Original Article

ANTIOXIDANT AND PHOTOPROTECTIVE POTENTIAL OF *Moringa oleifera* LAM (Moringaceae)

POTENCIAL ANTIOXIDANTE E FOTOPROTETOR DE Moringa oleifera *LAM (Moringaceae)*

Janine Mailho GIMENIS[1]; Amanda Costa GOMES[1]; Valter Henrique Marinho dos SANTOS[2]; Paulo Cesar FERREIRA[3]; Camila Areias OLIVEIRA[4]; André Rolim BABY[5]; Regildo Márcio Gonçalves da SILVA[6]

1. Mestre Farmacêutico, Universidade Estadual Paulista - UNESP, Instituto de Química de Araraquara, Araraquara, SP, Brasil; 2. Doutor Biólogo, Atina Ativos Naturais, Pouso Alegre, SP, Brasil; 3. Doutor Farmacêutico; 4. Professor, Doutor, Universidade de São Paulo, Departamento de Farmácia, São Paulo, SP, Brasil; 5. Mestre Farmacêutico; 6. Professor, Doutor, Universidade Estadual Paulista, Faculdade de Ciências e Letras de Assis, Assis, SP, Brasil. regildos@yahoo.com.br

ABSTRACT: *Moringa oleifera* Lam. (Moringaceae) is a plant with several biological activities and therapeutic properties. However, the complete knowledge about its pharmacological, biological and ecological effects, and about the active components present in each vegetable part are not still completely elucidated. This study aimed to evaluate the antioxidant and photoprotective activities of different extracts from leaves and flowers of *M. oleifera*. These activities were assessed through *in vitro* tests, DPPH radical scavenging method, iron ion chelating effect (FRAP), lipid peroxidation (TBARS), nitric oxide scavenging method and assessment of the activity against the lipid peroxidation through hemolytic method. The photoprotective activity was assessed through spectrophotometric analysis and through *in vitro* test with Labsphere. It was also determined the extract's phenolic content and total flavonoid through spectrophotometry and HPLC. The obtained results demonstrated that this species have components with antioxidant and photoprotective potential mainly in the extracts obtained from fresh leaves and flowers. Therefore, it was possible to verify that *M. oleifera* has potential to be used as source of antioxidant components with photoprotective activity mainly due to the presence of phenolic components and among these, the flavonoids.

KEYWORDS: Flavonoids. Formulations. Plant-based cosmetics. *Moringa oleífera.* Sunscreen. Vegetables extracts.

INTRODUCTION

Moringa oleifera Lam., a plant species belonging to the Moringaceae family, has been used as food, in traditional medicine and in industrial applications (ABDULKARIM et al.,2005; ANWAR et al.,2007; SILVA et al.,2013; ARANTES et al.,2015). *M. oleifera* is native to eastern Africa and southern India, introduced to different regions around the world including Africa, Americas and Caribbean Islands for similar usage as a food component and herbal medicine (BEZERRA et al.,2004; MUHL et al.,2011). The leaves, flowers and immature pods of this plant are used as highly nutritive supplements with many pharmacological properties (BEZERRA et al.,2004; ANWAR et al.,2007; MUHL et al.,2011; SILVA et al.,2013; ARANTES et al.,2015; ARORA; ONSARE, 2015). Moreover, *M. oleifera* have long been recognized by the Ayurvedic and Unani systems of medicine for prevention and treatment of several diseases, e.g., gastric ulcers, skin diseases, hay fever, fatigue and bronchitis (ANWAR et al.,2007; SIXT-DANIELL et al.,2011; VONGSAK, 2013).

The *M. oleifera* leaf extract have been reported to exhibit antioxidant activity both *in vitro* and *in vivo*, due to abundant phenolic acids and flavonoids (EILERT et al.,1981; SANCHEZ-MACHADO et al.,2006). However, the studies conducted so far did not evaluate the photoprotective potential of extracts from this species.

The antioxidant components are responsible for reestablishing the equilibrium of redox metabolism and reduce the surplus levels of free radicals to prevent oxidative stress. This stress can lead to molecular and cellular damage that can promote the emergence of different diseases and premature aging (POURZAND; TYRRELL, 1999; MORITA; KRUTMANN, 2000; JAIN; JAIN, 2010). The increased production of free radicals can be of endogenous origin such as aerobic respiration, inflammation, hydrogen peroxide metabolism and cytochrome P450 enzymes, and exogenous origin such as diet, medication, smoking, pollution and ionizing radiation (mainly ultraviolet exposure) (BONDY; NADIERI, 1994).

Excessive exposure to ultraviolet radiation leads to increased free radicals and reduces endogenous antioxidants, promoting damage to

Received: 14/09/17
Accepted: 15/06/18

membrane lipids, proteins, carbohydrates and nucleic acids by oxidation and modification of these molecules. Such damage can lead to formation of skin cancer (photocarcinogenesis) and directly influence the process of aging (photoaging) (SEITE et al., 2000; DUALE et al.,2010; WANG et al.,2010). Recent studies have shown that active substances found in vegetables can present active components with photoprotective action. Among these are vitamins C and E, tannins, alkaloids and flavonoids (VIOLANTE et al.,2009). This effect is related to the structural similarity between synthetic chemical filters and active ingredients extracted from plants, since the ultraviolet absorption has been observed when using plant extract in pharmaceuticals and cosmetics, indicating a possible anti-sunburn action (RAMOS et al.,1996).

Among the sunscreens active components rich in flavonoids, ethanolic extracts show two peaks absorption at 240-280nm and 300-550nm wavelengths, indicating a potential for absorbing UV radiation, whose wavelength is between 290 - 320 (UVB) and 320-400 (UVA) (VIOLANTE et al.,2009). Studies have shown that flavonoids inhibit enzymes that are involved in free radicals production and act as chelating agents against metal involved in the metabolism of oxygen (BROWN et al.,1998). Therefore, the current study was undertaken to evaluate the antioxidant activity of different extraction forms of *M. oleifera*'s leaves and flowers correlating the way of prepare for best use in ethnopharmacology.

MATERIAL AND METHODS

Plants materials

The leaves and flowers of *M. oleifera* were collected from specimens present in the Universidade Estadual Paulista (UNESP-Brazil) (22°32'20''S and 50°22'60''W). A voucher specimen has been deposited in the Herbarium Assisense (HASSI) under number of 760. The leaves and flowers were cleaned and a portion was dried at 40º C for 24h. The dried samples were ground and passed through a sieve (20 mesh). The powder went kept sealed and protected from luminosity in containers until use. Another portion of fresh sample was used for squeezing and obtaining extracts from the fresh plant parts.

Preparation of extract

Several extraction methods were performed using water, hydroethanolic solution 70% (v/v) and absolute ethanol as solvents. Each extraction was repeated several times until exhaustion. Each method was done in triplicate.

Preparation of fresh and lyophilized aqueous extract

Fresh leaves and flowers of *M. oleifera* were pounded into processor with distilled water at a ratio of 1:10 (w: v) for 5 minutes and filtered, obtaining fresh aqueous extract. A volume of aqueous extracts were frozen and freeze-dried to obtain the dry extracts. The extracts were used in the tests.

