
Evaluation of the, *in vitro*, photoprotective capacity of *Moringa oleifera* oil for its use in sunscreen formulation

Avaliação da capacidade fotoprotetora, in vitro, do óleo de Moringa oleifera para uso em formulação de filtro solar

Gabriela Cristina Ferreira Mota¹; Kamila de Fátima Anunciação Campos¹; Lucas Resende Dutra Sousa²; Tatiane Roquete Amparo³; Paula Melo de Abreu Vieira²; Adriana Akemi Okuma⁴; Tânia Marcia Sacramento Melo¹ and Viviane Martins Rebello dos Santos^{1*}

¹Departamento de Química, Instituto de Exatas e Ciências Biológicas, Universidade Federal de Ouro Preto, Câmpus Morro do Cruzeiro, Ouro Preto, Minas Gerais, Brasil.

²Laboratório de Morfopatologia, Núcleo de Pesquisas em Ciências Biológicas, Universidade Federal de Ouro Preto, Câmpus Morro do Cruzeiro, Ouro Preto, Minas Gerais, Brasil.

³Programa de Pós-graduação em Ciências Farmacêuticas, Escola de Farmácia, Universidade Federal de Ouro Preto, Câmpus Morro do Cruzeiro, Ouro Preto, Minas Gerais, Brasil.

⁴Departamento de Química, Centro Federal de Educação Tecnológica de Minas Gerais, Brasil

*Autor correspondente. E-mail: vivianesantos@ufop.edu.br

Recebido: 24/06/2021; Aceito: 01/12/2021

ABSTRACT

Moringa oleifera Lam is an Indian plant with applications in the agricultural and medical fields. The assets development capable of increasing the efficiency of sunscreens, mainly those of plant origin, due to their natural benefits, represents an increasing demand for cosmetology. The present study aims to identify by CG-MS the constituents of the most active oil and to evaluate the photoprotective capacity of *Moringa* oil, and its action in sunscreen formulations. Extracts of the oils from the *Moringa* seeds were evaluated for the Sun Protection Factor (SPF) observing the highest result for the dichloromethane extract. This extract showed low cytotoxic potential for human fibroblasts and it was incorporated into a sunscreen. The extract increased the SPF of the sunscreen and its effect may be related to fatty acids identified by GC-MS. The results showed the benefit of *Moringa* oil as a vegetable active in the sunscreen formulations by increasing the SPF of sunscreens in a natural and sustainable way.

Keywords: *Moringa oleifera*; extrato de óleo vegetal; Sun Protectors; fatty acids

RESUMO

Moringa oleifera Lam. é uma planta indiana com aplicações nas áreas agrícolas e médica. O desenvolvimento de ativos capazes de aumentar a eficiência dos filtros solares, principalmente os de origem vegetal, devido aos seus benefícios naturais, representa uma demanda crescente por cosmetologia. O presente estudo tem como objetivo identificar por CG-MS os constituintes do óleo mais ativo e avaliar a

capacidade fotoprotetora do óleo de Moringa, e sua ação em formulações de filtros solares. Os extratos dos óleos das sementes de Moringa foram avaliados quanto ao Fator de Proteção Solar (FPS), observando-se o maior resultado para o extrato de diclorometano. Este extrato apresentou baixo potencial citotóxico para fibroblastos humanos e foi incorporado a um protetor solar. O extrato aumentou o FPS do protetor solar e seu efeito pode estar relacionado aos ácidos graxos identificados por GC-MS. Os resultados mostraram o benefício do óleo de Moringa como ativo vegetal nas formulações de protetores solares por aumentar o FPS dos protetores solares de forma natural e sustentável.

Palavras-chave: *Moringa oleifera*; vegetable oil extract; Protetores solares; ácidos graxos

