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Original Article

# Evaluation of antioxidant, photoprotective and antinociceptive activities of *Marcetia macrophylla* extract: potential for formulation of sunscreens

## Avaliação da atividade antioxidante, photoprotetora e antinociceptiva de *Marcetia macrophylla*: potencial formulação fotoprotetora

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**Abstract**

The antioxidant, photoprotective and antinociceptive *Marcetia macrophylla* active extract was investigated as an active ingredient in a sunscreen cream formulation. Thus, the *M. macrophylla* extract showed $IC_{50}$ of 3.43 mg/ml of the antioxidant (DPPH· scavenging test) and Sun Protection Factor of 20.25 (SPF/UV-B, at 250 µg/ml) and UV-A of 78.09% (photobleaching *trans*-resveratrol test). The antinociceptive activity was superior to all standards tested using the *in vivo* acetic acid-induced writhing test (99.14% at the dose of 200 mg/kg) and the high-performance liquid chromatography coupled with diode array detector and mass spectroscopy multi-stage (HPLC-DAD-MS/MS) enabled the structural characterization of the quercetin-3-*O*-hexoside, quercetin-3-*O*-pentoside and quercetin-3-*O*-desoxihexoside. The pharmaceutical formulation containing the *Marcetia macrophylla* crude active extract was prepared and the physicochemical tests (organoleptic characteristics, pH analysis and centrifugation), the *in vitro* UVB (sun protection factor, SPF) and UVA (β-carotene) using the spectroscopic method were investigated. The formulation showed satisfactory results concerning the physicochemical parameters evaluated and active against the UV test. Thus, *M. macrophylla* showed biological activities with potential use in pharmaceutical preparations.

**Keywords:** *Marcetia macrophylla*, endemic plant, biological activity, flavonoid, phytoformulation.

**Resumo**

O extrato bruto de *Marcetia macrophylla* mostrou atividade antioxidante, fotoprotetora e antinociceptiva, sendo em seguida investigado como ingrediente ativo em uma formulação fotoprotetora. Assim, o extrato de *M. macrophylla* apresentou atividade antioxidante com IC50 de 3,43 mg/mL (teste de sequestro do DPPH·) e Fator de Proteção Solar de 20,25 (FPS/UV-B, 250 µg/mL) e UV-A de 78,09% (teste de fotobranqueamento do trans-resveratrol). A atividade antinociceptiva usando o teste in vivo de contorções abdominais induzidas por ácido acético foi superior a todos os padrões testados (99,14% na dose de 200 mg/Kg). A análise por cromatografia líquida de alta eficiência acoplada a detector de fotodiodos e espectroscopia de massas multi-estágio (CLAE-DAD-EM/EM) possibilitou a caracterização dos flavonoides quercetina-3-O-hexosídeo, quercetina-3-O-pentosídeo e quercetina-3-O-desoxihexosídeo. A formulação farmacêutica contendo o extrato ativo bruto de *Marcetia macrophylla* foi preparada e os testes físico-químicos (características organolépticas, análise de pH e centrifugação), o UVB in vitro (fator de proteção solar, FPS) e UVA (β-caroteno) foram investigados. A formulação apresentou resultados satisfatórios frente aos parâmetros físico-químicos avaliados e ativos contra UV. Assim, *M. macrophylla* apresentou atividades biológicas com potencial uso em preparações fitofarmacêuticas.

**Palavras-chaves:** *Marcetia macrophylla*, planta endemica, atividade biológica, flavonoides, fitoformulação.

## 1. Introduction

Flavonoid-rich extracts with potential antioxidant and anti-inflammatory properties are gaining popularity as skin-care products containing botanical ingredients (Perugini et al., 2002). Flavonoids inhibit lipid peroxidation and reduce the risk of cardiovascular diseases, effects related to their antioxidant activity, characterized by the

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ability to scavenge free radicals (Nichols and Katiyar, 2010; Souza et al., 2018; Hollman and Katan, 1999). In addition, these compounds also present antibacterial, antiviral, anti-inflammatory and anti-allergic action, as well as photoprotective activities (Marwah et al., 2007; Cook and Samman, 1996; El-Hawary et al., 2020). Thus, flavonoid-rich extracts stand out as a viable option for the development of pharmaceutical products, particularly sunscreens. Studies show that flavonoids produced by a plant are considered as an important factor as self-protection against ultraviolet light (Markham et al., 1998). That can be strengthened due to their structural similarity with chemical filters commercially used, which are susceptible to radiation absorption in the ultraviolet region, usually containing two maximum peaks in ultraviolet spectrum absorption (UV-B and UV-A regions) (Oliveira et al., 2016).