Preparation of hydroethanolic and ethanolic extracts

M. oleifera's leaves and flowers were dried at 40°C and pounded. The resulting powders were used to prepare the hydroethanolic and ethanolic extracts. The hydroethanolic extracts were prepared at a ratio of 1:10 (w/v) with ethanol 70% solution in distilled water by mechanical stirring for 24 hours. The extracts were filtered and concentrated on a rotary evaporator under vacuum. The resulting extracts were frozen and lyophilized to obtain dry extracts. The ethanolic extracts were prepared at a ratio of 1:10 (w/v) ethanol PA by mechanical stirring for 24 hours. After that, they were filtered and taken to rotary evaporator and posteriorly taken to desiccator to obtain the dry extracts. (CHOI et al., 2016).

Preparation of sunscreen formulations

The oil-in-water emulsions were prepared using an anionic emulsifier (Ammonium Acryloyldimethyltaurate / VP Copolymer). From this emulsion, there were prepared three samples: Mo-0 emulsion (without extract and sunscreen addition), Mo-1 emulsion (without sunscreen and extract added) and Mo-2 (with sunscreen and *M. oleifera* hydroethanolic extract).
The qualitative and quantitative composition of the formulations are described in Table1.

Test of antioxidant activity

DPPH radical scavenging test

The antioxidant activity of crude extract and fractions were determined by the H^+ donor ability to the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma, USA), according to the *in vitro* methodology (BLOIS, 1958). The experiment was performed in triplicate using a solution made of 1 mL of acetate buffer (pH 5.5 and 100 mM), 1.25 mL of ethanol P.A., 250µL of DPPH solution and 50 mL of samples. The extracts reacted with DPPH radical for a period of 30 min under low light and were then subjected to an UV-vis spectrophotometer (Femto-600 Plus) at an absorbance of 517 nm

(BRAND-WILLIANS et al.,1995; DI MAMBRO; FONSECA, 2005). The calculation of antioxidant activity was performed according to the formula: Antioxidant Activity (%) = [(control-sample)/control] x100. The antioxidant activity of the extract or fraction can be seen by the degree of discoloration of the reagent after the 30 min required for the reaction to attain a plateau. Gallic acid (Vetec-Fine Chemicals, Brazil) was used as a standard.

Table 1. Qualitative and quantitative composition of the formulations for photoprotective efficacy

Composition[a]	Concentration % (w/w) Formulation codes		
	Mo-0	Mo-1	Mo-2
Ammonium Acryloyldimethyltaurate / VP Copolymer	3	3	3
Propylene Glycol	5	5	5
Disodium EDTA	0.15	0.15	0.15
BHT	0.05	0.05	0.05
Phenoxyethanol (and) methylparaben (and) ethylparaben (and) butylparaben (and) propylparaben (and) isobutylparaben	0.7	0.7	0.7
Butyl Methoxydibenzoylmethane	3	-	3
Ethylhexyl PABA	8	-	8
Ethylhexyl methoxycinnamate	7.5	-	7.5
Hydroethanolic extract of M. oleifera	-	2	2
Water	b	b	b

[a]Qualitative composition was reported in accordance with INCI (International Nomenclature of Cosmetic Ingredient), [b]Enough to complete 100%.

Ferric reducing antioxidant power (FRAP)

The FRAP reagent was prepared as required by mixing 25 mL acetate buffer (300 mM, pH 3.6), 2.5 mL TPTZ solution (10 mM in HCl solution (40 mM)), and 2.5 mL $FeCl_3$ solution (20 mM). Sample (1000, 2000, 3000, 5000 and 10000µg/mL) or Trolox standard (25-500 µM/L) 90 µl was mixed with 270 µL of distilled water and 2.7 mL freshly prepared FRAP reagent and incubated at 37 °C by 30 min. Maximum absorbance values were reader at 595 nm. The results were expressed as micromoles of Trolox equivalents (TE) per gram of dry extract or fraction (BENZIE; STRAIN, 1996).

Nitric oxide scavenging

Nitric oxide (NO) scavenging assay was carried by using sodium nitroprusside (SHAHRIAR *ET AL.*,2013). This can be determined by the use of the Griess reaction. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline PBS (pH 7.4) was mixed with 0.5 mL of extracts at various concentrations (3000, 5000 and 10000 µg mL^{-1}) and incubated at 37°C for 150 minutes. After incubation the mixture was maintained 60 minutes at room temperature. The absorbance was measured at 540 nm. The percentage of inhibition was calculated according to the following equation concerning sodium nitrite calibration curve (y=0.0052x+0.0349).

Lipid peroxidation assay (TBARS)

A modified thiobarbituric acid reactive species (TBARS) assay using egg yolk homogenates as lipid-rich media was performed to measure the formation of lipid peroxide (AWAH et al.,2010; RUBERTO; BARATTA, 2000). Malondialdehyde (MDA), a secondary end-product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogenic with maximum absorption at 532 nm. Egg homogenate (500 µl) of 10%, v/v in phosphate buffered saline (pH 7.4) and 100 µl of sample (3000, 5000 and 10000 µg mL^{-1}) were added to 1 mL of the hydrochloride 2.2"Azobis (2-amidinopropane) dihydrochloride (AAPH) (0.12M) to induce lipid peroxidation. The mixture was incubated for 30 minutes at 37ºC. After incubating and cooling at room temperature, it was added 0.5 mL of trichloroacetic acid (ATA) (15%) and 0.5 mL of TBA (0.6%). The mixture was incubated at 97ºC for 15 minutes. After incubation, centrifugation was performed with 1 mL of n-butane (2783 rpm for 10 minutes). Then, the reading of the supernatant was performed at 532 nm. Inhibition (%) of lipid peroxidation was calculated using the equation: Inhibitory activity (%) = $[(A_C-A_S)/A_C]x100$ where A_C is the absorbance of the control, and A_S is the absorbance of the sample.

Inhibition oxidative hemolysis human erythrocyte

Ethics Committee of the Universidade Estadual Paulista - UNESP approved all the procedures in this study protocol and all subjects involved gave a written informed consent (no 833.386, approved in 16/09/2014). For this study, blood was obtained from healthy donors who were non-smokers and not receiving any pharmacological treatment and collected in tubes containing EDTA anticoagulant. Every experiment was performed daily with the erythrocytes that came from the same donors. Anticoagulated blood was centrifuged during 5 min at 2500 rpm, plasma and the white cell layer (buffy coat) were discarded after and the remaining erythrocytes were washed three times with phosphate buffered saline (PBS). An erythrocyte suspension was prepared at 10% concentration in PBS. AAPH (2,2′-azobis (2-amidinopropane) hydrochloride solution was prepared using the same buffer at the moment of its use, and also kept away from the light. The extract was dissolved in 50% stock-solution of propylene glycol, and later diluted in PBS at the time of use. The incubation of erythrocyte suspension with AAPH in the presence or absence of extracts at different concentrations was performed in a 37ºC water-bath with a gentle shaking motion and in the absence of light for 6h. Positive (100% hemolysis) and negative controls were incubated with and without AAPH, respectively, included in the assay to examine a protective effect of extracts.

