Rutina como fotoestabilizadora de protetores solares de amplo espectro

Rutin as photostabilizer for broad spectrum sunscreens

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RESUMO
A associação dos filtros butil metoxidibenzoilmetano (BMBM) e octil metoxicinamato (EHMC) é amplamente utilizada em formulações farmacêuticas, no entanto, é capaz promover alteração na absorção espectral após a exposição à radiação UV. A adição de substâncias naturais em formulações de filtro solar tem sido explorada em relação à eficácia fotoprotetora. O principal objetivo deste trabalho foi avaliar o potencial do flavonoide rutina como uma substância fotoestabilizadora do EHMC e da BMBM. As amostras foram avaliadas antes e após a exposição à radiação UV para fotoproteção in vitro e as interações moleculares por 1H RMN, DSC, TG e análise qualitativa da supressão do estado de energia singleto. A adição de rutina nas formulações contendo BMBM e EHMC promoveu aumento na preservação do FPS in vitro de 53,9% para 65,8 (0,1% rutina) e 70,8% (1,0% rutina). As análises de DSC e TG da rutina associada à EHMC e BMBM foram indicativas de interação entre o flavonoide e os filtros. A razão trans/cis para EHMC melhorou de 5,5 ± 0,1 para 12,6 ± 0,4 com a adição de rutina. A supressão do estado singleto indicou que um dos mecanismos envolvidos na fotoestabilização é a supressão do estado excitado singleto. Esses resultados podem contribuir para o desenvolvimento de formulações de filtros solares de amplo espectro com maior segurança e eficácia.

Palavras chave: fotoestabilização, fotoproteção, flavonoide, rutina, alteração molecular

ABSTRACT
The combination of butyl methoxydibenzoylmethane (BMBM) and octyl methoxycinnamate (EHMC) is widely used in pharmaceutical formulations but may exhibit alteration in spectral absorption following exposure to UV radiation. The addition of natural substances in sunscreen formulations has been explored regarding photoprotective efficacy. The main objective of this research was to evaluate the potential of rutin as a photostabilizer substance of EHMC and BMBM. The samples were evaluated before and after exposure to UV radiation to in vitro photoprotection and molecular interactions by 1H NMR, DSC, TG and qualitative analysis of the suppression of singlet energy state. The addition of rutin in the formulations containing BMBM and EHMC promoted an increase in the preservation of in vitro SPF of 53.9% to 65.8 (0.1% rutin) and 70.8 % (1.0% rutin). The DSC and TG curves of rutin showed interaction between the flavonoid and filters. The trans/cis ratio for EHMC improved from 5.5 ± 0.1 to 12.6 ± 0.4 with rutin addition. The suppression of the singlet state indicated that one of the mechanisms involved in the photostabilization is suppression of singlet excited state. These results can contribute to the development of broad-spectrum sunscreens formulations with increased safety and efficacy.

keywords: photostabilization, photoprotection, flavonoid, rutin, molecular alteration

1 INTRODUCTION

The mechanism of the UV radiation absorption involves the absorption of a photon whose energy is high enough to promote an electron from a lower energy level to a higher energy level. This energy state is called the singlet excited when not involves other properties such as spin. Once in the excited state, the molecule has several available pathways such as return to the ground state, possibly emitting fluorescence or energy thermally, or undergoes some type of photoreaction. The other pathways can be decays to a less energetic excited state called triplet excited state, in which the electros cease to be spin paired. The lower energy excited state turns revert to ground state in either a radiative or nonradiative decay, or it can also undergo photochemistry (MOYAL, 2012; KIKUCHI et al., 2012; SISA, 2010; BONDA, 2008).

Structural transformation, like tautomerization of butyl methoxydibenzoylmethane (BMBM) and isomerization of octyl methoxycinnamate (EHMC), or degradation may to lead to a substantial decrease in UV protection efficacy (MOYAL, 2012). The most commonly employed strategy for elevate the photostability of the potounstable UV filters is the addition of photostabilizing agents, substances acting as quenchers of sunscreen excited states (PANGNAKORN et al., 2007; BONDA, 2011; SCALIA & MEZZENA, 2010).

Flavonoids, such as many photostabilizers UV filter, may transfer or accept light energy to or from other molecules and can act as quenchers. In flavonoids, the molecular structure responsible for absorbing UV energy is called chromophore, usually associated with delocalized π-electrons in conjugated systems (TOMAZELLI, et al., 2018; SISA et al., 2010; KIMBROUGH, 1997).

In the last years, the photoprotection improvement by flavonoids, as rutin, has received considerable additional attention (MARTINS et al., 2020; SCALIA & MEZZENA, 2010; COQUENET et al., 2008). Flavonoids are widely distributed among the plant kingdom. Among a large number of flavonoids, rutin is remarkable for its expressive properties to antagonize the increase in capillary fragility, to reduce high blood pressure, to protect plants against direct and indirect influences of UV radiation and to show antioxidant actions against a wide range of free radicals. In addition, not been detected in vitro cutaneous penetration of the rutin incorporated in a cosmetic emulsion (VELASCO et al., 2018; Bondarev & Knyukshto 2013). The chromophoric nature of flavonoids, such as rutin, are similar of UV organic filters and can be responsible for prevention of UV radiation damage to a variety of plants. These characteristics make the

rutin an important candidate for application in development of photostable sunscreens. (FREIRE, et al., 2021; BONDAREV & KNYUKSHTO, 2013; ZVEZDANOVIC et al., 2012; SISA et al., 2010).

The aim of this work was to evaluate the act of rutin in the reducing of the molecular structural changes of two UV photounstable filters; octyl methoxycinnamate (EHMC) (UVB filter) and butyl methoxydibenzoylmethane (BMBM) (UVA filter) by induce UV radiation. Nuclear Magnetic Resonance (NMR) investigated the rutin influence in the BMBM tautomerism and EHMC isomerism. In addition, the molecular interaction, among UV filters association and rutin, also were observed by Differential Scanning Calorimetry (DSC) and thermogravimetry (TG), as well the qualitative analysis of suppression of the state singlet energy. The photostability of formulations containing the BMBM, EHMC and rutin were evaluated by diffuse reflectance spectrophotometry with sphere integration before and after artificial UV exposition. For comparison purposes, formulations containing bis-ethylhexyloxyphenol methoxyphenyl triazina, a broad-spectrum UV filter, were also prepared and examined.