Sunscreen is a cosmetic preparation intended to come into contact with the skin and lips, with the sole or main purpose of protecting it against UV-B and UV-A radiation by absorbing, scattering or reflecting radiation. The sun protection factor (SPF) is a numeric value used to evaluate the time during which the skin is protected against sunburn caused by UV-B radiation by administering a particular sunscreen (Mukherjee et al., 2011). The SPF is a set value obtained by dividing the Minimal Erythema Dose in a skin protected by a sunscreen (MEDp) and the Minimal Erythema Dose on the same skin when unprotected (MEDup).

The Melastomataceae family is one of the largest of the Angiosperms. It has around 166 genera and approximately 4,500 species with pantropical distribution (Renner, 1993). It is chemically characterized by means of the biosynthesis of tannins (Yoshida et al., 2010), steroids (Crevelin et al., 2006; Calderon et al., 2002) and especially flavonoids (Leite et al., 2012a). The *Marcetia* genus stands out with 28 species described in Brazil. The state of Bahia has about 85% of *Marcetia* species and it is considered as the diversity center of this genus (Santos and Silva, 2005). A previous study carried out by our research group showed the antimicrobial activity and the characterization of the flavonoids in several *Marcetia* species collected in the Brazilian semiarid region (Leite et al., 2012b).

In this work, we describe the evaluation of the antioxidant, photoprotective and antinociceptive activities of the *M. macrophylla* extract and the preparation of a sunscreen formulation containing this active extract.

## 2. Materials and Methods

### *2.1. Plant material*

*Marcetia macrophylla* Wurdack was collected in Rio-de-Contas (Chapada Diamantina, state of Bahia, Brazil). A voucher specimen was deposited in the Herbarium of the Department of Biology in the State University of Feira de Santana (HUEFS) with the following number: 99625. All procedures for the access to genetic patrimony and associated traditional knowledge were carried out and the project was registered in SisGen (ABB3E50).

### *2.2. Preparation of the crude extracts*

The dried and powdered leaves (1680 g) were submitted to maceration at room temperature with hexane (4 L) for 72 h, which was further filtrated. The solvent was removed to furnish the plant material defatted. An aliquot was macerated with ethanol (3 L) for 72 h. After that, it was filtrated and the ethanol was removed under reduced pressure using a rotaevaporator apparatus, furnishing the *Marcetia macrophylla* ethanolic extract (MME). The other aliquot was macerated with EtOAc (2 L) and MeOH (2 L) at room temperature, successively, for 72 h. The solvents were removed under reduced pressure to yield the *Marcetia macrophylla* ethyl acetate (MMA, 47.3 g) and methanolic (MMM, 53.2 g) extracts, respectively.

### *2.3 Chemical and instruments*

TLC was performed on aluminum sheets of silica gel 60 (Merck) and TLC detection was provided by UV absorption at 254 and 360 nm. The analysis of high-performance liquid chromatography coupled to a photodiode detector (HPLC-DAD) (EZChrom Elite), equipped with a VRW HITACHI L 2130 pump and a HITACHI L-2300 VRW diode array detector was obtained by means of the Merck-Hitachi® LaChron Elite chromatograph. The results were acquired and processed using the EZChrom Elite software, 20-µL injection volume. In this process, we used a LiChrospher® (C18 reverse phase; 5 mm, 250 x 4.6 mm) column. Samples and mobile phase were filtered through cellulose acetate membranes with pore size of 0.22 micrometers. The samples and standard sample were dissolved in methanol. The analysis was made under the following conditions: mobile phase to two-phase system: A (0.1% $H_3PO_4$ in $H_2O$ - acidified water) and B (methanol) gradient: time 0 min - 20 min (75% A and 25% B to 100% B), 20 min - 24 min (100% B) 24 min - 25 min (75% A and 25% B); 25 min - 35 min (75% A and 25% B). Reading was carried out at 280 nm.

ESI-MS spectra were acquired on an Esquire 3000 Plus (Bruker Daltonics) spectrometer equipped with an ESI source in the positive ion mode. High-purity nitrogen gas was used as collision, nebulizer and auxiliary heated gas. The ESI interface conditions were as follows: spray voltage, 4500 V; declustering potential, C 40 V; focusing potential, C120 V; nebulizer gas flow, 1.5 L/min; auxiliary gas flow, 3 L/min; temperature, 180 °C. Samples were introduced via a syringe pump at a flow rate of 4 µl/ min. The collision energy was set at 20 eV and mass data were processed with DataAnalysis 3.2.