INTRODUCTION

Moringa oleifera is a plant, belonging to the Moringaceae family, native to northern India, and with much potential in technological development for the cosmetic industries, possessing biochemical components with antiseptic and anti-inflammatory properties (Abdulkarim et al., 2005; Anwar et al., 2007; Falowo et al., 2017). *Moringa oleifera* has various applications in agriculture, medicine, cosmetology and biodiesel, being considered an exceptional source of vitamins A, B and C, with a high protein content coming from the oil of its seeds, leaves and branches (Anuniação et al., 2004) The oil extracted from the seeds, known as ben oil or behen oil, is applied as a fixative for the perfume and cosmetic industries, as well as in the production of soap (Anuniação et al., 2004; Nascimento et al., 2009; Almeida et al., 2019; Almeida et al., 2020). Moreover, Brazil is the fourth greatest consumer of cosmetic products in the world, however, in the scientific literature only a few studies about the use of *Moringa* in cosmetic formulations in the country were verified. Despite *Moringa* oil has already been used in cosmetics, there are no scientific studies about its evaluation on sun protector formulations. Sun filters with vegetable origin components have great interest in the cosmetic industries, tending to the use of sustainable cosmetics from natural raw materials with photo protective activity or the capacity in potentiating the Sun Protection Factor (SPF) (Nascimento et al., 2009). The efficacy of a sun filter is evidenced by the SPF, therefore, quantifying the protection that a certain synthetic or natural product can offer, in terms of exposure, compared to unprotected exposure. Therefore, the present work aimed to evaluate and determine the sun protection factor in vitro, by means of a spectrophotometric method, from the *Moringa oleifera* seed oil for the incorporation process into the formulation systems of commercial sunscreens.

MATERIALS AND METHODS

General procedures

Solvents were purchased from Sigma Aldrich and used without further purification. UV-Vis Ultraviolet readings were performed on the Bel Engineering UV-M51 Spectrophotometer. Gas-chromatography coupled to mass spectrometry (GC-MS) on a GC-MS D5975 Agilent apparatus, equipped with Agilent J&W HP-5MS advanced GC column (30 m x 250 µm x 0.25 µm particle size). The cytotoxicity assay was performed using Human fibroblasts MRC-5 cells, cultivated in RPMI 1640 medium (Sigma®).

Plant material

The seeds were purchased from Arbocenter, through the website www.sementesarbocenter.com.br on April 3, 2019. The seeds were collected at Araçatuba – SP, Brazil from the 2019 crop (01/31/2019) at latitude 20° 56' 19 72" and longitude 50° 40' 6 17".

Extraction

Moringa oleifera seeds were benefited by separating the seeds from the husks and its impurities. Later, the seeds were put to dry in the oven for 4 hours at a temperature of 60-70°C, and then milled. The seed oil extractions were performed in a Soxhlet extractor apparatus using different organic solvents (hexane, dichloromethane and ethyl alcohol P.A.) for a period of 8 to 10 hours for each extraction. After the extractions, the solvent was rotaevaporized obtaining extracts (hexane, dichloromethane and ethanolic (Falowo et al., 2017).

***In vitro* determination of the sun protection factor (SPF) of the extracts**

The absorption readings of the extracts were made using the UV/Vis Spectrophotometer to determine the maximum absorbance in the ultraviolet regions A and B (UVA and UVB). Solutions in concentrations from 0.1 to 0.5 mg/mL were prepared using the specific solvent of each extraction at dilution. The scanning was performed in triplicate between the wavelengths 200 to 800 nm in the UV spectrophotometer for all the extract solutions, using the quartz bucket with an optical path of 1.0 cm and the solvent of each extraction as white in the respective reading. Through the Mansur method equation, it was possible to determine the Solar Protection Factor (SPF) value of each extraction concentration (Mansur et al., 1986). The absorption readings were taken between 290 and 320 nm (UVB region) and with intervals of 5 nm (Gonçalves, et al., 2019; Mansur et al., 2016).

Incorporation of the extracts into the sunscreen

The dichloromethane extract was incorporated in the photoprotective formulation with Permulem TR-1 UVA-UVB 5% Gel with filter. The extract was solubilized in ethanol and propylene glycol with the proportion of 1:1 and incorporated to Permulem TR-1 UVA-UVB 5% Gel with filter. This mixture was placed on a stirring plate in the presence of a magnet to assist in the incorporation process. The mixture remained under the stirring plate for a period of 20 to 30 minutes. The final composition of the formulation was 1% ethanolic extract, 10% ethanol, 10% propylene glycol and TR-1 Permulem Gel q.s.p. 100%. Soon afterwards, the formulation was weighed and diluted in a mixture of ethanol / propylene glycol, with the proportion of 1:1 until a concentration of 0.04 g / mL was obtained. A solution containing only TR-1 UVA-UVB 5% Permulem Gel was also made a positive control.