2 MATERIAL AND METHODS

2.1 CHEMICALS

Octyl methoxycinnamate (EHMC) (2-ethylhexyl (2E)-3-(4-methoxyphenyl)prop-2-enoate), and bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT) (2,2′-[6-(4-methoxyphenyl)- 1,3,5-triazine-2,4-diyl] bis{5-[(2-ethylhexyl)oxy]phenol}) were obtained from Basf (São Paulo, Brazil) and the butyl methoxydibenzoylmethane (BMBM) (1-(4-tert-butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione), was obtained from DSM, (São Paulo, Brazil). The deuterated solvents with tetramethylsilane, chloroform-d3, (CDCL3 99.8%) and dimethylsulfoxide-d6 was purchased from Cil (São Paulo, Brazil); internal reference standard (IS) dimethyl sulfone, purity of 98%, and (2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Sao Paulo, Brazil).

Emulsified system was developed as O/W (oil/water) emulsion, containing following ingredients: acqua, propylene glycol, capriylic/capric triglyceride and phenoxyethanol (and) methylparaben (and) ethylparaben (and) propylparaben (and) butylparaben purchased from Mapric (São Paulo, Brazil); ammonium acryloyldimethyltaurate/vp copolymer purchased from Pharmaspecial (São Paulo,

Brazil); and the UV sunscreen filters EHMC, BMBM and BEMT. The rutin purchased from Pharmanostra (São Paulo, Brazil).

2.2 SUNSCREENS PHOTOSTABILITY EVALUATION

In this study, the formulations were developed with 10.0% w/w EHMC, varying amounts of BMBM (0.0, 2.5 and 5.0%) and rutin (0.0, 0.5 and 1.0 % w/w), according to factorial design DOE 32. Control formulation without rutin added to the higher quantities of the filters BMBM and EHMC with the addition of BEMT (5.0% w/w) was also examined for comparative propose. The in vitro UV protection and photostability influence of rutin in formulations was evaluated by diffuse reflectance spectrophotometry with integrated sphere (LabSphere®, UV-2000S® UV Transmittance Analyzer) before and after artificial sun-light exposition. For this, 0.75 µg cm-2 each sunscreen formulation were spread onto a 5.0 cm2 area of a polymethylmethacrylate (PMMA) (Helioplate ® HD 6) rough plate from Tecnotests Produtos e Serviços (São Paulo, Brazil). Three plates were prepared for each product to be tested. The product film was allowed to dry in the dark under ambient conditions (22±2 º C) for 20 min before analysis.

The dose used for irradiation of the samples was 2.7 kJ m-2, the irradiance was 0.20 W m-2, measured at 340 nm, approximately 208 W m-2 (300-800nm), in Weather-Ometer with an Osram XBO 150/1 xenon lamp. In order to mimic solar UV radiation was used the boro/boro filter.

2.3 ACCELERATED STABILITY OF SUNSCREENS FORMULATIONS

This study employs extreme temperature conditions. The effects of temperature variation may assist in the identification of signals instability of emulsions as phase separation, loss viscosity, precipitation and aggregation.

To evaluate the stability of sunscreens formulations all the samples were stored in opaque polyethylene tubes under following temperatures: 45.0 ºC ± 0.5 °C; 25.0 °C ± 2.0 °C under direct and indirect sunlight exposure; 5.0°C ± 0.5°C and thermal six cycling (24 hours at 45.0 ºC ± 0.5 °C and 24 hours at -5.0 ± 0.5°C). The sample were analyzed in triplicate and evaluation were assessed on t0 (48 hours after preparation, (defined as non-alteration reference) and on the 15th (BABY et al., 2007).

Photoprotection was evaluated as described in 2.2. The physicochemical stability of these emulsions was evaluated by mean pH determination, using the pH meter

(Quimis®) and the viscosity obtained with the ViscoStar® viscosimeter equipped whit a TR11 spindle, the rotation used was 30 rpm.

The antioxidant activity of the formulations was determined using the methanolic solutions of samples of equal concentrations in the presence of 100,0 µM solution of DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma) at a ratio of 1:7. After 20 minutes of reaction at room temperature (24.0 + 2.0 °C). The absorbance values were measured at 515.0 nm in a Evolution 600 UV-Vis spectrophotometer, Thermo Scientific, and converted into the percentage of free radical scavenging (HUBNER, et al., 2019; VELASCO et al., 2011; HUANG et al., 2005; BONDET et al., 1997).

2.4 EVALUATION OF RUTIN INFLUENCE IN EHMC ISOMERIZATION AND BMBM THAUTOMERISM BY ^{1}H NMR

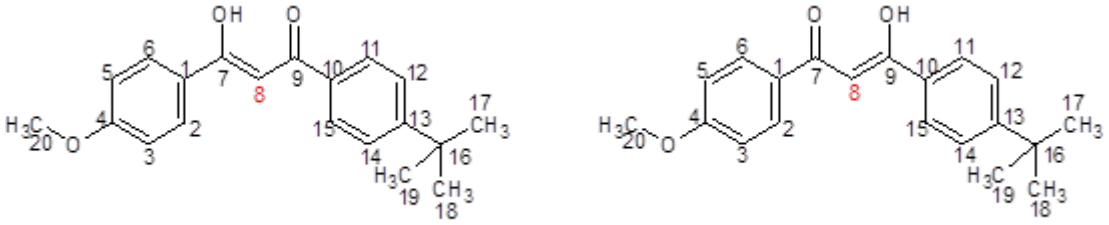
The UV filters chemical stability was monitored by 1H NMR. The analytical measurements were carried out with a Bruker (Rheinstetten/Karlsruhe, Germany), DPX300® Spectrometer with a 5 mm multinuclear probe at 300.13 MHz (1H), high-performance digital FT NMR. The following parameters were optimized for QNMR: 30º pulse, pre-acquisition delay of 5 µs, 32 K data points, relaxation delay of 10 s, and a total of 32 scans. Chemical shifts were referenced internally to tetramethylsilane (TMS = 0.0). The NMR probe was maintained at 30.0°C throughout the measurements. Phase and baseline corrections were made manually. This manual mode was used also for the signal integration (choice of integration limits, generally without the 13C satellites, and if needed the BIAS and SLOPE-functions for the integral calculation due to improper baseline corrections). For statistical reasons, each measurement was repeated 3 times (WELLS et al., 2002; BATISTA et al., 2008).