### *2.4. Fractionation of the extract*

The MMM extract was chromatographed over silica gel 60 (70-230 mesh) eluted with organic solvent in an ascending order of polarity (Hex, AcOEt, EtOH and MeOH, successively). A total of 16 fractions were collected and the fractions were analyzed through TLC and HPLC-DAD. The fractions with flavonoid profiles were gathered and subjected to a new fractionation using Sephadex LH-20. Thus, the fractions 3 to 8 were re-organized and put together and characterized as a mixture of three flavonoids, named flavonoid-rich fraction.

*2.5. Total Flavonoid Content (TFC)*

The TFC of the extracts was determined by the method of reaction with aluminum chloride ($AlCl_3$), which when complexed with flavonoids absorb UV energy in 425 nm (Kalita et al., 2013). An analytical curve was performed with the quercetin standard at concentrations of 20, 40, 80, 160, 320 and 640 µg/ml followed by linear regression, where values are reported in micrograms of quercetin per microgram of extract. The calculation of linear regression using the least squares method generated the described equation, as well as its average linear correlation coefficient ($R^2$), which obtained a value of 0.9937, very close to 1, giving the method linearity (Mensor et al., 2001 ).

A 1 M solution of $AlCl_3$ (1%) and potassium acetate ($CH_3CO_2K$) was prepared. For the test sample, an ethanol solution of MME with a concentration of 2 mg/ml was prepared. Then the QUE solutions were added individually to 2-ml volumetric flasks; 0.1 ml of $AlCl_3$ solution; 0.1 ml of $CH_3CO_2K$ solution; 2.8 ml of distilled water and, last, the volumetric flask had its volume completed with EtOH. The same procedure was applied to the test samples. The blank volumetric flask was prepared without the $AlCl_3$ solution. The MME was left to rest for 30 minutes. Afterwards, the samples were read at 415 nm in an Evolution 220 UV/Vis spectrophotometer (Thermo Scientific®) (Gursoy et al., 2009). The absorbance of the MME solution was determined by subtracting the absorbance value of the quercetin equivalents (QUE) solution from the absorbance value of the blank. The concentration of total flavonoids was calculated from the construction of the quercetin calibration curve (1 mg/ml). The data were expressed as µg of QUE per 10 mg of ethanolic extract weight.

*2.6. DPPH free radical scavenging assay*

Aliquots of the samples were incubated in the absence of light and at room temperature with a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution. The absorbance values were measured at 518 nm and converted into the percentage of free radical sequestration values (% SRL) using the following formula: % SRL = [(absorbance of the control − absorbance of the sample)/absorbance of the control] × 100. The $IC_{50}$ values were calculated from a non-linear regression of the data (Dewanto et al., 2002).

*2.7. Sun Protection Factor (SPF)*

The sample was dissolved in ethanol to a final concentration of 12.5, 25, 50, 125 and 250 µg/µl. The sample absorbance was measured in UV-B wavelength range (290-320 nm), with 5-nm increments and 3 determinations were made at each point (Mansur et al., 1986). The SPF was calculated by applying Mansur´s equation: $SPF = CF \times {}_{290}\sum^{320}{}_{290} EE\ (\lambda) \times I(\lambda) \times abs\ (\lambda)$, where: CF (correction factor) = 10; EE ($\lambda$) is the erythemal efficiency spectrum; $I$ ($\lambda$) is the solar intensity spectrum; abs ($\lambda$) is the absorbance of the solution. The values of EE ($\lambda$) x $I$($\lambda$) are constant according to Mouffouk et al. (2020).

*2.8. UV-A blocking activity*

A 1.0 mg/ml hydroalcoholic solution of *trans*-resveratrol was prepared. Petri dishes of 4.5 cm diameter were filled with 5.0 µl of this solution. Each Petri dish was covered with a 0.04-g evenly spread layer of the extract to be tested for the UV blocking activity. Petri dishes with clean lids were used as control. The Petri dishes were placed inside the UV-A chamber and were irradiated with a radiation intensity of about 830 W/m² (between 320 and 400 nm) for a period of time (0-120 min). In predetermined intervals (20 min), a sample was collected and diluted at 1:10. The absorbance was measured spectrophotometrically (306 nm) using a UV-VIS Varian® (Cary 100 BIO) (Allan et al., 2009). The absorbance of a sample totally protected from light was also measured in predetermined intervals to assure that the degradation was induced by UV-A light; *trans* isomer, when exposed to light, becomes the *cis* making constant absorbance. This experiment was performed in triplicate.