Aliquots were removed every one hour, transferred to eppendorf tubes and centrifuged at 2500 rpm for 10 minutes and then was performed spectrophotometric reading of supernatant. Hemolysis was determined by reading the absorbance of hemoglobin at 540 nm. The test samples were done in triplicate for statistical analyzes and percentage hemolysis was calculated by the following equation: % Hemolysis=100.[(A_S-A_{NC})/(A_{PC}-A_{NC})] where A_S is the absorbance of the sample, A_{NC} is the absorbance of the control negative and A_{PC} is the absorbance of the control positive.

***In vitro* photoprotective efficacy**

Photoprotective efficacy was performed in vitro by reflectance spectrophotometry with an integrated sphere (RSIS) (UV-2000S Ultraviolet Transmittance Analyzer, Labsphere, USA). Sun Protection Factor (SPF) and critical wavelength were calculated. Samples were weighed and uniformly applied with a glove-coated finger on the rough side of PMMA (polymethyl methacrylate) plates, at a ratio of 1.3 mg/cm^2. The samples were then allowed to dry at room temperature for 20 min, protected from light. The analyses were carried out in replicates of three, and nine different points per plate were measured for each sample (VELASCO et al.,2008; COLIPA GUIDELINES, 2011; WANG AND LIM, 2011).

***In vitro* photostability assay**

After the in vitro photoprotection assay, sample plates with the sunscreens were irradiated for one hour using a solar simulator. Solar simulator emission was maintained at 580.08 W/m^2, corresponding to a UV irradiance of 55 W/m^2 (irradiation dose, 198 kJ/m^2). SPF and critical wavelength parameters were analyzed post-irradiation and compared with the pre-irradiation results. The analyses were prepared in replicates of three, and nine different points per plate were measured for each sample (VELASCO et al.,2008; SCALIA; MEZZENA, 2010; COLIPA GUIDELINES, 2011; WANG; LIM, 2011).

Phytochemical screening

Total phenols and flavonoids quantification

The quantification of total phenols and flavonoids was performed with the extract diluted in ethanol at concentrations of 100, 250, 500, 1000, 1500 and 2000μg.mL^{-1}. The Folin-Ciocalteu method was performed to determine total phenols (SINGLETON; ROSSI, 1965). For each 0.5 mL of extract at different concentrations it was added 5mL of distilled water and 0.25 mL of Folin-Ciocalteu reagent. After 3 minutes, 1mL of saturated Na_2CO_3 solution at 10% was added and the mixture was stored for 1 hour. The absorbance was measured at 725nm using a UV-Vis spectrophotometer (model: SP220, Biospectro, Brazil). All the tests were performed in triplicate and the results were expressed in mg of gallic acid per gram of extract.

For the extract's total flavonoids quantification the determination by UV-Vis spectrophotometer was performed and the samples were prepared based on flavonoids complexation with $AlCl_3$ (ZHISHEN et al.,1999). An aliquot of 250 μL of each different concentration of extract was mixed with 1.25 mL of distilled water and 75 μL of $NaNO_2$ solution at 5%. After 6 minutes, 150 μL of $AlCl_3$/H_2O solution at 10% was added. After 5 minutes, 0.5 mL of NaOH 1M solution was added and then the total volume was completed by adding 2.5 mL of distilled water. The samples were shaken in a vortex mixer and the absorbance was measured at 510 nm. All the tests were performed in triplicate

and the results were expressed in mg of rutin per gram of extract.

The total phenolic and flavonoid analyzes are generic quantitative tests for the determination of these compounds, which represent the presence or absence of these compounds in the evaluated extracts. This analysis is not directly related to the antioxidant activity of the extracts, therefore, the determination of total phenolics and flavonoids is correlated with the compounds present in the extracts, forming a preliminary phytochemical profile.

Analysis by high performance liquid chromatography (HPLC-PDA)

With aid of PDA detector performing scan in the spectral range of 200-600nm, spectra in UV region for eluted peaks were obtained. Peaks with absorption bands typical of flavonoids (Figure 1) which are recognized for presenting the Band II, with maximum wavelength in spectral range of 250-280nm, assigned to A-ring and Band I, with maximum length in spectral range of 300-320nm, assigned to B-ring, presenting higher incidence of molecules from group of flavones and flavonoids.

Polyphenol analysis by HPLC / PDA

The stock solutions of leaves and flowers were diluted in the proportions of 1:50 (v / v) to 1:20 (v / v) methanol, respectively. The system used was the DionexUltimate 3000 HPLC (Dionex, Idstein, Germany) coupled with a photodiode detector 3000 and the Ultimate Chormeleon software to quantify and qualify the phenolic components. A column (Acclaim® 120) was used, C18 5µm 120A (4.6 mm x 250 mm) for separation of components. The column was maintained at 40°C throughout the analysis and detection was performed at three wavelengths (225, 280, 300 and 340 nm). The sample injection volume was 10 uL. The mobile phase (A) was composed of acidified water with 1% phosphoric acid and the step (B) of methanol. The identification and quantification of components were performed by comparison of retention time and spectrum of purified standards (Sigma-Aldrich, Brazil) (ESCARPA; GONZÁLEZ, 2001).

Statistical analysis

The data are expressed as the mean±SD by measuring three independent replicates. Analysis of variance using one-way ANOVA followed by Tukey's test was performed to test the significance of differences between means obtained among the treatments at the $\alpha \leq 0.05$ level of significance using BioEstat software version 5.0.

RESULTS

Antioxidant activity

Table 2 shows the results obtained with the in vitro tests for antioxidant activity and determination of phenols and flavonoids of the hydroethanolic and ethanolic extracts prepared from dried leaves and flowers of *M. oleifera* and fresh dry and aqueous extracts of *M. oleifera's* leaves and flowers. The hydroethanolic and ethanolic extracts demonstrated dose-dependent activity at the highest concentration (10 mg/mL) to the DPPH test. The hydroethanolic extracts of leaves and flowers in the concentration of 5 to 10 mg / mL did not differ significantly from the standard Gallic acid. Similarly, in the FRAP test was observed that the higher values were obtained for the concentration of 10 mg/mL of hydroethanolic and ethanolic extracts of leaves and flowers. In determining the content of phenolics and total flavonoids for all extracts evaluated, there was no significant difference in the concentrations tested. However the ethanol extracts of leaf and flower at a concentration of 3 mg/mL showed the highest concentration of phenols and flavonoids. For fresh dry and aqueous extracts of *M. oleifera*'s leaves and flowers, it was found that the antioxidant activity behaved in dose dependence, and the highest observed value was 100.0% for the concentration, added to the fact that they did not differ statistically from control Gallic acid for the DPPH test. In determining the content of phenolics and total flavonoids all evaluated extracts also showed higher levels in the concentration of 100.0%.

Table 2. Antioxidant activity through DPPH radical scavenging method (%) and iron chelate (FRAP in mM) and determination of phenols (FT) and total flavonoids (FlT) in ethanolic (Eth) and hydroethanolic (HEth) extracts of dried leaves and flowers and aqueous extract of fresh leaves and flowers of *Moringa oleifera*.

