolic flavonoid compounds such as quercetin, carotenoids and several vitamins, among them ascorbic acid (Galati et al., 2003; Tesoriere et al., 2004; Ennouri et al., 2005). However, according to Peixoto Sobrinho et al. (2009) and Nascimento et al. (2011), there can be an appreciable variation in the content of total flavonoids, depending on the date and location of collection, humidity, light, temperature and other factors. This may explain the difference between the results of this study and those in the literature.

Tyrosinase is an enzyme widely distributed in nature, being present in plants, animals and microorganisms, and has a key role in the biosynthesis of melanin and other physiological processes (Faria et al., 2007). For the pharmaceutical industry, tyrosinase inhibitors are important in the treatment of certain dermatological diseases associated with hyperpigmentation caused by abnormal production of melanin (Souza, 2011). Therefore, tyrosinase inhibitors have been used in cosmetics for skin whitening and depigmentation after sunburn.

In the assay of tyrosinase inhibition performed in this study, the dried extract showed significant inhibitory activity (~11%), in comparison with the standard inhibitor, kojic acid, tested at the same concentration. However, Souza (2011), in a study of the Brazilian tree *Pouteria torta* (grown for the fruit abil), that an aqueous extract of its leaves at a concentration of 1 mg.mL^{-1} inhibited 87.60% of the activity of tyrosinase. Thus, when we compare our results with literature data, we realize that the inhibitory activity of the extract of *O. ficus-indica* was modest.

Bacteria such as *Staphylococcus aureus* and *Escherichia coli* stand out as common contaminants in cosmetics and in hospital facilities. These microorganisms have pathogenic strains resistant to common antimicrobial agents, making their occurrence a potential threat to health (Dantas, 2010). There is a growing desire among the public to use less aggressive products of natural origin or as close

to natural as possible. The cosmetic industry is, in turn, subject to consumer concerns, and also seeks to develop formulations containing natural products, for example, to conserve the end product (Packer & Luz, 2007). Thus, Packer and Luz claim that the presence of flavonoids in plants can provide natural microbicidal activity. However, even though *O. ficus-indica* has flavonoids, in this study the extract of this plant was unable to inhibit the growth of the two bacterial strains.

On the other hand, in the study of Salah-Fatnassi et al. (2009), it was shown that two species of *Opuntia* exhibited strong activity against the majority of tested fungi, including yeasts, among them *Candida albicans*. The sensitivity of the tested organisms varied from one extract to another and also between the two species of opuntia, the inhibition ranging from 0% to 72.72%.

The amount of flavonoids produced by a plant is considered an important factor in the protection of the plant against ultraviolet radiation. Thus, plant extracts containing flavonoids are capable of absorbing ultraviolet light, which suggests the possibility of using such extracts as sunscreens in photoprotective preparations for topical use (Nascimento et al., 2009; Souza et al., 2005).

Although the phytochemical screening demonstrated the presence of flavonoids and the literature indicates the use of *O. ficus-indica* in products for photoprotection (Sáenz-Hernández, 2001; Hamou, 2008), the present study found that the incorporation of the atomized extract of cladodes of *O. ficus-indica* into a cosmetic base neither showed any protective potential nor was it able to enhance the SPF of a formulation with a synthetic filter (Table 3). It is worth mentioning that, according to a resolution of the Brazilian food, drug and sanitary authority, Anvisa (Brasil, 2012), sunscreen products suitable for cosmetic use should provide an SPF ≥ 6.

These results can be explained by various studies that the efficacy of a sunscreen depends on the radiant energy absorption capacity attributed to its chromophoric groups, which is proportional to their concentration, the absorption bands and the wavelength of the absorption peak (Violante et al., 2008, Munhoz et al., 2012).

Other studies have shown that various plant products had flavonoids in their composition and absorbed in the ultraviolet region, but did not show any potential as natural sunscreens (Souza et al., 2005; Violante et al., 2008).

Given the foregoing, the use of natural products in formulations has always been accepted by consumers, so that the pharmaceutical industries are becoming more interested in adding plant extracts to the composition of their products. However, it is very important that these products are standardized and that the effects attributed to specific extracts are verified by means of scientific studies.

Hence, all the physical and chemical assays carried out in this study are valuable and their results can be used as guidelines for the quality control of atomized extract of O. ficus-indica.

Furthermore, we observe that, even though the phytochemical screening of the extract of *O. ficus-indica* indicated the presence of flavonoids, the assessment of its potential activities demonstrated that, under the standard conditions of the study, this extract did not exhibit antioxidant, photo-protective or antibacterial activity, whereas it did show moderate but significant inhibition of the enzyme tyrosinase.

Finally, the originality of this pharmaceutical study of *O. ficus-indica* may be emphasized, as it affords prospects of new studies on this species, which is so plentiful in northeastern Brazil.

ACKNOWLEDGMENT

We would like to thank the Brazilian Coordinating Council for the Improvement of Higher Education (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES/Brazil - PROCAD, Process n° 552652/2011-3) for the financial support. The State of São Paulo Research Foundation (FAPESP- Process n°: 2013/22212-0). The authors also are grateful to the Graduate Program in Pharmaceutical Sciences of UFPE and UEPB.

RESUMO

Caracterização do extrato nebulizado de Opuntia ficus-indica (L.) Mill. e avaliação da sua utilização farmacêutica

Diante das múltiplas potencialidades da *Opuntia ficus-indica* (L.) Mill. relatadas na literatura e ampla empregabilidade dos extratos secos na produção de fitoterápicos e fitocosméticos, este estudo objetivou caracterizar o extrato nebulizado dos cladódios de *O. ficus-indica* bem como verificar a composição fitoquímica e quantificar os fenóis totais. O estudo propõe ainda avaliar as atividades antioxidante, antimicrobiana, inibidora da enzima tirosinase e fotoprotetora do extrato visando à sua utilização farmacêutica. A caracterização físico-química foi realizada através de testes farmacopeicos, análise térmica e espectroscopia na região do infravermelho. A caracterização fitoquímica foi feita por CCD e a quantificação de fenóis por espectroscopia na região do visível (725 nm). A atividade antioxidante foi analisada pela técnica do sequestro do radical livre DPPH*, a atividade antibacteriana pelo método de difusão em ágar, a atividade inibidora da enzima tirosinase através da verificação da ação difenolase e a atividade fotoprotetora pelo método in vitro de Mansur. As análises farmacopeicas, espectroscópicas e térmicas permitiram caracterizar o extrato nebulizado de O. ficus-indica. Quanto à avaliação das potencialidades foi detectado que, nas condições do estudo, o extrato não apresentou atividade antioxidante, antimicrobiana e fotoprotetora. Contudo, demonstrou relativa capacidade inibitória da enzima tirosinase. Portanto, cabe então destacar a originalidade da pesquisa com a *O. ficus-indica* (L.) Mill. na área farmacêutica, dando perspectivas para novos estudos com essa espécie tão abundante e adaptada ao semi-árido brasileiro.

Palavras-chaves: Caracterização fitoquímica. *Opuntia ficus-indica* (L.) Mill. Atividade antimicrobiana. Atividade antioxidante. Inibição da Tirosinase.

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Received on March 18th, 2013.

Accepted on July 03rd, 2013.