*2.9. Antinociceptive activity*

Male adult albino Swiss mice (30-40 g) were used throughout this study. Experimental protocols and procedures were approved by the Animal Care and Use Committee of the Vale do São Francisco Federal University under the number 024240408. Mice were divided into seven groups of six mice each. Acetic acid (0.9% v/v) was administered i.p. in a volume of 0.1 ml/10 g. Vehicle (saline), morphine (10 mg/kg), acetylsalicylic acid (ASA 150 mg/kg), MME and MMM (100 and 200 mg/kg), were administered i.p. 30 min before the acetic acid injection. The amount of abdominal writhing produced in each group for the succeeding 10 min was counted and compared to the response in the control group (Silva et al., 2018). The antinociceptive activity was expressed as the inhibition percentage of abdominal contraction. ANOVA statistical analysis was performed followed by Dunnett's test using the GraphPad Prism 5.0 program.

*2.10. Preparation of sunscreen formulations*

The study employed the methods for pharmaceutical formulations described in the literature (Costa et al., 2015; Batistuzzo et al., 2002). Briefly, the polawax lotion was employed: Phase 1 (methylparaben 0.15%, glycerine 5% and deionized water q.s.p 100 g); Phase 2 (propylparaben 0.05%, liquid vaselin 3%, octyl stearate 4% and polawax 5%) and Phase 3 (imidazolidinyl urea). Composition of the sunscreen formulation: Phase 1 (methylparaben 0.15%, glycerine 5% and deionized water q.s.p 100 g); Phase 2 (propylparaben 0.05%, vaselin liquid 3%, octyl stearate 4% and polowax 5%) and Phase 3 (imidazolidinyl urea).

The vehicle (Polawax® lotion used for the incorporation of MME) was prepared by heating the emulsifying wax (phase 1) and water (phase 2) at 75 °C, separately. After that, phase 2 was poured into phase 1 under constant agitation, ending with the addition of the ethanolic extract (phase 3) and the mixing at a temperature below 40 °C. The standard formula consisted of a simple sunscreen emulsion of O/W containing benzophenone-3 as a chemical filter. The emulsion was prepared with the same method described for preparing the O/W emulsion without benzophenone-3. The extract was incorporated to the Polawax® lotion in the concentrations of 5, 10, 20 and 30% (w/w). Once formulated, the pH was measured and

adjusted with triethanolamine to pH 6.0 to 7.0, which is the desirable pH for a sunscreen.

*2.11. Physicochemical analysis of the formulations*

After they were prepared, the sunscreen formulations containing benzophenone-3 and different concentrations of MME (5, 10, 20 and 30%) were allowed to stand for 24 hours. Afterwards, the samples underwent the evaluation of organoleptic characteristics (25 °C), pH analysis (pH values were adjusted between 6.0 to 7.0) and centrifugation (30 minutes at 3,000 rpm at room temperature) (Brasil, 2014).

In addition to the physicochemical analysis of the formulations, the evaluation of the spreadability of formulations was performed using the methodology described by Cordeiro et al., 2013. In order to determine the spreadability of the samples, a circular glass plate (20 cm in diameter and 0.3 cm in thickness) was used, with a central hole of 1 cm in diameter, over another glass plate that was used as a support. Graph paper was fixed below the support plate. The sample was placed in the hole in the plate, leveling with the help of a spatula. The mold plate was removed so that the sample remained in the center of the support plate. In this way, a glass plate of predetermined weight was placed on the sample. After one minute, the covered area was calculated, measuring the diameter in two positions, vertically and horizontally, from the scale of the graph paper. The procedure was repeated with the addition of new plates, and the measurement was performed one minute after the addition of each new plate, until five plates were added (Lange et al., 2009).

*2.12. Determination of the SPF of the sunscreen formulations*

The MME was dissolved in ethanol to a final concentration of 12.5, 25, 50, 125 and 250 µg/ml. The SPF model used in this study was according to the methodology described in the literature (Mansur et al., 1986; Silva et al., 2016). The sample absorbances were measured in UVB wavelength range (290-320 nm), with 5-nm increments and 3 determinations were made at each point. The SPF was calculated by applying Mansur´s equation: $SPF = CF \times {}_{290}\sum^{320}{}_{290} EE(\lambda) \times I(\lambda) \times abs(\lambda)$, where: CF (correction factor) = 10; EE ($\lambda$) is the erythemal efficiency spectrum; $I$ ($\lambda$) is the solar intensity spectrum; abs ($\lambda$) is the absorbance of the solution. The values of EE ($\lambda$) x $I$($\lambda$) are constant according to Mouffuka et al., 2020. For the determination of the SPF of the sunscreen formulations, different contents of the extract (5, 10, 20 and 30%) were dissolved in ethanol to a final concentration of 0.2, 2.0, 5.0, 10, 15, 20, 30 and 50 mg/ml to evaluate the profile of the sunscreen formulations prepared.