Extracts		Concentration (µg/mL)	Leaves (dried) of *M. oleifera*				Flowers (dried) of *M. oleifera*			
			DPPH	FRAP[a]	FT[b]	FlT[c]	DPPH	FRAP[a]	FT[b]	FlT[c]
Alcoolic	HEth	3000	23.66±0.65a	26.14±0.49a	16.49±1.53a	171.54±4.72a	09.06±6.01a	25.09±0.65a	18.06±1.60a	32.84±7.22a
		5000	39.43±2.61b	24.57±0.29a	18.61±0.16a	191.88±8.22a	20.01±1.09b	23.80±0.12a	13.11±0.00a	103.55±6.31 b
		10000	74.33±0.58c	16.12±0.21b	16.16±1.33a	180.11±6.31a	46.67±2.58c	15.56±0.00a	11.61±0.80a	89.31±4.20c
	Eth	3000	18.25±5.11a	98.63±2.49c	25.47±0.56a	670.72±33.13b	41.24±0.88c	16.26±1.13a	32.14±1.21b	575.36±4.25 d
		5000	42.63±2.54b	28.13±0.58a	19.00±0.35a	509.11±4.20c	61.40±2.66d	19.55±0.24a	28.72±1.13b	536.88±2.54 d
		10000	56.47±5.98b	15.99±0.06b	16.52±0.39a	360.66±2.20d	63.31±2.07d	14.34±0.34a	21.05±0.36b	373.44±4.82e
Aqueous	Dried	10,0	7.76±1.65a	16.22±10.04a	23.64±1.73a	32.05±7.56a	13.3±2.7a	-	1.69±0.48a	15.11±0.48a
		50,0	32.03±1.17b	143.25±3.34b	90.86±1.27b	81.49±8.78b	43.55±5.31b	53.81±2.24a	38.64±3.75b	17.89±1.27a
		100,0	82.04±1.69c	155.29±1.60b	157.8±4.74b	152.33±7.26c	80.91±1.99c	160.6±1.92b	83.64±1.92c	24.00±1.66a
	Fresh	10,0	29.22±3.59b	66.22±2.0c	16.97±0.96a	-	13.24±2.63a	-	16.97±0.96d	-
		50,0	82.35±1.27c	148.81±2.74b	64.47±2.09c	129.00±0.83c	62.63±3.43b	117.33±4.0c	64.47±2.09c	45.11±3.94b
		100,0	74.62±10.48c	153.81±0.32b	107.8±6.73b	241.22±3.15d	93.23±1.14c	146.9±1.69b	107.8±6.73e	113.72±2.68c
Gallic Acid		80(µg/mL)	74.33±0.52c				74.33±0.52c			

Values are expressed as mean ±SE. Same letters within the same column indicate no significant differences among samples by Tukey test ($\alpha \leq 0.05$). [a]Percentage values of DPPH radical scavenging activity; [b]Values of total phenol levels (mg of gallic acid equivalent/g of extract); [c]Total flavonoid levels in quercetin equivalent per mg/g of extract

Table 3 shows the results of antioxidant activity by the method of TBARS and the kidnapping of NO radical to hydroethanolic extract of dry leaf and aqueous extract of fresh leaves and flowers. The highest activities were observed at the concentration of 10 mg/mL, to hydroethanolic extract, but there was no significant difference between the analyzed concentrations. The results of these tests for the aqueous extract of fresh leaves and flowers were observed at the concentration of 100%. As for the aqueous extract of fresh flowers the highest percentage of anti-lipid peroxidation activity was observed in the concentration of 50% and also, the concentration of 100% showed greater sequestration of NO ion.

The TBARS and NO tests were performed for the hydroethanolic extract of the leaves because it was the extract that presented better results in the previous tests.

Table 3. Inhibition of lipid peroxidation (TBARS) and NO radical scavenging at different concentration of hydroethanolic extract and fresh leaves and flowers aqueous extract of *Moringa oleifera*.

Extract		Concentrations (µg mL^{-1})	Leaves	
			TBARS- Inhibitory activity (%)	% NO radical scavenging
Alcoolic	HEth	3000	64.36±02.55a	75.59±0.51a
		5000	54.40±10.57a	76.55±1.67a
		10000	56.13±21.60a	77.58±0.22a

Aqueous	Concentration (%)	Leaves		Flowers	
		TBARS- Inhibitory activity (%)	% NO radical scavenging	TBARS- Inhibitory activity (%)	% NO radical scavenging
Fresh	10%	12.59±0.35a	74.76±3.31a	15.25±0.76a	74.18±2.80a
	50%	28.12±0.64b	74.44±2.08a	27.70±0.54a	73.03±3.86a
	100%	52.57±1.05c	75.01±1.99a	22.68±1.37a	74.35±3.11a

Values are expressed as mean ±SE. Same letters within the same column indicate no significant differences among samples by Tukey test ($\alpha \leq 0.05$)

The analysis of the antioxidant activity by hemolytic method is shown in Figure 1. It shows that the hydroethanolic leaf extract has the potential to inhibit the hemolytic action AAPH for all concentrations tested, describing an action dependent concentration profile. The concentration of 1mg/mL showed the highest percentage inhibition of hemolysis hour for the third and fourth hour.

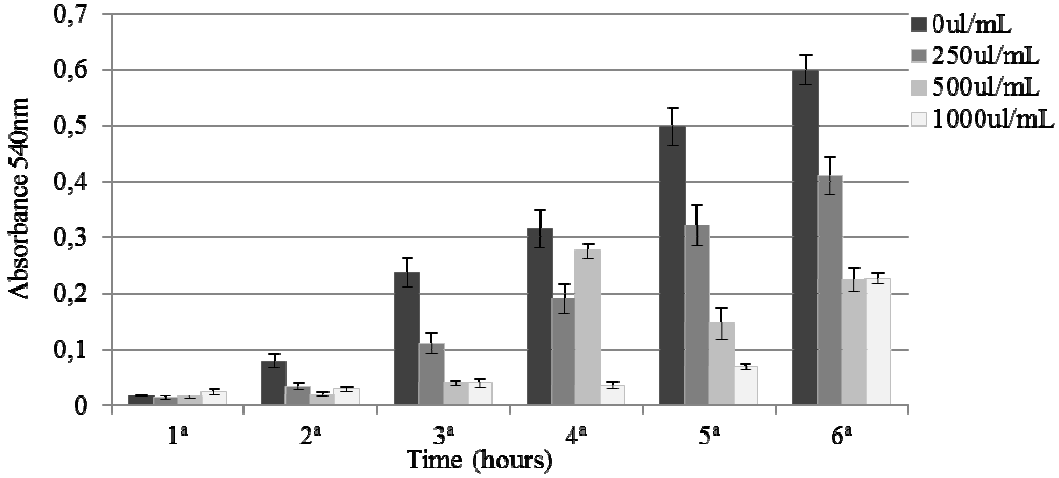

Figure 1. Antioxidant activity through hemolysis of *Moringa oleifera*'s dried leaves hydroethanolic extract during 6 hours of incubation and measured in spectrophotometer at 540nm.

Evaluation of photoprotective potential

Determination of the maximum wavelength and the maximum absorbance of the hydroethanolic extract of *M. oleifera*'s leaves.