***In vitro* determination of the SPF after incorporation of the dichloromethane extract**

The UV-Vis Ultraviolet absorption readings, the incorporation and the Permulem TR-1 UVA-UVB 5% Gel with isolated filter, were made using the Genesys 10S Spectrophotometer to determine the maximum absorbance in the ultraviolet regions A and B (UVA and UVB). There were two solutions prepared: a) a solution of Permulem Tri UVA-UVB 5% Gel with filter (positive control) and b) solution of Permulem TR-1 UVA-UVB 5% Gel formulation with dichloromethane extract, in all solutions, ethyl alcohol was used (Mansur et al., 1986; Mansur et al., 2016). The scanning of solutions a and b were performed in triplicate between the wavelengths 200 to 800 nm in the UV Spectrophotometer, using the quartz bucket with optical path of 1.0 cm and the ethyl alcohol P.A as white in the respective reading. Through the equation of the method by Mansur in the absorption readings between 290 and 320 nm, with intervals of 5 nm, it was possible to determine the Solar Protection Factor (SPF) value of each solution (Gonçalves, et al., 2019; Mansur et al., 1986; Mansur et al., 2016).

Cytotoxicity assay

Human fibroblasts MRC-5 cells, cultivated in RPMI 1640 medium (Sigma®), were distributed in 96-well microtiter plate using a density of 5×10^4 cell/well and after they were incubated at 37°C with 5% of

CO₂ for 24 h. Cells were treated with the sample dissolved in RPMI 2% DMSO, at concentrations ranging from 1000.0 to 62.5. The cell viability was evaluated using the sulforhodamine B assay (SRB). After 24 h incubation, the media was removed and cells were fixed with cold 20% trichloroacetic acid for 1h at 4°C. The microtiter plate was washed with distilled water and dried. Thereafter, fixed cells were stained for 30 min with 0.1% SRB dissolved in 1% acetic acid. The plate washed again with 1% acetic acid, again allowed to dry and 200 µL of 10 mM TRIS buffer (pH 10.5) were added to stain solubilization at room.

Gas-Chromatography coupled to Mass Spectrometry (GC-MS)

Volatile compounds were identified by GC-MS with GC column (30 m x 250 µm x 0.25 µm particle size). Helium as used as carrier gas (1.4 ml/min). The dichloromethane extract of the *Moringa oleifera* seeds (1.0 mg/mL) was injected (1.0 µL) on split less mode. The injector and detector were set at 290 °C. Column temperature, initially at 100 °C (1 min), was increased to 200 °C (5°C/min), following an enhancement of 10 °C/min until 290 °C, keeping at isothermal condition for ten minutes. Compound identification was performed based on relative retention time and comparison of mass spectra with NIST/2.0 library data.

RESULTS

After extraction from the seeds, a yield of 40% was obtained for the hexanic extract, 37% for the dichloromethane extract and 27% for the ethanolic extract.

Table 1 shows the SPF values obtained from the three extractions of oil (hexane, dichloromethane and ethyl alcohol) in natura from *Moringa* seeds. When scanning the extracts, the wavelengths with higher absorbance of ultraviolet radiation were 251 nm in the extraction with hexane, 228 nm in the extraction with dichloromethane and 205 nm in the extraction with ethyl alcohol, all with higher absorption in the UVC range. In the dichloromethane extract it is possible to notice significant absorbances in the UVB and UVA.

Table 1. SPF values of *Moringa oleifera* seed extracts with hexane, dichloromethane and ethyl alcohol P.A. solvents.

Concentrations (mg/mL)	SPF in Region UVB (290 – 320)		
	Extracts		
	Hexane	Dichloromethane	Ethyl alcohol
0.1	0.0444±0.4256	5.1004±0.1821	0.9099±0.0228
0.2	0.0819±0.2648	5.9072±0.1618	1.3490±0.4279
0.3	1.3178±0.6445	5.9267±0.0427	1.5041±0.8066
0.4	1.4929±0.1667	6.9077±0.0768	1,5744±0.7955
0.5	1.9865±0.4845	7.5544±0.0285	2,7039±0.6991

Table 2 shows an efficacy of the dichloromethane extract as active in the formulation.

Table 2. SPF values of the formulation with Permulem Tri UVA-UVB Gel 5% with the filter and the use of dichloromethane extract as actives in sunscreens.