*2.13. UV-A blocking activity*

A 1 mg/ml hydroalcoholic solution of *trans*-resveratrol was prepared. Petri dishes of 4.5 cm diameter were filled with 5 µl of this solution. Each Petri dish was covered with a 0.04-g evenly spread layer of formulation containing MME to be tested for its UV-A blocking activity. Petri dishes with clean lids were used as control. The Petri dishes were placed inside the UV-A chamber and were irradiated with a radiation intensity of about 830 W/m$^2$ (between 320 and 400 nm) for a period of time (0-120 min). In predetermined intervals (20 min), a sample was collected and diluted at 1:10. The absorbance was measured spectrophotometrically (306 nm) using a UV-VIS Varian® (Cary 100 BIO). The absorbance of a sample totally protected from light was also measured in predetermined intervals to assure that the degradation was induced by UV-A light; *trans* isomer, when exposed to light, becomes the *cis* making constant absorbance (Serafini et al., 2014).

*2.14. Statistical analysis*

The analyses were performed in triplicate and the results expressed as mean ± standard deviation (SD). Differences were considered significant when $p < 0.05$. Multiple comparisons between more than two groups were performed with one-way ANOVA supplemented with Tukey´s test. The data obtained were analyzed using the Graph Pad Prism® version 5.0.

## 3. Results and Discussion

*3.1. Obtaining the extracts*

Before the extraction of the secondary metabolites of *M. macrophylla* leaves for the preparation of extracts, the excess of waxes was removed using non-polar solvent. The Melastomataceae species, native from the Brazilian semiarid region, are known for bioproducing a considerate amount of waxes (Mimura et al., 1998). Afterwards, an aliquot of defatted plant material was added with ethanol as extractor solvent to yield the respective extract (MME) that was used for the antioxidant DPPH-test and sunscreen formulation. The ethanol was used in this step due to its low toxicity as this is a basic requirement for the development of topical pharmaceutical formulations containing plant extracts (Mohammad et al., 2018; Romanhole et al., 2020). In the other aliquot, the ethyl acetate (MMA) and methanol (MMM) were added successively as extractor solvents with the goal to yield extracts with different polarities to better evaluate the natural compounds with antinociceptive activity (Murugan and Parimelazhagan, 2013). In this case, one of the extracts can be concentrating more inactive molecule making the other extract more active. Finally, due to the good antinociceptive activity, the MMM was chosen for phytochemical investigation.

*3.2. DPPH and Photoprotective activity*

TFC was calculated as quercetin equivalent (QUE), showing 0.122 µg/ml of quercetin for every 10 mg of MME. The MME antioxidant activity was evaluated by the methodology of the scavenging of stable free radical DPPH at 250, 125, 50, 25, 10 and 5 mg/µl (Ignat et al., 2011) and compared to the BHT and AA standards (Table 1). The comparison of the free radical sequestration activity percentage values (% FRSA) between extract and standards can be seen as the presence of good antioxidant activity of the extract. The $EC_{50}$ values for crude extract, BHT

and ascorbic acid were 2.948 ± 0.625, 8.152 ± 0.365 and 1.857 ± 0.280 µg/µl, respectively. These results indicate that MME showed a higher antioxidant activity than did BHT.

With the methodology of Mansur et al. (1986), MME at concentrations of 250 and 125 µg/µl showed satisfactory sunscreen activity (20.25 and 10.32, respectively) (Figure 1). These results were higher than the minimum required by the Brazilian Health Regulatory Agency ANVISA (Brasil, 2012). When *trans*-resveratrol is exposed to UVA radiation, it undergoes degradation, which can be seen by the absorbance decreases during the time intervals (Wu et al., 2015). It was found that during the control test of exposition to UVA radiation, there was photobleaching of 40.49% *trans*-resveratrol. The plate was coated with MME and photobleaching was less pronounced; the end of the 120-min exposure conversion of *trans*-resveratrol to *cis*-resveratrol was 21.91%. This extract was shown to be capable of preventing UV-A radiation from permeating across a Petri dish lid (Figure 2).