A test was performed for the scanning spectrum of the hydroethanolic extract of the leaves. The estimated extract had a maximum absorbance between 1.6 and 2.0 in lengths from 290 to 400nm, being greater than the spectrophotometric profile described by the positive control (OMC - octyl methoxycinnamate) that presented maximum absorbance between 1.2 and 1.6.

***In vitro* determination of hydroethanolic extract of *M. oleifera*'s leaves photoprotective efficacy**

Figure 2-A illustrates the spectrophotometric profiles of the formulations with hydroethanolic extract of *M. oleifera* leaves without addition of UV filters (Mo-1) and containing the UV filters (Mo-2) compared to control (Mo-0 - no addition of extract and adding sunscreen). The Mo-1 sample showed no photoprotective effect, as expected since it is a UV filter-free formulation. It was also observed a synergistic effect of the combination of *M. oleifera* extract with the associated sun filter (Mo-2) compared preparation with only the chemical sunscreen (Mo-0).

Figure 2-B illustrates the absorption spectrum profiles of formulations with hydroethanolic extract of *M. oleifera* leaves (Mo-2 with sunscreen after 30 and 60 minutes) and control (Mo-0 without extract and with added sunscreen after 30 and 60 minutes) to determine the photostability. It was observed that the formulation with *M. oleifera* and adding UV filters, contributed to the stability of the formulation after 30 and 60 minutes of irradiation compared to the same formulation without the *M. oleifera*.

Determination of phenolic components by HPLC-PDA

In quantification and identification of polyphenolic components present in the extracts of the leaves and flowers of *M. oleifera* using HPLC / PDA it was possible to observe the presence of four components to the leaves: Chlorogenic Acid (238.95 µg/g), Gallic Acid (9.974 µg/g), Isoorientin (3,52 µg/g) and Rutin (1358,04 µg/g), and six for the flowers - Trans-ferulic Acid (44.42 µg/g), p-coumaric Acid (164.44 µg/g), Isoorientin (1,13 µg/g), Vitexin (1,88 µg/g), Orientin (0,66 µg/g) and Rutin (901,78 µg/g). In the leaves was observed highest concentration of rutin and chlorogenic acid components and for the most abundant flowers were rutin and p-coumaric acid.

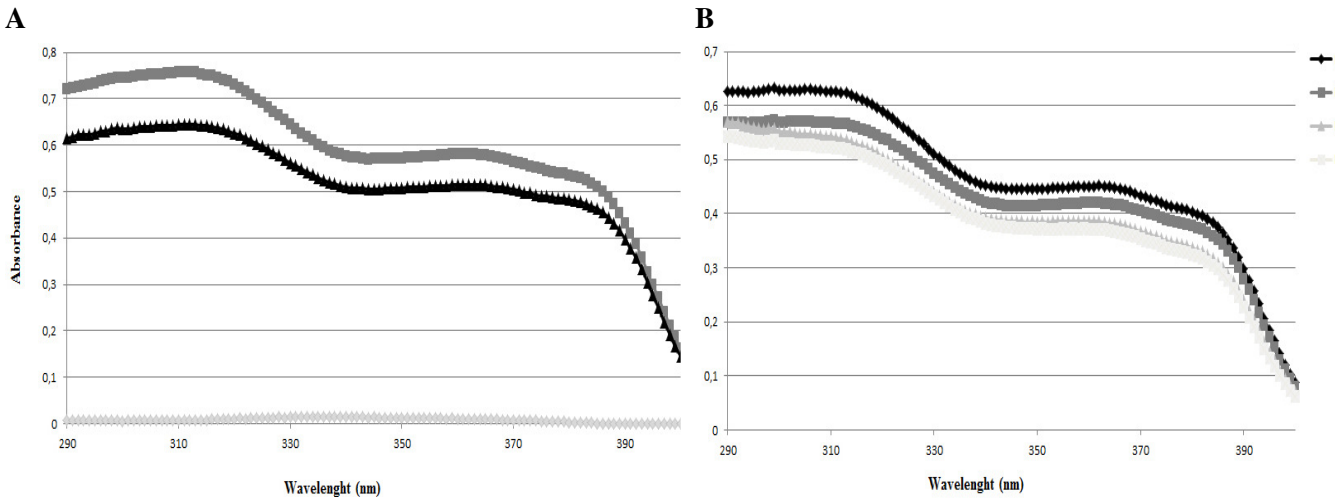

Figure 2. A - Absorbance spectra profiles of formulations containing *M. oleifera*'s leaves hydroethanolic extract (Mo-1 without sunscreen and Mo-2 with sunscreen) and control (Mo-0 without extract and with sunscreen) for analysis of photoprotective activity. **B** - Absorbance spectra profiles of formulations containing *M. oleifera*'s leaves hydroethanolic extract (Mo-2 with sunscreen after 30 and 60 minutes) and control (Mo-0 without extract and with sunscreen after 30 and 60 minutes) for photostability analysis.

DISCUSSION

Currently, different studies about the use of *Moringa oleifera* species, both as food and as preventive and treatment against different diseases, have shown that this species presents several phenolic components with nutritional and medicinal properties with significant impact in both human and animal health (GHASI et al.,2000; WATERMAN et al.,2014). In this study, we demonstrated that *M. oleifera* represents an important source of antioxidant components present within its leaves and flowers, regardless of how the extract is obtained, when evaluated through DPPH radical scavenging method and the iron chelate effect (FRAP) (SINGH et al.,2009; VERMA et al.,2009; MOYO et al.,2012; ALHAKMANI et al.,2013; RODRÍGUEZ-PÉREZ et al.,2015). However, these studies evaluated the extracts and antioxidant components from vegetable parts (leaves, flowers, fruits and seeds) separately and with unique acquisition methodology. Thus, the present study differs, since it is based on the evaluation of different vegetable parts (leaves and flowers) and acquisition methodologies of the extracts and consequently antioxidant components, mainly on the highest evaluated concentration (10 mg/mL).

Given the proven antioxidant potential of *M. oleifera*'s extracts, this study determined the phenolic and total flavonoid contents. The results were according with previous experiments carried, in which they have highlighted the presence of different phenolic and flavonoid components as constituents of the analyzed extracts (ATAWODI et al.,2010; SREELATHA, S. AND PADMA, 2011; COPPIN et al.,2013; JAISWAL et al.,2013).

In addition to the antioxidant evaluations carried out in this study, we did the analysis of the extracts with highest antioxidant activity, about lipid peroxidation, through TBARS method and Nitric Oxide radical scavenging assay. So it was possible to observe a higher activity in the extracts from the leaves of *M. oleifera*, and these results are according with the findings in the literature (MOYO et al.,2012). As regards the assessment of the antioxidant activity through the hemolithic method, the hydroethanolic extract from *M. oleifera*'s leaves has presented a protective effect against AAPH inducted hemolysis during the whole incubation period. These results are according with the studies in the literature. The concentration of 500µ inhibited less than the concentration of 250µ. In the incubation and centrifugation processes there may be variations between the concentration of hemoglobin present in the aliquot. In this way we will have differences in the percentage of hemolysis (YANG et al.,2006; PHRUEKSAN et al.,2014).