FPS in Region UVB (290 – 320)	
Gel with filter- Permulem Gel, 70%, UVA-UVB 5%	10.22±3.16
Dichloromethane Extract of the Moringa seeds	7.55±0.13
Formulation: Dichloromethane Extract with Gel Permulem, UVA-UVB 5%	21.36±0.02

The GC-MS results showed that the most abundant compounds are 6-Octadecenoic acid and 9-Octadecenal, (Z) and the majority are phytosterols and fatty acids between the volatile compounds in dichlorometanic extract (Table 3).

Table 3. Volatile compounds identified in dichlorometanic extract of the *Moringa oleifera* Lam. seeds by GC-MS.

Compound	t_r	% (volatile compounds)
(1) Hexadecanoic acid	21.17	4.34
(2) 8-Octadecenoic acid, methyl ester	23.36	2.80
(3) 6-Octadecenoic acid	23.95	36.72
(4) Ethyl Oleate	24.23	3.53
(5) Oleic acid	24.30	1.01
(6) 9-Octadecenoic acid (Z)- 2-hydroxy-1-(hydroxymethyl)ethyl ester	26.95	0.81
(7) 9-Octadecenal, (Z)	27.39	18.63
(8) Docosanoic acid, methyl ester	27.82	0.71
(9) Squalene	30.19	0.82
(10) alpha-tocopherol	33.10	1.26
(11) Campesterol	34.41	2.34
(12) Stigmasterol	34.87	2.92
(13) gamma-Sitosterol	35.75	4.75

DISCUSSION

This high yield of low polarity extracts is corroborated by previous data that indicate that *Moringa oleifera* seeds contain about 40% of oil, which consists mainly of fatty acids (3) of the table 3. In the Table 1 the extract with dichloromethane presented higher SPF value being the best natural photoprotective agent, in an isolated way, at these concentrations. In the table 2 the results observed show the potentiation of the SPF of Permulem Gel TR-1 UVA-UVB 5% with filter by the addition of Moringa seed dichloromethane extract. The positive result shows the benefit of using this oil in sunscreen formulations, increasing the Sun Protection Factor by almost triple the amount, only by adding a vegetable oil that promotes sustainable development. Besides the potentiation of the photoprotection of the Permulem Gel, the Moringa oil also has other properties favorable to the cosmetic industry.

Moreover, the cytotoxicity assay with human fibroblasts indicated low toxic potential of dichloromethane extract since its cytotoxic concentration for 50% of the cells was above the concentration that promotes photoprotective activity. The safety indicators of Moringa oil is reinforced by its application in some cosmetic products already related: use in hair (capillary oils, finishers of hair, creams and moisturizing masks) and skin (firming creams for face and neck, oils and moisturizing lotions for hands) containing specific protein fractions that protect the skin and hair threads from environmental influences and combat premature skin aging, besides conditioning and strengthening the hair (Almeida et al., 2017).

Recent studies have shown that fatty acids are promising candidates for the protection of the skin through a variety of mechanisms, which reinforces that the volatile compound in greater quantity (6-Octadecenoic acid) identified in the present work (Table 3) could be the main responsible for the increased photoprotective activity (Pilkington et al., 2011; Vasconcelos et al., 2016).

CONCLUSION

Moringa presents several utilities for the cosmetic industry as a plant, in all its extension, with potential for further studies of its properties. Formulations of sun protectors can be potentiated by the use of natural oils, diminishing skin irritations by the exaggerated use of sun filters and maintaining or increasing depending on the quantity used, the value of the Sun Protection Factor. In this research, the importance of using natural substances such as *Moringa oleifera* seeds oil in the formulations of the sun protectors was demonstrated, since a significant increase in SPF (Sun Protection Factor) values by potentiating the oil as an active for the product was observed without increasing the quantity of sun filter.

ACKNOWLEDGMENTS

We are grateful to the Universidade Federal de Ouro Preto-UFOP and CEFET-MG-Campus Belo Horizonte.

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**, v.93, p.253-263, 2005.
- ALMEIDA, C.B.L.; SÁ, A.C.C.; CARVALHO, R.C.D.; ALMEIDA, E.S.; Estudo prospectivo da moringa na indústria de cosméticos. **Caderno de Prospecção, Salvador**, v.10, p. 905-918, 2017.
- ALMEIDA, W.A.D.A.S.; DOS SANTOS, A.A.; PENIDO, R.G.; CORREA, DA G.C.H.S.; DO NASCIMENTO, A.M.; ANDRADE, A.L.; SANTOS, V.R.; CAZATI, T.; AMPARO, T.R.; DE SOUZA, G. H.B.; FREITAS, K.M.; DOS SANTOS, O.D.H.; SOUSA, L.R.D.; DOS SANTOS, V.M.R.; Photoprotective activity and increase of SPF in sunscreen formulation using lyophilized red propolis extracts from Alagoas. **Revista Brasileira de Farmacognosia**, v.29, n.3, p. 373- 380, 2019.