### *3.3. Antinociceptive activity*

The results of MMA and MMM are shown in Table 2. It was observed that there was a statistically significant decrease in the amount of writhing in the groups treated with extracts compared to the negative control. The inhibition of contortions of MMM at a concentration of 100 mg/kg, 94.19%, was similar to the standard morphine (95.04%) and greater than the acetylsalicylic acid (84.88%). Comparing the concentration of 200 mg/kg, this extract was superior to all standards, with 99.14% inhibition of writhing. The MMA had its activity proved to be lower than the standards tested. It was also observed that increasing doses resulted in increased response in both extracts tested.

### *3.4. HPLC-DAD and HPLC-MS/MS analysis*

The MME was fractionated using $SiO_2$ open-column chromatography (for more detail, see experimental) to

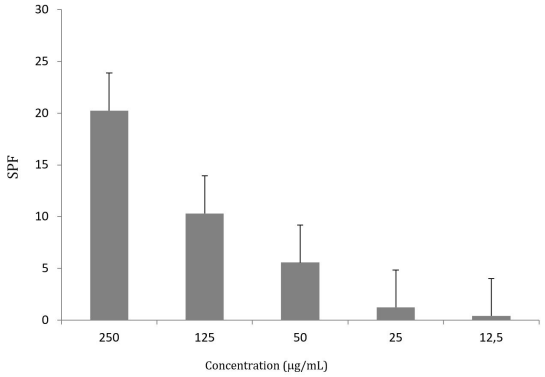


**Figure 1.** SPF profile of the sunscreens containing *Marcetia macrophylla* crude extract.

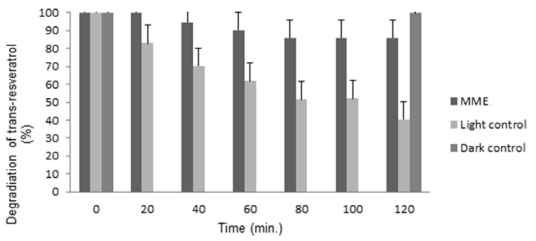


**Figure 2.** Activity against UVA radiation of the *Marcetia macrophylla* ethanolic extract (MME).

**Table 1.** Antioxidant activity of the *Marcetia macrophylla* crude ethanolic extract (MME).

| | Free Radical Sequestration Activity (FRSA, %) | | | | | |
|---|---|---|---|---|---|---|
| | 250* | 125 | 50 | 25 | 10 | 5 |
| MME | 95.61 | 94.60 | 93.61 | 92.99 | 56.17 | 17.85 |
| BHT** | 94.64 | 94.07 | 86.58 | 57.16 | 30.20 | 18.38 |
| AA*** | 97.03 | 96.62 | 96.98 | 96.57 | 74.32 | 24.60 |

*µg/ml. **Butylated hydroxytoluene. ***Ascorbic acid.

**Table 2.** Antinociceptive evaluation of the *Marcetia macrophylla* ethyl acetate (MMA) and methanolic extract (MMM) on acetic acid-induced writhing in mice.

| Groups | Dose (mg/kg) | No. of writhing | % Inhibition |
|---|---|---|---|
| Control | --- | 20.17 ± 3.40 | --- |
| MMA | 100 | 7.17 ± 3.30** | 64.45 |
| | 200 | 7.00 ± 3.04** | 65.29 |
| MMM | 100 | 1.17 ± 0.75** | 94.19 |
| | 200 | 0.17 ± 0.17** | 99.15 |
| Morphine | 10 | 1.00 ± 1.00** | 95.04 |
| Acetylsalicylic acid | 150 | 2.67 ± 2.67** | 84.66 |

Values are mean ± S.E.M, n= 6. **p < 0.01 significantly different from control (ANOVA followed by Dunnett's test).

furnish a fraction containing three glycosylated flavonols in the mixture: quercetin-3-*O*-hexoside **(1)**, quercetin-3-*O*-pentoside **(2)** and quercetin-3-*O*-desoxihexoside **(1)**. The structural characterization of these known compounds was performed through high-performance liquid chromatography coupled to diode array detector (HPLC-DAD) (UV spectrum: compound **1**: $\lambda_{max:}$ 256/354 nm; **2**: $\lambda_{max:}$ 256/346 nm and **3**: $\lambda_{max:}$ 256/356 nm) and multi-stage mass spectroscopy (HPLC-MS/MS) {compound **1**: *m/z* 465.2 $[M+H]^+$, 303.1 ($[M+H^+]$ - 162 Da), **2**: *m/z* 435.0 $[M+H]^+$, 302.9 ($[M+H^+]$ - 132 Da) and **3**: *m/z* 449.0 $[M+H]^+$, 302.9 ($[M+H^+]$ - 146 Da)}. The Figure 3 showed the HPLC chromatogram and the respective chemical structures. These results were in accordance with data of literature (Simirgiotis et al., 2015; Cabrera, 2005).

