Revista Brasileira de Farmacognosia 28 (2018) 692–696

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Extract from byproduct *Psidium guajava* standardized in ellagic acid: additivation of the *in vitro* photoprotective efficacy of a cosmetic formulation

Lívia P.G. Milani*, Nathália O.S. Garcia, Mariana C. Morais, Andressa L.S. Dias, Nayara L. Oliveira, Edemilson C. Conceição

Laboratório de PD&I de Bioprodutos, Faculdade de Farmácia, Universidade Federal de Goiás, Goiânia, GO, Brazil

ARTICLE INFO

Article history:
Received 20 March 2018
Accepted 11 August 2018
Available online 15 September 2018

Keywords:
Guava
Sun protection factor
Antioxidant activity
Synergism

ABSTRACT

The fruits of guava, *Psidium guajava* L., Myrtaceae, are cultivated as food and used in agroindustries, generating byproducts or waste that represent environmental problems and require adequate destination. However, these byproducts present high levels of secondary metabolites and have been awakened interest regarding to its reusing. The extract was standardized in ellagic acid concentration by high performance liquid chromatography. The additivation capacity in the *in vitro* photoprotective efficacy of guava byproduct extract standardized in ellagic acid was verified as a result of its incorporation in cosmetic formulations, comparing it with a standard product. The extract presented synergy with the chemical UV filter (ethylhexyl methoxycinnamate), enhancing the solar protection factor of the phytocosmetic in 17.99%. Besides that, it was possible to show its antioxidant activity and the presence of secondary metabolites such as phenols and flavonoids. According to the results, it is possible to claim that the extract from the guava's agroindustrial byproducts present potential to be studied and reused, applying on the development of innovative products intended to the photoprotection care.

Introduction

The sun is a natural source of electromagnetic radiation and it is responsible for innumerable benefits for the human health and welfare. However, depending on the time and exposition frequency, it can cause severe and chronic harmful effects to the skin. Among the possible damages, can be cited: erythema, immune disorder, mutagenic changes, an increase of free radicals levels generated on the skin and decrease of natural antioxidat system efficacy (Flor et al., 2007; Dal'Belo, 2008). Thus, in order to avoid these effects, effective and low-cost primary prevention actions are needed such as the correct and adequate application of photoprotectors products, which contain active ingredients denominated solar filters (INCA, 2010). These substances are able to protect the skin against the harmful effects caused by the ultraviolet radiation exposure (Violante et al., 2009).

A world tendency in cosmetic product development, especially in the photoprotection market, is the use of natural ingredients incorporated on innovative and efficient formulations committed with the environment. This tendency is reinforced by the population acceptance and also by the means of communication admitting that the feedstock from natural resources are safer and ecologically acceptable than the components with synthetic origin, once they are less aggressive to the environment (Violante et al., 2009; Polonini et al., 2011; Polonini et al., 2014). Therefore, in order to turn this kind of raw material into sunscreen active ingredient, it is necessary that they present themselves with the molecule composition structurally similar to the chemical filters, containing chromophores groups and aromatic rings, besides compounds with antioxidants activities. Some metabolites classes that fit these requirements belong to derivatives of hydroxycinnamic acids, lignans and phenolic groups such as the flavonoids and the tannins (Guaratini et al., 2009; Violante et al., 2009; Costa et al., 2015). Thus, once proved the capacity to absorb solar radiation and its antioxidant activity, these new ingredients can provide a wider photoprotection than the synthetic UV filters due to their ability to scavenger free radicals generated on the skin after sun exposure (De Souza et al., 2013; Jarzycka et al., 2013; Polonini et al., 2014; Mansur et al., 2016).

A promising source of natural ingredients with reusable potential is the waste generated by the food processing industries.

* Corresponding author.
E-mail: livia.pg@posgrad.ufg.br (L.P. Milani).

https://doi.org/10.1016/j.bjp.2018.08.005

Important secondary metabolites in its composition can be studied and used on the development of high added value products, contributing with the decreasing of impacts on the environment and increase of economic return. Even considering environmental problems, industrial byproducts can be potential sources of bioactive compounds with antioxidant and antibacterial activity (Melo, 2010).