Given the different evidence of antioxidant activity found on *M. oleifera*'s extracts though this study, we carried out for the first time assessment of *M. oleífera*'s photoprotective potential, since the chronic or repeated exposure to ultraviolet radiation promotes photoaging and photocarcinogenesis (GUINEA et al.,2012). We observed that *M. oleifera's* extracts has photoprotective potential, once they have presented spectral absortion in the range of UV (290-400nm). Besides that, a synergistic effect was observed in the hydroethanolic extract from the leaves in association with chemical UV filters, also increasing photostability of the formulations evaluated in this study. The correlation between antioxidant activity and photoprotective effect must be considered, since studies demonstrated that UVB radiation generates reactive oxygen species (ROS), reacting with photosensitive molecules resulting in an imbalance between the ROS and promotes damage to molecular structures such as DNA, proteins and lipids (OBERLEY 2002; MUKHTAR 2003; PETROVA et al.,2011).

In addition to these results, the extracts from *M. oleifera*'s leaves and flowers were also assessed through chromatography (HPLC-PAD) and the calibration curves were set with different patterns of phenolic and flavonoid components. Analyses showed different phenolic classes present in the leaves, which were not found in the flowers, as well these presented unique phenolic classes in their composition. However, the rutin quantification, as major component in both extracts, was observed. This finding may be directly correlated with the antioxidant and photoprotective activities highlighted in this study, because experiments carried has demonstrated that the rutin flavonoid presents such potentials and activities (OLIVEIRA et al.,2015; OLIVEIRA et al.,2016).

CONCLUSION

M. oleifera has a potential to be used as source of components with antioxidant and photoprotective activities, as well as, a photostabilizing adjuvant for sunscreen systems, mainly due to the presence of phenolic components, and among them, the flavonoids.

ACKNOWLEDGMENTS

The authors thank UNESP and CAPES (National Council for the Improvement of Higher Education) for financial support.

RESUMO: *Moringa oleifera* Lam. (Moringaceae) é uma planta com várias atividades biológicas e propriedades terapêuticas. No entanto, o conhecimento completo sobre seus efeitos farmacológicos, biológicos e ecológicos, e sobre os componentes ativos presentes em cada parte vegetal não são ainda completamente elucidados. Este estudo teve como objetivo avaliar as atividades antioxidantes e fotoprotetoras de diferentes extratos de folhas e flores de *M. oleifera*. Estas atividades foram avaliadas através de testes *in vitro*, método de eliminação de radicais DPPH, efeito de quelação de íons de ferro (FRAP), peroxidação lipídica (TBARS), método de eliminação de óxido nítrico e avaliação da atividade contra a peroxidação lipídica através do método hemolítico. A atividade fotoprotetora foi avaliada através de análise espectrofotométrica e através de teste *in vitro* com Labsphere. Também foi determinado o conteúdo fenólico do extrato e o flavonoide total através de espectrofotometria e HPLC. Os resultados obtidos demonstraram que esta espécie possui componentes com potencial antioxidante e fotoprotetor principalmente nos extratos obtidos a partir de folhas frescas e flores. Por conseguinte, foi possível verificar que a *M. oleifera* tem potencial para ser utilizado como fonte de componentes antioxidantes com atividade fotoprotetora principalmente devido à presença de componentes fenólicos e entre estes, os flavonoides.

PALAVRAS-CHAVE: Flavonoides. Formulações. Cosméticos à base de plantas. *Moringa oleifera*; Protetores solares. Extratos vegetais.

REFERENCES

ABDULKARIM, S. M.; LONG, K.; LAI, O. M.; MUHAMMAD, S. K. S.; GHAZALI, H. M. Some physico-chemical properties of *Moringa oleifera* seed oil extracted using solvent and aqueous enzymatic methods. **Food Chemistry**., Chiba, v. 93, p. 253-256. November. 2005. https://doi.org/10.1016/j.foodchem.2004.09.023

ALHAKMANI, F.; KUMAR, S.; KHAN, S. A. Estimation of total phenolic content, in-vitro antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. **Asian Pacific Journal of Tropical Biomedicine**., Jeddah, v. 3, p. 623-627. August. 2013. https://doi.org/10.1016/S2221-1691(13)60126-4

ANWAR, F.; LATIF, S.; ASHRAF, M.; GILANI, A. H. *Moringa oleifera*: A food plant with multiple medicinal uses. **Phytotherapy Research**., Nápoles, v. 21, p. 17-25. January. 2007. https://doi.org/10.1002/ptr.2023

ARANTES, C. C.; PATERNIANI, J. E. S.; RODRIGUES, D. S.; HATORI, P. S.; PIRES, M. S. G. Diferentes formas de aplicação da semente de *Moringa oleifera* no tratamento de água. **Revista Brasileira de Engenharia Agrícola e Ambiental**., Campina Grande, v. 19, p. 266–272, 2015. https://doi.org/10.1590/1807-1929/agriambi.v19n3p266-272

ARORA, S.; ONSARE, J. G. In vitro antimicrobial evaluation and phytoconstituents of *Moringa oleifera* pod husks. **Industrial Crops and Products.,** North Dakota, v. 52, p. 125–135, 2014. https://doi.org/10.1016/j.indcrop.2013.09.016

ATAWODI, S. E.; ATAWODI, J. C.; IDAKWO, G. A.; PFUNDSTEIN, R. H.; WURTELE, G.; BARTSCH, H.; OWEN, R. W. Evaluation of the Polyphenol Content and Antioxidant Properties of Methanol Extracts of the Leaves, Stem, and Root Barks of *Moringa oleifera* Lam. **Journal of Medicinal Food**., Prague. v. 13, p. 710-716, Jun. 2010. https://doi.org/10.1089/jmf.2009.0057

AWAH, F. M.; UZOEGWU, P. N.; OYUGI, J. O.; RUTHERFORD, J.; IFEONU, P.; YAO, X.J.; FOWKE, K. R.; EZE, M. O. Free radical scavenging activity and immunomodulatory effect of Stachytarpheta angustifolia leaf extract. **Food Chemistry**., Chiba, v. 119, p. 1409-1416. April. 2010.

BENZIE, I.F.; STRAIN, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. **Analytical Biochemistry**., Canada, v. 239, p. 70–6. Jul. 1996. DOI: 10.1006/abio.1996.02922

BEZERRA, A. M. E.; MOMENTE, V.G.; MEDEIROS, S. Germinação de sementes e desenvolvimento de plântulas de moringa (*Moringa oleifera* Lam.) em função do peso da semente e do tipo de substrato. **Revista da Associação Brasileira de Horticultura**, Brasília, v. 22, p. 295-299. Junho. 2004. http://dx.doi.org/10.1590/S0102-05362004000200026.