### *3.5. Sunscreen formulations*

#### *3.5.1. Physicochemical analysis*

In the macroscopic analysis, one could observe that all formulations were homogeneous with the characteristic odor of MME. A more fluid consistency was observed in the concentrations of 20 and 30% of the extract. The color of the samples was determined by the extract color (dark brown) varying in intensity when a greater proportion of the extract was incorporated into the Polawax® lotion.

Macroscopic and pH analysis are important parameters because they may indicate problems concerning the physicochemical stability of the formulation interfering with the quality of the final product. The pH results of the formulations are shown in Table 3. Once formulated, the pH was measured and adjusted with triethanolamine to pH 6.0 to 7.0, which is the desirable pH for sunscreen. Low pH values can be related to the appearance of cumulative skin irritation (Reddy and Grace, 2016). All formulations were subjected to the centrifugation test, which showed that the samples did not separate or precipitate phases, thus indicating that the formulations are suitable for future stability testing. The non-appearance of visible oil droplets in the formulation samples after the centrifugation test ensures the uniformity of the dose applied to the skin; otherwise, photoprotection would not be seen as effective (Dario et al., 2016; Wang et al., 2014).

In the study of spreadability of formulations containing MME (Figure 4), one could observe that the increase in the printed weight of the samples did not result in a significant increase in the spreadability of the formulations, though the spreadability values were statistically different. Also, there was a reduction when compared to the Polowax® lotion

#### *3.5.2. SPF determination*

The *in vitro* SPF testing means are frequently used as a substitute for expensive and ethically questionable *in vivo* assays, which require the participation of many test subjects. Studies have demonstrated that the results obtained by *in vitro* methods compare favorably with those *in vivo* (Tabrizi et al., 2003). In order to evaluate the SPF, a pharmaceutical base was prepared (Polawax® emulsion); after that, different concentrations (5, 10, 20 and 30%) of the *Marcetia macrophylla* crude extract were added

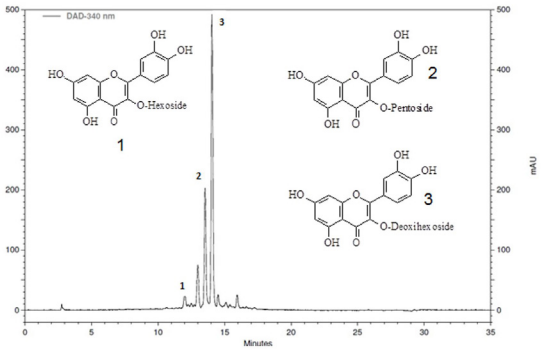


**Figure 3.** HPLC-DAD chromatogram of the flavonoid-rich fraction obtained from *Marcetia macrophylla* methanolic extract (MMM): peak 1 (11.4 min): quercetin-3-*O*-hexoside; peak 2 12.0 min): quercetin-3-*O*-pentoside and peak 3 (12.5 min): quercetin-3-*O*-deoxihexoside. Insert the respective chemical structure of the each peak.

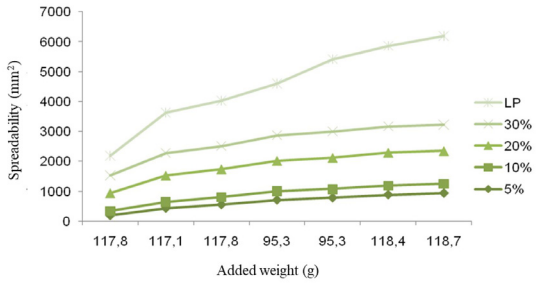


**Figure 4.** Emulsion spreadability (25 ºC) containing EMS in different concentrations.

**Table 3.** pH values before and after correction with triethanolamine.

| Formulation (%) | pH before correction | pH after correction* |
|---|---|---|
| 5 | 5.11 ± 0.041[a] | 6.41 ± 0.180 |
| 10 | 5.24 ± 0.045[a] | 6.73 ± 0.145 |
| 20 | 4.62 ± 0.070[b] | 6.71 ± 0.265 |
| 30 | 4.48 ± 0.062[c] | 6.45 ± 0.065 |

a, b, c: the means with different letters within the same column present significant difference between them ($p < 0.05$) according to Tukey´s test. *There was no significant difference between them ($p < 0.05$).