Fruits of *Psidium guajava* L., Myrtaceae, popularly known as guava, are widely used in agro-industries, generating byproducts or waste. However, it contains compounds with biological activities that have aroused the interest in relation to their reuse (Leong and Shui, 2002; Iha et al., 2008; Kong et al., 2010). Among the metabolites present in the fruit and in the waste of the guava, it can be highlighted the ellagic acid, a phenolic compound that has been the target of several studies (Oliveira et al., 2012; Díaz-de-Cerio et al., 2016; Kamath and Skariyachan, 2017; Blancas-Benitez et al., 2018). Its main biological activity is the *in vivo* and *in vitro* antioxidant activity (Dalvi, 2008; Kilic et al., 2014; García-Niño and Zazueta, 2015; Liu et al., 2018; Verotta et al., 2018).

Besides agroindustries byproducts represent a serious environmental problem and generate costs to the industry, these wastes can became potential source of rich extracts with natural origen that present the capacity to absorb energy on wavelengths of the ultraviolet radiation spectrum and also have potential antioxidant activity (Nascimento et al., 2009), therefore the aim of this study was to evaluate the extract of *P. guajava* byproduct standardized in ellagic acid, regarding the additivation of the photoprotective effectiveness of a cosmetic formulation containing a chemical filter and verifying its antioxidant activity.

Materials and methods

Plant material

The *Psidium guajava* L., Myrtaceae, byproducts, were provided by Predilecta Alimentos LTDA, sited in São Lourenço do Turvo District, Matão-SP. After the collecting, the samples were kept in plastic bags and frozen.

Liquid extract preparation

The fruit material, obtained as a byproduct from the fruit processing, was unfrozen during 48 h in ambient temperature and, thereafter, dehydrated in a drying oven with forced air circulation at 60 °C, until constant mass. Then, the dried material was ground in a knife and hammer mill and packaged away from light and humidity, under refrigeration.

The liquid extract was obtained by the extraction method of percolation (Farmacopeia Brasileira, 2010), using as extractor solvent a mixture of water and ethyl alcohol in (1:1) (v/v) with solid/solvent proportion 1:5 (w/v), according to the phenolic compounds extractive method optimization through Box–Behnken experimental design described by Garcia (2015). Initially the dried plant material and the extractor solvent remained in contact during 24 h in maceration. Then, it was initiated the percolation process. After the extract passed thought the filter and returned to percolator 10 times, the extract was all drained from the percolator and the liquid extract produced was concentrated in a forced ventilation equipment during two days at room temperature (25 °C). The obtained extract was kept in a shelter from the light and humidity under refrigeration.

Characterization of the concentrated liquid extract

Total phenols content

The concentrated liquid extract phenolic compounds quantification was proceeded according to the adapted methodology proposed by Hagerman and Butler (Mole and Waterman, 1987), using tannic acid as a pattern. It was prepared a methanolic solution of tannic acid (1 mg/ml) and aliquots (0.05–0.35 mg ml^{-1}) were used to build the calibration curve. The extract was diluted in methanol (1:3, v/v), in triplicate. The aliquots of the tannic acid standard and sample were added in test tubes containing a sodium lauryl sulfate/triethanolamine solution and ferric chloride solution. The test tubes were agitaded and left static for 15 min and the absorbance of the samples was verified on 510 nm in a spectrophotometer.

Total flavonoid content

The concentrated liquid extract flavonoids quantification was held according to the spectrophotometric method described by Rolim et al. (2005), with modifications. The standard used for the construction of calibration curve was rutin, prepared in methanol:acetic acid solution 0.02 M (99:1) using different concentrations (0.005–0.03 mg ml^{-1}). The extract was added in sample tubes containing methanol:acetic acid solution 0.02 M (99:1), in triplicate. The absorbance of the samples were verified on 361 nm.

Ellagic acid quantification by HPLC

The quantification of ellagic acid in the concentrated liquid extract was made by high performance liquid chromatography (HPLC). The analysis were proceeded in chromatograph (Waters®, HPLC Alliance®) with photodiode array detector (PDA) 2998, Enpower 2.0 Software, Zorbax column Eclipse XDB-C18 (Agilent Technologies®) (4.6 mm × 250 mm × 5 μm) and oven operating in 30 °C. The mobile phase was composed by acetonitrile, methanol and phosphoric acid solution 0.5% with flow gradient (Table 1) of 1.0 ml min^{-1}. The injection volume was 10 μl with 30 min of running and detection in wavelength of 254 nm for the ellagic acid.

The standard concentration was obtained through the regression-equation resulted by the linear regression of a calibration curve, correlating the chromatographic peak area referred to the ellagic acid pattern injection (Sigma Aldrich®, 95%) and its concentration in the sample.