Tautomerism equilibrium of BMBM was evaluated by the integral of resonance signals at 4.72 ppm (keto form) and 7.18 ppm (enol form) of the protons at C-8 (Figure 1.a) were manually integrated and values obtained were compared. In addition, for estimation of EHMC isomeric content both the resonance signals at 6.44 (E-isomer) and 5.83 ppm (Z-isomer) of the protons at C-7 (Figure 1.b) were manually integrated and the integral values obtained were compared before and after UV exposition (TROSSINI et al., 2015; HOLZGRABE et al., 2005).

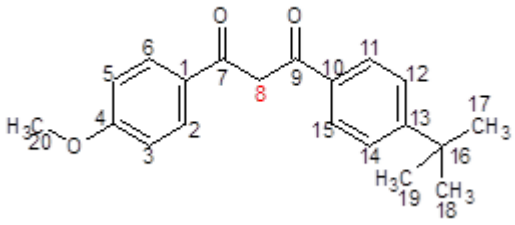
The filters BMBM and EHMC were evaluated alone or in combination in DMSOd6,, with or without rutin by comparing the 1H NMR spectra before and after irradiation. Samples were UV-irradiated whilst contained in the quartz cuvettes at three

different doses (720, 3600 and 5760 mJ cm-2). All samples were exposed to the solar simulator, which consist of a 150 W Xenon Arc Lamp, Oriel; 100 mW cm-2 of radiance. The emission was measure with a reference cell and meter, Oriel® Reference Cells, Newport.

Figure 1. UV filters structure (a) BMBM (enol en Keto forms) and (b) EHMC (*E –trans* and *Z-cis* isomer) (PATTANAARGSON & LIMPHONG, 2001; MTURI & MARTINCIGH, 2008 and DENCAUSSE *et al.*, 2008; TROSSINI *et al.*, 2015).

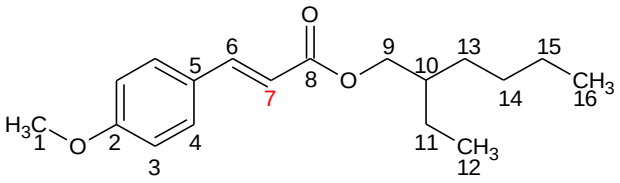


enol form

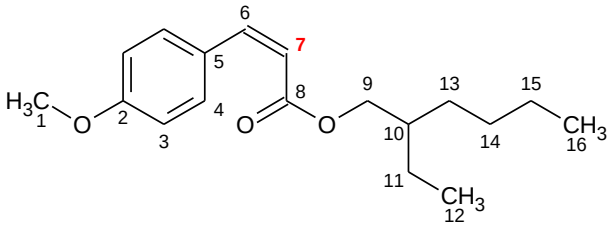


keto form

(a)



trans isomer



cis isomer

(b)

2.5 DIFFERENTIAL SCANNING CALORIMETRY (DSC) AND THERMOGRAVIMETRY (TG) UV FILTERS AND RUTIN INTERACTIONS

The DSC curves were obtained by equipment TA 2920 (TA-Instruments®, New Castle, DE, EUA), in a sealed aluminum pan with a mass between 2 and 3 mg, under a nitrogen flow of 50 mL.min^{-1} and at a rate of 10 and 5 °C min^{-1}. The temperature range is between 25 and 400 °C. The DSC cell was calibrated, before the tests, employing the indium metal standard substance content of 99.94 % (T_{fusion} = 156.6 °C; ΔH_{fusion} = 28.7 J g^{-1}) (ARAUJO, 2009).

The TG curves were obtained by equipment Thermobalance TGA / DTA 7200 SII Nano technoogy® in the temperature range between 25.0 and 600.0 °C under dynamic N_2 atmosphere, 100 mL min^{-1} , the heating rate of 5.0 and 10 °C min^{-1} using Pt crucible and sample mass of about 5 mg. Before testing, blank curves were obtained to evaluate the system's base line and verified using the instrument calibration is a sample of calcium oxalate monohydrate (ARAUJO, 2009).

2.6 QUALITATIVE ANALYSIS OF SUPPRESSION OF THE STATE SINGLET ENERGY

The qualitative analysis of the suppression of the singlet energy state BMBM and EHMC was performed by observing fluorescence of the solutions presented by the filters in methanol alone or combined with the addition of rutin or not. The benzoate phenylethyl was used as a negative control by being a photochemically inert compound. The solutions (10 µL) were transferred to a paper cromatography coated with silica gel. After evaporation of the solvent, the filters were fixed in paper cromatography and the reading was performed under supply of 365 nm. The response was considered positive when the samples showed decreasing fluorescence intensity of each other and less than the negative control (KAWAKAMI *et al.*, 2017; BONDA *et al.*, 2010; HALLSTAR INNOVATIONS CORP, 2010).

3. RESULTS AND DISCUSSION

3.1 SUNSCREENS PHOTOSTABILITY EVALUATION

The *in vitro* UV protection and photostability influence of rutin in formulations was evaluated by diffuse reflectance spectrophotometry with integrated sphere. The main finding of this analysis was that the addition of rutin afforded statistically significant differences in the SPF *in vitro*, before irradiation, only for the formulation without

BMBM. After irradiation, the addition of rutin improved the photostability for every groups [BMBM 0.0% (w/w); BMBM 2.5% (w/w); BMBM 5.0% (w/w)] SPF *in vitro* was maintained higher than samples without ruitn. These results are showed in Table 1 and Figure 2. The BEMT addition as photostabilizer improved the EHMC and BMBM photostability for the samples evaluated, such as observed by Chatelain & Gabard, 2001, Table 2.