for the obtaining of four photoprotective formulations. These sunscreens were diluted in eight serial dilutions to evaluate the SPF (Figure 5). For this study, it was considered that formulations with a SPF ≥ 6 had sunscreen activity, which corresponds to the minimum value necessary for a product to be considered as a sunscreen. It was found that the formulations containing the MME when diluted to 0.2 mg/ml showed SPF ≤ 6. The SPF values increased in proportion to the concentration of extract in each formulation. In concentrations between 10.0 to 50.0 mg/µl, all formulations containing ethanolic extract showed satisfactory photoprotective activity (SPF ≥ 6), also when

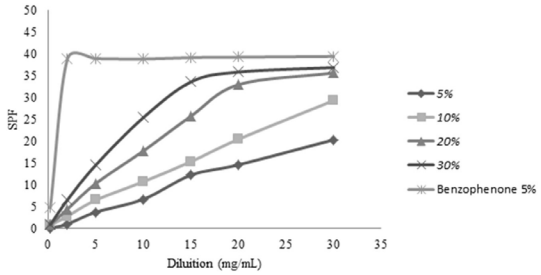


**Figure 5.** Profile of sunscreen formulations with *Marcetia macrophylla* crude extract.

compared to the standard formulation benzophenone-3. That confirms that the SPF is dependent on concentration. However, at a given concentration of absorption, saturation levels are reached and SFP remains almost constant.

The formulations at 30% at a concentration of 15.0 mg/µl and benzophenone 5% with 15.0 mg/µl were statistically equal. The same is true for the concentrations of 30.0 mg/ml and 50.0 mg/ml of the formulations with extract 20% and 30%. That indicates that the formulations may be suitable for very sensitive skin sunburn, because of their high sun protection, as demonstrated in the *in vitro* assay. Studies demonstrated that increased concentrations of the extracts in the emulsion result in an increase in the SPF. Creams containing flower hydroalcoholic extract of *Rosa damascema* increased SPF by 8.32 to 11.94, when the concentration of the extract was increased from 5% to 8% (Heinrich et al., 2004). The same was observed in the formulations containing the ethanolic extract of *M. macrophylla* (MME).

*3.5.3. UV-A blocking activity*

The formulations containing different concentrations (5, 10, 20 and 30%) of the MME were shown to have the ability to protect a photolabile solution (*trans*-resveratrol) from UVA radiation. When *trans*-resveratrol is exposed to UVA radiation, it undergoes degradation, which can be seen by the absorbance decreases during the time intervals. The plate was coated with MME and photobleaching was less pronounced in the end of the 120-min exposure conversion of *trans*-resveratrol to *cis*-resveratrol.

The evaluation of the photoprotective profile of formulations with MME, in the t20, was between 92.33 - 83.44% and in the half of the experiment (t60), the content of the trans-SVR was between 73.53 - 71.29%, which gradually declined to a range of 59.65 - 58.56% (t120), i.e., a figure about 20% higher than the CC, in the same time interval, 40.49%. The good photoprotective results of the formulations are confirmed. MME in the concentrations studied allowed one to infer that the photoprotection against UVA radiation comes from the studied samples. Studies developed to evaluate the photodegradation of sunscreens under exposure to natural and synthetic UVA radiation, UVA lamps, showed that both radiations offer a similar rate of photodegradation of the tested filters, even though the artificial UVA radiation is more fluent than the natural ones.

## 4. Conclusion

The crude extracts of *Marcetia macrophylla* showed antioxidant, photoprotective and antinociceptive activities. The chemical analysis showed the presence of three glycosylated flavonoids derivative of aglycone quercetin. The investigation of the sunscreen profiles revealed that the extract contains good sun protection factor. However, after the incorporation into an emulsion, a high amount of the extract is necessary to produce a sun protector against UVB radiation (SPF > 6). Among the formulations studied, the best SPF was obtained at a 30% concentration. The extract also showed good ability of photoprotection against UVA. The formulation base presented adequate physical and chemical parameters compatible with the extract, being promising for future stability tests with the extract *of Marcetia macrophylla.*

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## Conflict of interest

The authors declare that there is no conflict of interest.

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