Antioxidant activity determination

The antioxidant activity in concentrated liquid extract was performed following the methodology based in the scavenger of DPPH (2,2-difenil-1-picril-hidrazil) free radical from the antioxidants present in the sample (MAPA, 2007). It was prepared a methanolic solution of DPPH 0.06 mM. The extract and the ellagic acid standard, used as positive control in the test, were diluted in methanol. The negative control was constituted of a solution of 3.9 ml of the radical solution DPPH and 100 μl of methanol.

Table 1
Mobile phase gradient.

Time (min)	Acetonitrile (%)	Methanol (%)	Phosphoric acid 0.5% (%)
0	10	20	70
15	10	20	70
17	15	40	45
22	15	40	45
24	10	20	70
30	10	20	70

Table 2
Composition of the cosmetic formulations.

Components (INCI)[a]	Concentration (%, w/w)	
	Reference	Phytocosmetic
Phase A		
Aqua	79.35	69.35
Disodium EDTA	0.10	0.10
Glycerin	2.00	2.00
Phase B		
Cetearyl alcohol	2.50	2.50
Ceteareth-20	0.70	0.70
Potassium cetyl phosphate	2.00	2.00
Acrylates/C10–30 alkyl acrylate crosspolymer	0.20	0.20
C12–15 alkyl benzoate	2.00	2.00
Shea butter ethyl esters	2.00	2.00
BHT	0.05	0.05
Ethylhexyl methoxycinnamate	8.00	8.00
Phase C		
Methylchloroisothiazolinone (and) methylisothiazolinone	0.10	0.10
Cyclomethicone	1.00	1.00
P. guajava byproduct extract	–	10.00

[a] INCI, International Nomenclature of Cosmetic Ingredients; –, raw material not added.

Throughout the solutions, were prepared test tubes containing aliquots from 20 to 100 μl of extract solutions and ellagic acid in 3.9 ml of the DPPH solution 0.06 mM, completing the final volume of 4 ml with methanol. The tubes were homogenized and the absorbance was verified after 10 min on 515 nm. The analysis was performed in triplicate and the results were expressed by IC_{50}.

Solar protection factor (SPF) in vitro

The determination of the solar protection factor of the *P. guajava* byproduct extract standardized on ellagic acid was performed in two different cosmetic formulations containing the chemical filter ethylhexyl methoxycinnamate. The cosmetic formulation containing only chemical filter was denominated "reference" and the formulation containing the chemical filter and the extract standardized as additive was denominated "phytocosmetic" (Table 2).

The emulsions manipulation followed the procedure: phases A and B were prepared separately and heated until temperature between 75 and 80 °C. In the sequence, phase A was poured into phase B gradually, homogenizing in a mechanical stirrer. After the emulsification, the cooling process was started and the phase C was added in temperature under 40 °C, still upon agitation. In the end, the formulations were kept still for 24 h to finish the emulsification process.

The determination of solar protection factor (SPF) on the cosmetic formulations was performed *in vitro* by diffuse transmittance spectrometry, according to the ISO 24443:2012 methodology (ISO, 2012). For each formulation, it was prepared four polymethylmethacrylate plates (PMMA), where the samples were applied in the quantity nearly to 1.3 mg cm^{-2} and manually spread, until obtain a uniform film. Afterwards, the plates with the applied samples were kept still for at least 30 min, sheltered from the light and in a drying room with controlled temperature.

The mean absorbance spectrum of the samples on the plates was obtained on the range from 290 to 400 nm, in an interval of 1 in 1 nm, on spectrophotometer (Labsphere, UV-2000S). As negative control, it was evaluated plates with glycerin. Five specters were obtained from each sample, in different points of the plate, to estimate the SPF *in vitro*.

Results and discussion

Characterization of the concentrated liquid extract

Total phenols and flavonoids content

The *P. guajava* byproduct concentrated liquid extract presented 2.19% ± 0.05 and 1.81% ± 0.04 of the total phenols and flavonoids content, respectively. The phenolic compounds and the flavonoids are widely found in the vegetal kingdom and can be associated with biological activities of the plant, being of great interest, its quantification in the vegetal material (Carvalho et al., 2009; Couto et al., 2009; Silva et al., 2016). Structurally these compounds present aromatic rings and have the capacity to absorb ultraviolet radiation, besides neutralizing the free radicals formed by the oxidative process. These properties make them potential raw materials to be incorporated in innovative phytoproducts, widely searched by cosmetic industries, capable of minimizing the damages caused by the solar radiation (Munhoz et al., 2012; Polonini et al., 2014; Silva et al., 2016).