ALMEIDA, W.D.A.S.; SOUSA, L.R.D.; ANTUNES, A.S.; AZEVEDO, A.S.; DO NASCIMENTO, A.M.; AMPARO, T.R.A.; SOUZA, G.H.B.; DOS SANTOS, O.D.H.; ANDRADE, A.L.; CAZATI, T.; VIEIRA, P.M.A.; BUENO, P.C.P.; DOS SANTOS, V.M.R.; Green Propolis: In Vitro Photoprotective and Photostability Studies of Single and Incorporated Extracts in a Sunscreen Formulation. **Revista Brasileira de Farmacognosia**, v.30, n.3, p. 436-443, 2020.

ANUNCIACÃO, K.F, SOUSA, L.R.D.; AMPARO, T.R.; SOUZA, G.H.B.; VIEIRA, P.M.A.; BREGUEZ, G.S.; DOS SANTOS, V.M.R.; MELO, T.M.S.; Avaliação da Atividade Antioxidante e Fenóis Totais dos Óleos Extraídos das Sementes de *Moringa oleifera* Lam. **Revista Virtual de Química**, v.12, n.1, p. 148-154, 2020.

ANWAR, F.; LATIF, S.; ASHRAF, M.; GILANI, A.H.; *Moringa oleifera*: a food plant with multiple medicinal uses. **Phytother Res.**, v.21, p.17-25, 2007.

FALOWO, A.B.; MUKUMBO, F.E.; IDAMOKORO, E.M.; LORENZO, J.M.; AFOLAYAN, A.J.; MUCHENJE, V.; Multi-functional application of *Moringa oleifera* Lam. in nutrition and animal food products: A review. **Food Res. Int.**, v.106, n.4, p.317-334, 2017.

GONÇALVES, M.C.; DOS SANTOS, V.M.R.; TAYLOR, J.G.; PERASOLI, F.B.; DOS SANTOS, O.D.H.; RABELO, A.C.S.; JUNIOR, R.J.V.; COSTA, D.C.; CAZATI, T.; Preparation and characterization of a quercetin-tetraethyl ether-based photoprotective nanoemulsion. **Química Nova**, v.42, p.365-370, 2019.

MANSUR, J.S.; BREDER, M.V.R.; MANSUR, M.C.A.; AZULAY, R.D.; Determinação do fator de proteção solar por espectrofotometria. **An. Bras. Dermatol.**, v.61, p.121-124, 1986.

MANSUR, M.C.P.R.; LEITÃO, S.G.; COUTINHO, C.C.; VERMELHO, A.B.; SILVA, R.S.; PRESGRAVE, O.A.F.; LEITÃO, A.C.; LEITÃO, G.G.; JUNIOR, E.R.; SANTOS, E.P. *In vitro* and *in vivo* evaluation of efficacy and safety of photoprotective formulations containing antioxidant extracts. **Revista Brasileira de Farmacognosia**, 2016

NASCIMENTO, C.S.; NUNES, L.C.C.; DE LIMA, A.A.N.; JUNIOR S.G.; NETO, P.J.R.; Incremento do FPS em formulação de protetor solar utilizando extratos de própolis verde e vermelha. **Revista Brasileira de Farmacognosia**, v.90, n.4, p.334-339, 2009.

PILKINGTON, S.M.; WATSON, R.E.; NICOLAOU, R.L.E.; Omega-3 polyunsaturated fatty acids: Photoprotective macronutrients. **Experimental Dermatology**, v. 20, n.7, p.537-543, 2011.

VASCONCELOS, R.O.; BUSTOS, S.A.; GONZALEZ, J.R.; SOARES, W.; BARBOSA, M.D.; CHAMMAS, R.; VOTTO, A.P. DE S.; TRINDADE, G.S.; Interaction between omega 3 PUFA and UVB radiation: Photoprotective effect in normal and tumoral murine melanocytes? **Journal Photochemistry Photobiology B**, 164, p.361-368, 2016.