BLOIS, M. S. Antioxidant Determinations by the Use of a Stable Free Radical. **Nature.** v. 181, p. 1199-1200. April. 1958. https://doi.org/10.1038/1811199a0

BONDY AND NADERI, S. Contribution of hepatic cytochrome p450 systems to the generation of reactive oxygen species. ***Biochemical Pharmacology***., Kansas, v. 48, p. 155-159. July. 1994. https://doi.org/10.1016/0006-2952(94)90235-6

BRAND-WILLIAMS, W.; CUVELIER, M. E.; BERSET, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. **LWT Food Science and Technology**., Georgia, v. 28, p. 25-30, 1995. https://doi.org/10.1016/S0023-6438(95)80008-5

BROWN J. E.; KHODR H.; HIDER R. C.; RICE-EVANS C. Structural dependence of flavonoid interactions with Cu 2+ ions; implications for their antioxidant properties. **Biochemical Journal**., Great Britain, p. 1173–1178. Mar. 1998.

CHOI, EJ.; DEBNATH, T.; TANG, Y.; RYU, YB.; MOON, SH.; KIM, EK. Topical application of *Moringa oleifera* leaf extract ameliorates experimentally induced atopic dermatitis by the regulation of Th1/Th2/Th17 balance. **Biomedicine & Pharmacotherapy**., Korea, v. 84, p. 870-877. December 2016.

CHUMARK, P.; KHUNAWAT, P.; SANVARINDA, Y.; PHORNCHIRASILP, S.; MORALES, N. P.; PHIVTHONG-NGAM, L.; RATANACHAMNONG, P.; SRISAWAT, S.; PONGRAPEEPORN, K. S. The in vitro and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. Leaves. **Journal of Ethnopharmacology**., South Africa, v. 116, p. 439-46. Mar. 2008. https://doi.org/10.1016/j.jep.2007.12.010

COPPIN, J. P.; XU, Y.; CHEN, H.; PAN, M.; HO, C.; JULIANI, R.; SIMON, J. E.; WU, Q. Determination off lavonoids by LC/MS and anti-inflammatory activity in *Moringa oleifera*. **Journal of Functional Foods**., Canada, v. 5, 1892 – 1899, 2013.

COSMETICS EUROPE. **COLIPA Guidelines**. In vitro method for the determination of the UVA protection factor and ''critical wavelength'' values of sunscreen products, 2011.

DI MAMBRO, V. M.; FONSECA, M. J. V. Assays of physical stability and antioxidant activity of a topical formulation added with different plant extracts. **Journal of Pharmaceutical and Biomedical Analysis**., Arlington, v. 37, p. 287-295. Feb. 2005.

DUALE, N.; OLSEN, A.K.; CHRISTENSEN, T.; BUTT, S.T.; BRUNBORG, G. Octyl methoxycinnamate modulates gene expression and prevents cyclobutane pyrimidine dimer formation but not oxidative DNA damage in UV-exposed human cell lines. **Toxicological Sciences**., Tokyo, v. 114, p. 272–284. Jan. 2010.

EILERT, U.; WOLTERS, B.; NAHRSTED, A. The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stenopetala*. **Planta Medica**., Switzerland, v. 42, p. 55-61. May. 1981.

ESCARPA, A.; GONZÁLEZ, M. C. Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatografic and spectrophotometric methods. **Analytica Chimica Acta**., USA, v. 427, p. 119-127. January. 2001.

GHASI, S.; NWOBODO, E.; OFILI, J. O. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed wistar rats. **Journal of Ethnopharmacology**., South Africa, v. 69, p. 21–25. Jan. 2000.

GUINEA, M.; FRANCO, V.; ARAUJO-BAZÁN, L.; RODRÍGUEZ-MARTÍN, I.; GONZÁLEZ, S. In vivo UVB- photoprotective activity of extracts from comercial marine macroalgae. **Food Science and Technology International**. v. 50, p. 1109-1117. March. 2012. https://doi.org/10.1016/j.fct.2012.01.004

JAIN, S. K. AND JAIN, N. K. Multiparticulate carriers for sun-screening agents. **International Journal of Cosmetic Science**., London, v. 32, p. 89–98. Apr. 2010.

JAISWAL, D.; RAI, P. K.; MEHTA, S.; CHATTERJI, S.; SHUKLA, S.; RAI, D. K.; SHARMA, G.; SHARMA, B.; KHAIR, S.; WATAL, G. Role of *Moringa oleifera* in regulation of diabetes-induced oxidative stress. **Asian Pacific Journal of Tropical Medicine**., USA, v. 426-432. Jun. 2013.

KHONGRUM, J.; WATTANATHORN, J.; MUCHIMAPURA, S.; THUKHUM-MEE, W.; THIPKAEW, C.; WANNANON, P.; TONG-UN, T. *Moringa oleifera* Leaves Extract Attenuates Neuropathic Pain Induced by Chronic Constriction Injury. **American Journal of Applied Sciences**., China, v. 9, p. 1182-1187, 2012. https://doi.org/10.3844/ajassp.2012.1182.1187

KUMBHARE M. R.; GULEHA V.; SIVAKUMAR T. Estimation of total phenolic content, cytotoxicity and in-vitro antioxidant activity of stem bark of *Moringa oleifera*. **Asian Pacific Journal of Tropical Disease**., p. 144-150. April. 2012. https://doi.org/10.1016/S2222-1808(12)60033-4

MORITA, A. AND KRUTMANN, J. Ultraviolet A radiation-induced apoptosis. **Methods Enzymology**., USA, v. 319, p. 302–309, 2000.

MOYO, B.; OYEDEMI, S.; MASIKA, P. J.; MUCHENJE, V. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. **Meat Science**., Australia, v. 91, p. 441–447. Aug. 2012.

MUHL, Q. E.; DU TOIT, E. S.; ROBBERTSE P. J. *Moringa oleifera* (horseradish tree) leaf adaptation to temperature regimes. **International Journal of Agriculture and Biology**., Pakistan, v. 13, p. 1021–1024, 2011.

MUKHTAR, H. Eat Plenty of Green Leafy Vegetables for Photoprotection: Emerging Evidence. **Journal of investigative Dermatology Symposium Proceedings**. 121. Aug. 2003. https://doi.org/10.1046/j.1523-1747.2003.12377.x

OBERLEY, T.D. Oxidative damage and cancer. **American Journal of Pathology**., Columbia, v. 160, p. 403-408. Feb. 2002.

OLIVEIRA, C. A.; PERES, D. A.; GRAZIOLA, F.; CHACRA, N. A. B.; ARAÚJO, G. L. B.; FLÓRIDO, A. C.; MOTA, J.; ROSADO, C.; VELASCO, M. V. R.; RODRIGUES, L. M.; FERNANDES, A. S., BABY, A. R. Cutaneous biocompatible rutin-loaded gelatin-based nanoparticles increase the SPF of the association of UVA and UVB filters. **European Journal of Pharmaceutical Sciences**., Denmark, v. 81, p. 1–9. Jan. 2016.

OLIVEIRA, C. A.; PERES, D. A.; RUGNO, C. M.; KOJIMA, M.; PINTO, C. A. S. O.; CONSIGLIERI, V. O.; KANEKO, T. M.; ROSADO, C.; MOTA, J.; VELASCO, M. V. R.; BABY, A. R. Functional photostability and cutaneous compatibility of bioactive UVA sun care products. **Journal of Photochemistry and Photobiology B:Biology**., Canada, v. 148, p. 154–159. Jul. 2015.