Table 1. *In vitro* Photoprotection efficacy evaluation, emulsions before and after UV irradiation. Active ingredients: EHMC (10.0% w/w) and three-level of BMBM (0.0, 2.5 and 5.0% w/w.) and rutin (0.0, 0.5 and 1.0 % w/w), according to factorial design *DOE* 3^2

Sample Identification	SPF *in vitro* maintenance	
	SPF *in vitro* before irradiation	(%) Maintenance after irradation
BMBM- R-	7.3 ±0.6[b]	77.4±7.4[b]
BMBM- R0	6.0±1.0[b]	100.0±0.0[a]
BMBM- R+	9.3±0.6[a]	93.0±6.1[a]
p (Teste de Tukey)	0.004	0.004
BMBM0 R-	12.3±0.6a	48.7±2.2b
BMBM0 R0	13.7±1.2a	78.3±5.8a
BMBM0 R+	13.3±0.6a	72.5±4.0a
p (Teste de Tukey)	0.196	<0.001
BMBM+ R-	16.7±1.5a	53.9±1.4b
BMBM+ R0	18.3±1.5a	65.8±5.4a
BMBM+ R+	16.0±1.0a	70.8±4.2a
p (Teste de Tukey)	0.182	0.006

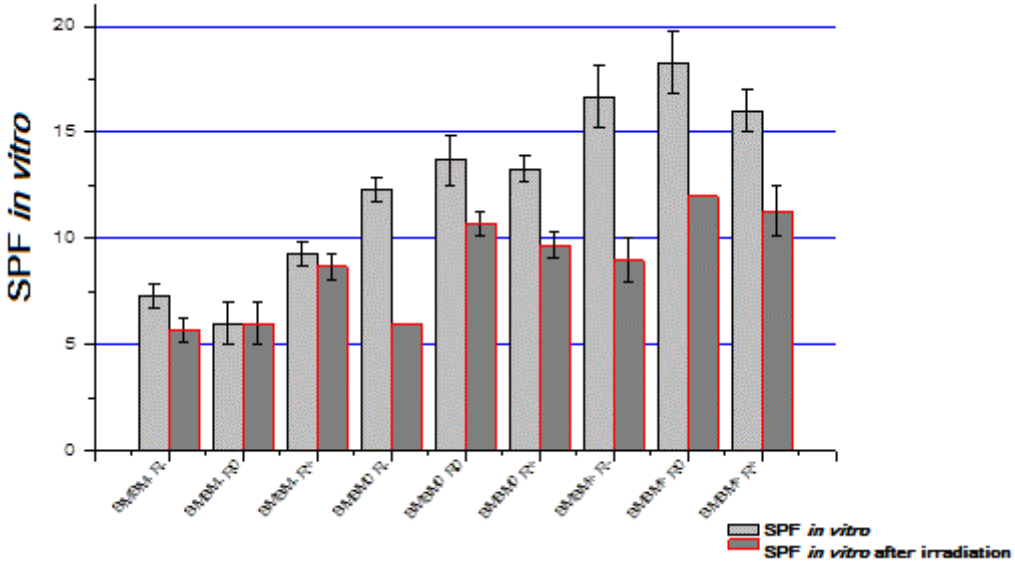
Legend: BMBM = BUTYL METHOXYDIBENZOYLMETHANE; R = rutin; BMBM- = BMBM 0.0% (w/w); BMBM0 = BMBM 2.5% (w/w); BMBM+ = BMBM 5.0% (w/w); R - = Rutin 0% (w/w); R0 = Rutin 0.1% (w/w) e R+ = Rutin 1.0% (w/w); Mean of 3 determinations ± standard deviation; SPF = Solar Protection Factor. Different letters overlapped in the same column, for each group (BMBM-, BMBM0, BMBM+), represent statistically different data according to the Tukey test ($p<0.05$).

Table 2. Comparative results of the SPF *in vitro* maintenance after UV radiation exposition, for samples: BMBM+R-; BMBM+R0; BMBM+R+ and BEMT5.

Sample Identification	SPF *in vitro* maintenance after UV radiation exposition, 192 kJ m^{-2} total dose, 209.2 W m^{-2} irradiance
BMBM+R-	53.9±1.4[b]
BMBM+R0	65.8±5.4[a]
BMBM+R+	70.8±4.2[a]
BEMT5	69.8±2.8[a]

Legend: BMBM = BUTYL METHOXYDIBENZOYLMETHANE; R = rutin; BMBM- = BMBM 0.0% (w/w); BMBM0 = BMBM 2.5% (w/w); BMBM+ = BMBM 5.0% (w/w); R - = Rutin 0% (w/w); R0 = Rutin 0.1% (w/w) e R+ = Rutin 1.0% (w/w); BEMT5 = BEMT 5.0% (w/w). Mean of 3 determinations ± standard deviation; SPF = Solar Protection Factor. Different letters overlapped represent statistically different data according to the Tukey test ($p<0.05$).

Figure 2. Rutin and BMBM influence, SPF *in vitro* at different concentration levels according factorial design *DOE* 3^2, before and after UV irradiation. Active ingredients: EHMC (10.0% w/w) and three-level of BMBM (0.0, 2.5 and 5.0% w/w.) and rutin (0.0, 0.5 and 1.0 % w/w).



Legend: BMBM = BUTYL METHOXYDIBENZOYLMETHANE; R = rutin; BMBM- = BMBM 0.0% (w/w); BMBM0 = BMBM 2.5% (w/w); BMBM+ = BMBM 5.0% (w/w); R - = Rutin 0% (w/w); R0 = Rutin 0.1% (w/w) e R+ = Rutin 1.0% (w/w); Mean of 3 determinations ± standard deviation; SPF = Solar Protection Factor

3.2 ACCELERATED STABILITY OF SUNSCREENS FORMULATIONS

At the end of the stability test, all tested formulations were stable because the observed variations did not damage the functional performance (photoprotection *in vitro* and antiradical activity) of any of them. The results indicated that BMBM 5.0% (p/p) with rutin 1.0% (p/p) formulation showed better performance against the BMBM 5.0% (p/p) with rutin 0.0% (p/p) formulations and BMBM 5.0% (p/p) with rutin 0.1% (p/p). Additionally, *in vitro* photostability results of the formulation BMBM with rutin 1.0%

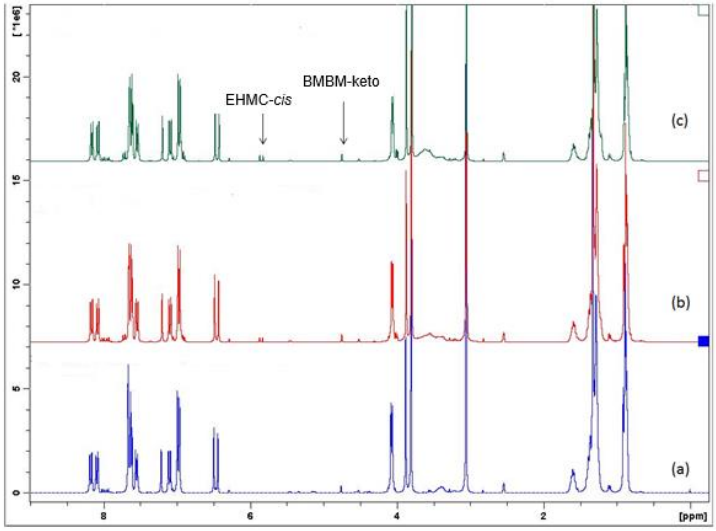
(p/p) were comparable to those obtained for the formulation BEMT5, with the advantage of a high antiradical activity in the formulation added with rutin.

The 45.0 ± 2.0 ºC condition caused statistically significant changes for pH values and UVA/UVB ratio. For the BMBM+R+ formulation there was a decrease in the pH value from 6.5 to 6.3 (3.0%). In this condition, the UVA/UVB ratio parameter was changed for the BMBM+R+ and BEMT5 formulations, which showed an increase from 0.779 to 0.805 and from 0.803 to 0.837, respectively. The addition of rutin in photoprotective formulations, both in the lowest concentration (0.1% w/w) and the highest (1.0% w/w) significantly influenced the results obtained in the inhibition of the DPPH• radical. The rutin-free formulations, BMBM+R- and BEMT5, presented low DPPH• radical inhibition values, respectively 7.8 and 13.6%, since the formulations added with 0.1 and 1.0% rutin showed radical inhibition values 72.4 (BMBM+R-) and 90.6% (BMBM+R+) no statistically significant changes were recorded for this parameter during the stability evaluation.

3.3 EVALUATION OF RUTIN INFLUENCE IN EHMC ISOMERIZATION AND BMBM THAUTOMERISM BY ^{1}H NMR

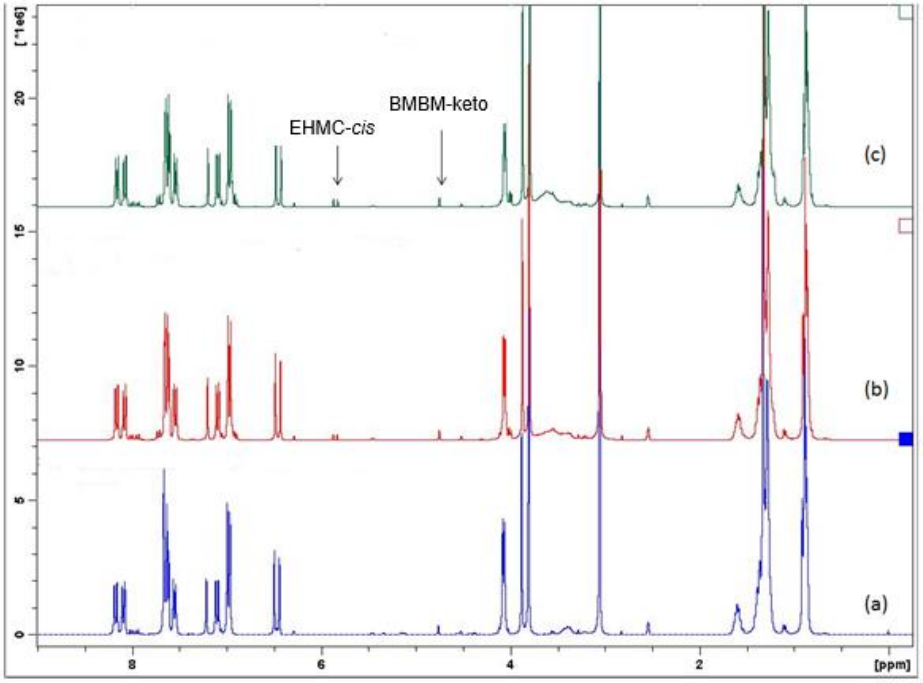
The main changes in the ^{1}H NMR spectra of BMBM and EHMC filters were observed by the addition of rutin in solution containing the two filters. It was observed that the *cis/trans* EHMC increased by 5.5 ± 0.1 (without addition of rutin) to 12.6 ± 0.4 (with the addition of rutin). Observing these results, we can infer that rutin acted decreasing the photoisomerization of EHMC when it was in the presence of BMBM. This result agrees with the results obtained in sunscreens photostability evaluation, that it was observed that the presence of rutin in photoprotective formulations containing the two filters BMBM and EHMC showed higher stability to UV radiation, assessed by analyzing the SPF *in vitro* and λc. However, the reason enol/ keto BMBM filter when in solution with the filter EHMC decreased value of 24.0 ± 3.0 (without rutin) to 13.1 ± 0.5 (with rutin), the approximated value observed for solutions that did not contain the EHMC, Figure 3 and 4.

Figure 3. [1]H NMR spectra of BMBM and EHMC filters before and after UV irradiation



Legenda: BMBM = BUTYL METHOXYDIBENZOYLMETHANE, EHMC = ETHYLHEXYL METHOXYCINNAMATE (a) = non-irradiated; (b) = 3600 J cm^{-2} e (c) = 5760 J cm^{-2}. Highlights the selected signals for the quantification of the BMBM keto forms and EHMC *cis* isomer.

Figure 4.[1]H NMR spectra of BMBM and EHMC filters with rutin before and after UV irradiation,



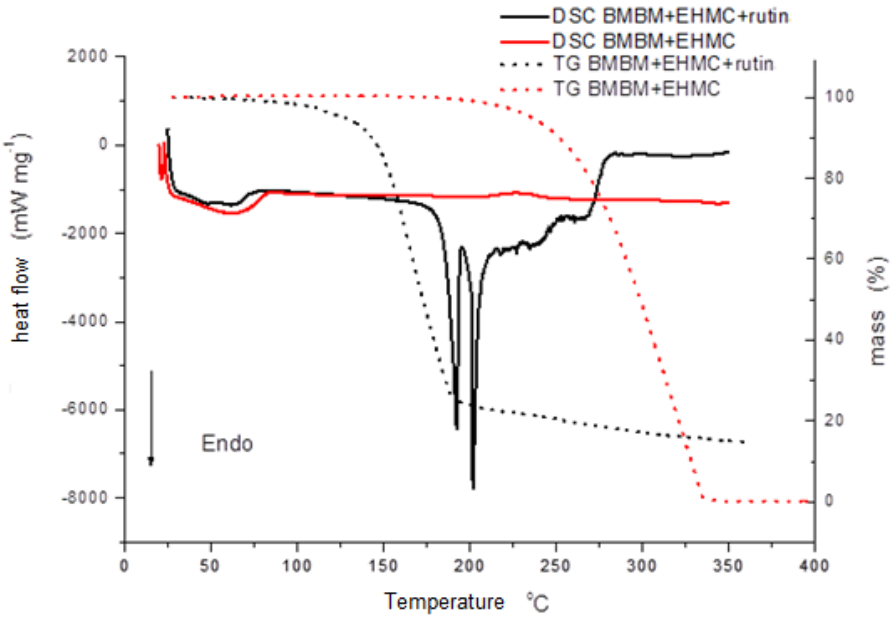
Legend: BMBM = BUTYL METHOXYDIBENZOYLMETHANE, EHMC = ETHYLHEXYL METHOXYCINNAMATE (a) = non-irradiated; (b) = 3600 J cm^{-2} e (c) = 5760 J cm^{-2}. Highlights the selected signals for the quantification of the BMBM keto forms and EHMC cis isomer.