PETROVA, A.; DAVIDS, L.M.; RAUTENBACH, F.; MARNEWICK, J.L. Photoprotection by honeybush extracts, hesperidin and mangiferin against UVB-induced skin damage in SKH-1 mice. **Journal of Photochemistry and Photobiology B:Biology**., Canada, v. 103, p. 126-139. May. 2011.

PHRUEKSANAN, W.; YIBCHOK-ANUN, S.; ADISAKWATTANA, S. Protection of *Clitoria ternatea* flower petal extract against free radical-induced hemolysis and oxidative damage in canine erythrocytes. **Research in Veterinary Science.** Italy, v. 97, p. 358–364. Oct. 2014.

POURZAND, C. AND TYRRELL, R. M. Apoptosis, the role of oxidative stress and the example of solar UV radiation. **Photochemistry Photobiology**., Canada, v. 70, p. 380–390. Oct. 1999.

RAMOS, M. F. S.; SANTOS E. P.; BIZARRI, C. H. B.; MATTOS, H. A.; PADILHA, M. R. S.; DUARTE, H. M. Preliminary studies towards utilization of various plant extracts as antisolar agents. **International Journal of Cosmetic Science**., London, v. 18, p. 87-101, 1996.

RODRÍGUEZ-PÉREZ, C.; QUIRANTES-PINÉB, R.; FERNÁNDEZ-GUTIÉRREZ, A.; SEGURA-CARRETERO, A. Optimization of extraction method to obtain a phenolic compounds-rich extract from *Moringa oleifera* Lam leaves. **Industrial Crops and Products**., North Dakota, v. 66, p. 246–254, 2015.

RUBERTO, G.; BARATTA, M.T. Antioxidant activity of selected essential oil components in two lipid model systems. **Food Chemistry**., Chiba, v. 69, p. 167–174, 2000.

SANCHEZ-MACHADO, D. I.; LOPEZ-CERVANTES, J.; VAZQUEZ, N. J. R. High-performace liquid chromatography method to measure α- and γ-tocopherol in leaves, flowers and fresh beans from *Moringa oleifera*. **Journal of Chromatograph**., Belgium, v. 1105, p. 111-114. August. 2006.

SCALIA, S.; MEZZENA, M. Photostabilization effect of quercetin on the UV filter combination, butyl methoxydibenzoylmethane–octyl methoxycinnamate. **Journal of Photochemistry and Photobiology B-Biology**., Canada, v. 86, p. 273–278. Nov. 2010.

SEITE, S.; COLIGE, A.; VIVENOT, P.P.; MONTASTIER, C.; FOURTANIER, A.; LAPI_ERE, C.; NUSGENS, B. A full-UV spectrum absorbing daily use cream protects human skin against biological changes occurring in photoaging. **Photodermatology, Photoimmunology and Photomedicine**., Boston, v. 16, p. 147–155. Aug. 2000.

SHAHRIAR, M.; HOSSAIN, I.; SHARMIN, F.A.; AKHTER, S.; HAQUE, A.; BHUIYAN, M.A. In vitro Antioxidant and Free Radical Scavenging Activity of *Withania Somnifera Root*. **Journal of Pharmacy**., UK, v. 3, p. 38–47. Mar. 2013.

SILVA, T. C. S.; NUNES, T. P.; COSTA, D. G.; LIMA, L. A. L. C.; SILVA, G. F.; OLIVEIRA JUNIOR , A. M. Utilização de sementes de *Moringa oleifera* Lam como alternativa para produção de biodisel. **Revista Geintec**., Brasil, v. 3, p. 012-025, 2013. D.O.I.:10.7198/S2237-0722201300020002

SINGH, B. N.; SINGH, B.R.; SINGH, R.L.; PRAKASH, D.; DHAKAREY, R.; UPADHYAY, G.; SINGH, H.B. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. **Food and Chemical Toxicology**., Spain, v. 47, p. 1109–1116. Jun. 2009.

SINGLETON, V. L.; ROSSI, J. A. JR. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **American Journal of Enology and Viticulture**., Washington, v. 16, p. 144-158. January. 1965.

SIXL-DANIELL, K.; SIXL, W.; SIXL, G.; FUCHS, W. On the use of *Moringa oleifera* as a medicinal plant in India and the Philippines. **LV. Évfolyam**. p. 57– 63, 2011.

SREELATHA, S. AND PADMA, P. R. Modulatory effects of *Moringa oleifera* extracts against hydrogenperoxide-induced cytotoxicity andoxidative damage. **Human & Experimental Toxicology**., Finland, v. 30, p. 1359–1368. Dez. 2011.

VELASCO, M. V. R.; SARRUF, F. D.; SALGADO-SANTOS, I. M. N.; HAROUTIOUNIAN-FILHO, C. A.; KANEKO, T. M.; BABY, A. R. Broad spectrum bioactive sunscreens. **International Journal of Pharmaceutics**., France, v. 363, p. 50–57. Nov. 2008.

VERMA, A. R.; VIJAYAKUMAR, M.; MATHELA, C. S.; RAO, C. V. In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. **Food and Chemical Toxicology**., Spain, v. 47, p. 2196–2201. Jun. 2009.

VIOLANTE, I. M. P.; SOUZA, I. M.; VENTURINI, C. L.; RAMALHO, A. F. S.; SANTOS, R. A. N.; FERRARI, M. Avaliação in vitro da atividade fotoprotetora de extratos vegetais do cerrado de Mato Grosso. **Revista Brasileira de Farmacognosia**., Brasil, v. 1, p. 452-57. Jun. 2009.

VONGSAK, B.; SITHISARNA, P.; MANGMOOLB, S.; THONGPRADITCHOTEC, S.; WONGKRAJANGC, Y.; GRITSANAPANA, W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. **Industrial Crops and Products**., North Dakota, v. 44, p. 566– 571. January. 2013.

WANG , S.; LIM, H. Current status of sunscreen regulation in the United States; food and drug administration's final rule on labeling and effectiveness testing. **Journal of the American Academy of Dermatology**., Charleston, v. 65, p. 863–869. August. 2011.

WANG, S. K.; BALAGULA, I.; OSTERWALDER, U. Photoprotection: a review of the current and future technologies. **Dermatology and Therapy**., UK, v. 23, p. 31–47. January. 2010.

WATERMAN, C.; CHENG, D. M.; ROJAS-SILVA, P.; POULEV, A.; DREIFUS, J.; LILA, M. A.; RASKIN, I. Stable, water extractable isothiocyanates from *Moringa oleifera* leaves attenuate inflammation in vitro. **Phytochemistry**., France, v. 103, p. 114–122. April. 2014.

YANG, H.; CHEN, S.; CHANG, N.; CHANG, J.; LEE, M.; TSAI, P.; FU, H.; KAO, W.; CHIANG, H.; WANG, H.; HSEU, Y. Protection from oxidative damage using Bidens pilosa extracts in normal human erythrocytes. **Food and Chemical Toxicology**., Spain, v. 44, p. 1513–1521. Apr. 2006.

ZHISHEN, J.; MENGCHENG, T.; JIANMING, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. **Food Chemistry**., Chiba, v. 64, p. 555-559. March. 1999.