3.4 DIFFERENTIAL SCANNING CALORIMETRY (DSC) AND THERMOGRAVIMETRY (TG) UV FILTERS AND RUTIN INTERACTIONS

From the data obtained from analysis of DSC curves and TG of UV filter samples, isolated and combined, it was possible to infer that there was interaction between BMBM

and EHMC filters with rutin, Figure 5. This result is in agreement with literature data obtained by various analytical techniques showed that these two filters associated with chemical instability and showed that this behavior is modified by the addition of flavonoids such as quercetin and kaenpferia purified extract of galangal (MOYAL, 2012; GONZALEZ; 2011; SCALIA & MEZZENA, 2010; GONZALEZ, PECHKO & KALAFSKY, 2007). The sunscreen thermal analyzes techniques have been reported in the literature, but the interaction of the UV filters was usually studied by analytical techniques such as high-performance liquid chromatography, UV spectrometry, nuclear magnetic resonance among others, but there is no evaluation information DSC and TG. Data available in the literature reported that adding flavonoids to UV filters could positively change the performance in photoprotection efficacy and photostability (SCALIA & MEZZENA, 2010; VELASCO *et al.*, 2008, VELASCO *et al.*, 2011). The agreement of test results of UV filters interaction, BMBM and EHMC with rutin, DSC and TG, with literature data suggest a practical application for these analysis techniques in the development of photoprotective formulations. The techniques have as main advantage the use of low volume samples and the absence of use of solvents.

Figure 5.DSC and TG curves of UV filter binary mixture, BMBM and EHMC (1:1) and ternary mixture BMBM, EHMC and rutin (1:1:1)

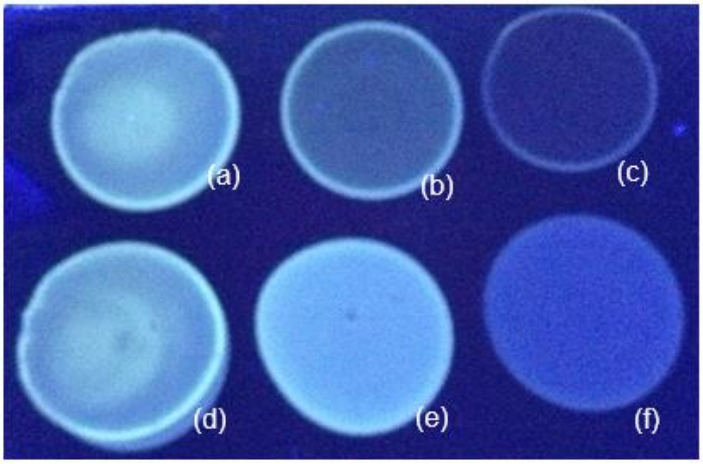


Legend: BMBM = BUTYL METHOXYDIBENZOYLMETHANE; EHMC = ETHYLHEXYL METHOXYCINNAMATE

3.5 QUALITATIVE ANALYSIS OF SUPPRESSION OF THE STATE SINGLET ENERGY

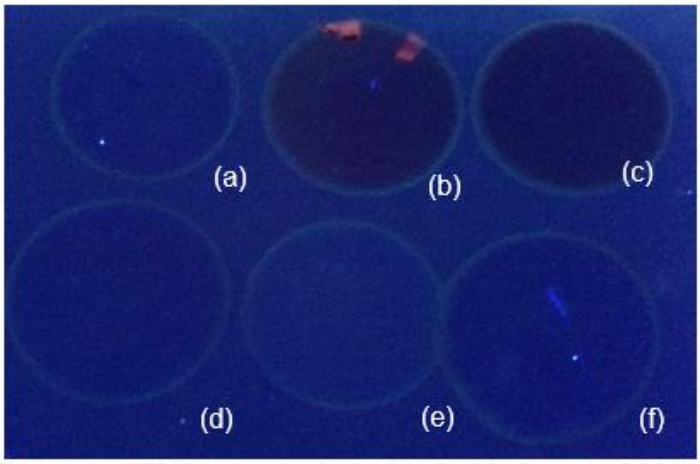
The power transfer, both as singlet - singlet as triplet - triplet, is an important mechanism for the photostabilization of organic sunscreens (SCALIA & MEZZENA, 2010; BONDA, 2010). Scalia and Mezzena, 2010, suggested the triplet - triplet energy transfer as a possible photostabilization mechanisms of BMBM and EHMC filters by quercetin because they have singlet energy state similar values. Qualitative evaluation of the suppression of the singlet excited state of BMBM and EHMC filters, found that rutin had the ability to suppress the singlet energy state of these filters and we can infer that one of the mechanisms involved in the routine involves photostabilization the transfer of singlet - singlet energy, Figure 6, 7 and 8.

Figure 6.Qualitative analysis of suppression of the state singlet energy UV filter BMBM by rutin.



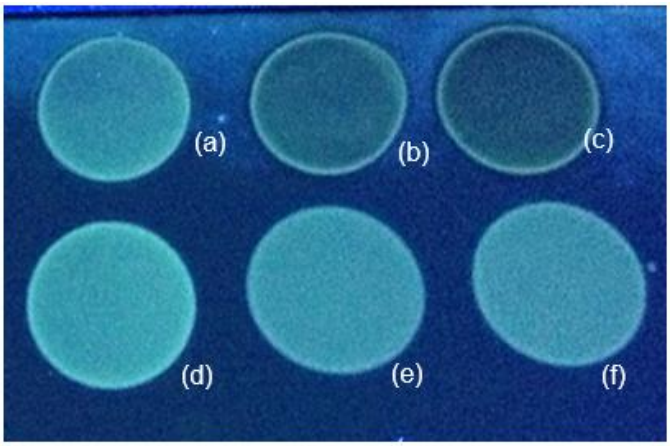
Legend: Test solution BMBM and rutina: (a) 2.0:0.0; (b) 2.0:1.0 and (c) 2.0:2.0. Negative control solution BMBM and phenylethyl benzoate: (d) 2.0:0.0; (e) 2.0;1.0 e (f) 2.0:2.0.

Figure 7.Qualitative analysis of suppression of the state singlet energy UV filter EHMC by rutin



Legenda:Test solution EHMC and rutin: (a) 2.0:0.0; (b) 2.0:1.0 and (c) 2.0:2.0. Negative control solution EHMC and phenylethyl benzoate: (d) 2.0:0.0; (e) 2.0;1.0 e (f) 2.0:2.0.

Figure 8.Qualitative analysis of suppression of the state singlet energy UV filter BMBM and EHMC by rutin.



Legend: Test solution BMBM, EHMC and rutin: (a) 1.0:1.0:0.0; (b) 1.0:1.0:1.0 and (c) 1.0:1:0:2.0. Negative control solution BMBM, EHMC and phenylethyl benzoate: (d) 1.0:1.0:0.0; (e) 1.0:1.0:1.0 and (f) 1.0:1.0:2.0.

4 CONCLUSION

The development, evaluation of the in vitro photoprotective efficacy and evaluation of the photostability of photoprotective formulations with or without rutin made it possible to verify that the presence of rutin cooperates in the maintenance of photoprotection when exposed to artificial UV radiation.

From the evaluation of the results of the thermal analysis tests, we observed that the rutin underwent alterations promoted by the addition of sunscreens. By 1H NMR analysis it was possible observed the molecular changes in the filters by exposure to UV radiation, highlighting the reduction of EHMC photoizomerization in the presence of BMBM, when the combination of filters was added with rutin. The qualitative analysis of singlet oxygen suppression suggests the suppression of the singlet excited state of the BMBM filter with the addition of rutin, which acted by absorbing excess energy and promoted greater stability of the filters when subjected to UV radiation.

REFERENCES

ARAUJO, G. L. B. Caracterização no estado sólido dos polimorfos de tibolona. São Paulo, 2009. 202p. Tese de doutorado - Faculdade de Ciências Farmacêuticas - Universidade de São Paulo.

BABY, A. R.; MIGLIATO, K. F.; MACIEL, C. P. M.; ZAGUE, V. PINTO, C. A. S. O.; SALGADO, H. R. N.; KANEKO, T. M.; VELASCO, M. V. R. Accelerated chemical stability data of O/W fluid emulsion containing the extract of Trichilia catigua Adr. Juss (and) Ptychopetalum olacoides Bentham. Brazilian Journal of Pharmaceutical Sciences, v.43, n.3, p.405-412, 2007.

BATISTA, I. A. S. A.; GONÇALVES, M. I. A.; SINGH, A. K.; HACKMANN, E. R. M.; SANTORO, M. I. R. M. Quantitative determination of dimethylaminoethanol in cosmetic formulations by Nuclear Magnetic Resonance Spectroscopy. Journal of AOAC International, v.91, n.6, p.1303-1308, 2008.

BONDA, C. Research pathways to photostables sunscreens. Cosmetics and Toiletries, v.123, n.2, p.149-260, 2008.

BONDA, C.; PAVLOVIC, A.; HANSON, K.; BARDEEN, C. singlet quenching proves faster is better for photostability. Cosmetics And Toiletries, v.125, n.2, p.40–48, 2010.

BONDA, C.; ZHANG, J. Photostabilization of retinol and retinyl palmitate by ethylhexyl methoxycrylene. Cosmetic & Toietries. v.126, n.1, p.40-48, 2011.

BONDAREV, S. L.; KNYUKSHTO V. N. Fluorescence and phosphorescence of rutin. Journal of Luminescence, n. 142, p. 236-240, 2013.

BONDET, V.; BRAND-WILLIAMS, W.; BERSET, C. Kinetics and mechanisms of antioxidant activity using the DPPH* free radical method. Lebensmittel-Wissenschaft und-Technologie, v.30, n.6, p.609-615, 1997.

CHATELAIN E.; GABARD B. Photostabilization of Butylmethoxydibenzoylmethane (Avobenzone) and Ethylhexyl methoxycinnamate by Bis-ethylhexyloxyphenol methoxyphenyl triazine (Tinosorb S), a new UV broadband filter. Photochemistry and Photobiology, v.74, n.3, p.401-406, 2001.

COQUENET, B.; COUTEAU, C. PAPARIS, E.; COIFFARD, L. J. M. Quercetin and rutin as potencial sunscreen agents: determination of efficacy by an in vitro method. Journal of Natural Products, v.71, n.6, p.1117-1118, 2008.

DENCAUSSE, L.; GALLAND, A.; CLAMOU, J. L.; BASSO, J. Validation of HPLC method for quantitative determination of Tinosorb®S and three other sunscreens in a high protection cosmetic product. International Journal of Cosmetic Science, v.30, n.5, p.373-382, 2008.

FREIRE; T. B.; RODRIGUES JÚNIOR, O.; PINTO C. A. S. O.; ESCUDEIRO; C. C.; BABY; A. R.; VELASCO, M. V. R. New model of biological membrane (shed snakeskin) for studies of antioxidant activity in photoprotective formulation.. Brazilian Journal of Development, v.7, n.4, p 43214-43236; 2021

GONZALEZ, H. D. Photostability of sunscreen combinations containing avobenzone exposed to natural and artificial ultraviolet light. Journal of American Academy Dermatology, v.64, n.2, supl.1, 2011.

GONZALEZ, H. D.; PECHKO, A. P.; KALAFSKY, R. Photostable sunscreen compositions and methods of stabilizing. United States Patent, n. 6.440.402 B1, 2007.

HALLSTAR INNOVATIONS CORP. (Chicago, IL). Craig Bonda. Test method for determining compounds capable of quenching electronic singlet state excitation of photoactive compounds. United States Patent, n. 7776614B2, 2010.

HOLZGRABE, U.; DEUBNER, R.; SCHOLLMAYER, C.; WAIBEL, B. quantitative nmr spectroscopy — applications in drug analysis. Journal of Pharmaceutical and Biomedical Analysis, v.38, n.5, p.806-812, 2005.

HUBNER A.; SOBREIRA, F., VETORE NETO, A., PINTO, C.A.S. O.; DARIO, M. F., DÍAZ, I. E. C., LOURENÇO, F. R.; ROSADO, C.; BABY, A. R.; BACCHI; E. M. The Synergistic Behavior of Antioxidant Phenolic Compounds Obtained from Wine making Waste's valorization, Increased the Efficacy of a Sunscreen System. Antioxidants, v.8, n.11, p. 530; 2019.

HUANG, D.; OU, B.; PRIOR, R. L. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, v. 53, n. 6, p.1841-1856, 2005.

KAWAKAMI, MÁXIMO, L.N.; FONTANEZI, B.B.; DA SILVA, R. S., GASPAR, L.R. Diethylamino hydroxybenzoyl hexyl benzoate (DHHB) as additive to the UV filter avobenzone in cosmetic sunscreen formulations-Evaluation of the photochemical behavior and photostabilizing effect. The European Journal of Pharmaceutical Sciences, v. 1, n. 99, p. 299-309, 2017.

KIKUCHI, A.; HATA, Y., KUMASAKA, R., NANBU, Y.; YAGI, M. Photoexcited singlet and triplet states of a uv absorber ethylhexyl methoxycrylene. Photochemistry and Photobiology, n. p. 89: 523–528, 2012.

KIMBROUGH, D. R. The Photochemistry of Sunscreens. Journal of Chemical Education. V. 74, n. 1, p. 51 1997.

MARTINS, T. E. A.; PINTO, C. A. S. O.; OLIVEIRA, A. C.; VELASCO, M. V. R.; GUITIÉRREZ, A. R. G.; RAFAEL, M. F. C.; TARAZONA, J. P. H.; RETUERTO-FIGUEROA, M. G. contribution of topical antioxidants to maintain healthy skin - A Review. Scientia Pharmaceutica, v. 88, n. 27, p. 1-17, 2020.

MOYAL, D. The development of efficient sunscreens. Indian Journal of Dermatology Venereology and Leprology, v.78, n.7, p.31-34, 2012.

MTURI, G. J.; MARTINCIGH, B. S. Photostability of the sunscreening agent 4-tert-butyl-4-methoxydibenzoylmethane (avobenzone) in solvents of different polarity and proticity. Journal of Photochemistry and Photobiology A: Chemistry, v. 200, n.2-3, p.410-420, 2008.

PANGNAKORN, P.; NONTHABENJAWAN, R.; EKGASIT, S. THAMMACHAROEN, C.; WANICHWECHARUNGRUANG, S. P. Monitoring 2-ethylhexyl-4-methoxycinnamate

photoisomerization on skin using attenuated total reflection fourier transform infrared spectroscopy. Applied Spectroscopy, v.61, n.2, p. 193-198, 2007.

PATTANAARGSON, S.; LIMPHONG, P. Stability of octyl methoxycinnamate and identification of its photo-degradation product. International Journal of Cosmetic Science, v.23, n.3, p.153-160, 2001

SCALIA, S.; MEZZENA, M. Photostabilization effect of quercetina on the UV filter combination, butyl methoxydibenzoylmethane octyl-methoxycinnamate. Photochemistry and Photobiology, v.86, n.2, p.273-278, 2010.

SISA, M.; BONNET, S. L.; FERREIRA, D.; VAN DER WESTHUIZEN, H.; Photochemistry of Flavonoids, Molecules, n.15, p. 5196-5245, 2010.

TOMAZELLI, L. C.; RAMOS, M. M. A.; SAUCE, R.; CÂNDIDO, T. M.; SARRUF, F. D. PINTO, C. A. S. O.; OLIVEIRA, C. A. ; ROSADO; C.; VELASCO, M. V. R.; BABY, A. R. SPF enhancement provided by rutin in a multifunctional sunscreen. International Journal of Pharmaceutics, v.552, n. 1–2, p. 401-406, 2018.

TROSSINI, G. H. G.; MALTAROLLO; V. G.; D'A. GARCIA,R.; PINTO C. A. S. O.; VELASCO; M. V. R.; HONORIO, K. M.; BABY, A. R. Theoretical study of tautomers and photoisomers of avobenzone by DFT methods. Journal of Molecular Modeling, v.21, n.12, p. 319, 2015.

VELASCO, M. V. R.; SAUCE, R.; OLIVEIRA; C. A.; PINTO, C. A. S.O.; MARTINEZ, R. M.; BAAH, S.; ALMEIDA, T. S., ROSADO, C.; BABY, A. R. Active ingredients, mechanisms of action and efficacy tests of antipollution cosmetic and personal care products. Brazilian Journal Pharmaceutical. Science, v.54, n. Special, 2018.

VELASCO, M. V. R.; BALOGH, T.S.; PEDRIALI, C. A.; SARRUF, F.D.; PINTO, C. A. S. O.; KANEKO, T. M.; BABY, A. R. Novas metologias analíticas para avaliação da eficácia fotoprotetora (in vitro) - revisão. Journal of Basic and Applied Pharmaceutical Sciences, v. 32, n. 1, p. 27-34, 2011.

VELASCO, M. V. R.; BALOGH, T. S.; PEDRIALI, C. A.; SARRUF, F. D.; PINTO, C. A. S. O.; KANEKO, T. M.; BABY, A. R. Associação da rutina com p-metoxicinamato de octila e benzofenona-3: avaliação in vitro da eficácia fotoprotetora por espectrofotometria de refletância. Latin American Journal of Pharmacy, v. 27, n. 1, p.23-27, 2008.

WELLS, R. J.; HOOK, J. M.; AL-DEEN, T. S.; HIBBERT, D. B. Quantitative Nuclear Magnetic Resonance (QNMR) spectroscopy for assesing the purity of technical grade agrochemicals: 2,4-dichorophenoxyacetic acid (2,4-D) and sodium 2,2-dichloroproprionate (Dalapon sodium). Journal of Agricultural and Food Chemistry, v. 50, n. 12, p. 3366-3374, 2002.

ZVEZDANOVIĆ, J. B.; STANOJEVIĆ; J. S.; MARKOVIĆ, D. Z.; CVETKOVIĆ, D. J. Irreversible UV-induced quercetin and rutin degradation in solution studied by UV spectrophotometry and HPLC chromatography. Journal Serbian Chemical Society. v. 77, n. 3, p. 297–312, 2012.