Some phenolic compounds found in *P. guajava* residues are isovanilic acid, vanillic acid, siringeic acid, gallic acid, hydroxycinnamic acids and other compounds (Melo, 2010). Iha et al. (2008) confirmed the presence of the flavonoids quercetin, epicatechin, β-carotene and rutin in *P. guajava* fruits (Vinayagam et al., 2018). Sousa et al. (2011a) determined the flavonoids and phenolic contents in tropical fruit pulp residues which the values found for *P. guajava* byproducts were: 1.06 μg g^{-1} of flavonoids and 24.63 mg/100 g in the equivalent of gallic acid for total phenolic content.

Ellagic acid quantification by HPLC

The quantification of ellagic acid in the *P. guajava* byproduct liquid extract by HPLC was determined by the calibration curve obtained for the ellagic acid standard (Fig. 1), correlating the peak area referent to the marker and its concentration in the sample. Fig. 2 showed the chromatograms referent to the ellagic acid standard and *P. guajava* byproduct liquid extract overlapping, confirming the presence of the marker in the extract.

The concentration of ellagic acid found was 4.86 ± 0.16 μg ml^{-1}. This value can be used as reference to quality control on the production of the *P. guajava* byproduct standardized extract. The ellagic acid is a derivate of the ellagitannin that has brought interest in researches for its important biological functions, highlighting the antioxidant capacity, with action mechanisms associated in oxidation stress reduction (Abe et al., 2010).

Antioxidant activity determination

The results were expressed on the concentration needed to reduce the initial quantity of DPPH in 50% (IC_{50}) (MAPA, 2007; Sousa et al., 2011b) and for the *P. guava* byproduct extract the IC_{50} was 19.80 μg ml^{-1} and for the ellagic acid used as standard, 7 μg ml^{-1}.

The IC_{50} of the standardized extract presented an inferior value compared to the positive control, the standard ellagic acid, which

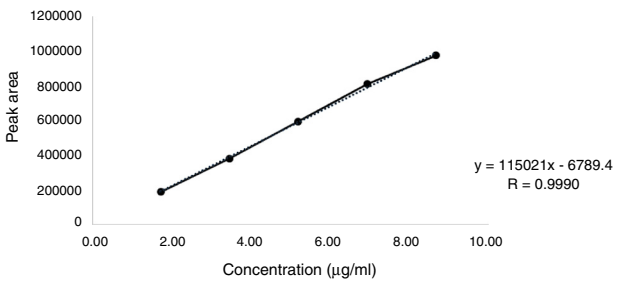

Fig. 1. Calibration curve of the ellagic acid standard.

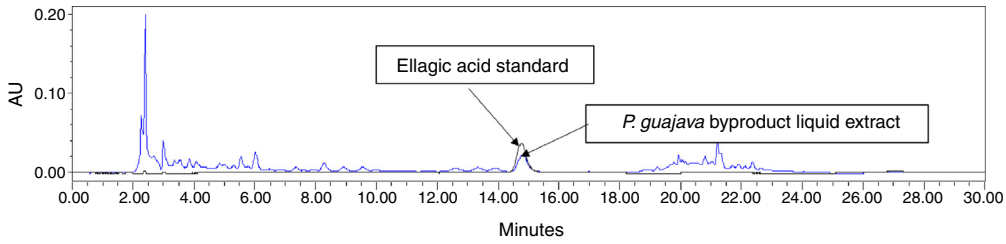

Fig. 2. Overlapping of chromatograms in 254 nm obtained with the injection of samples referent to the ellagic acid standard (black) and *Psidium guajava* byproduct liquid extract (blue).

is known by its potent antioxidat activity. However, it is important to highlight that the extract was produced from an agro-industrial residue holding a complex nature and also passed through different stages of processing at food processing industries. So, it is possible to affirm that the result obtained suggests that the *P. guajava* byproduct extract has a relevant antioxidant activity.

The study performed by Sousa et al. (2011a), showed that the hydroalcoholic residue of the guava demonstrated higher antioxidant activity among the others evaluated waste samples, showing IC_{50} of $142.9 \pm 4.5\ \mu g\ ml^{-1}$, when compared with hydroalcoholic extracts of acerola ($308.07 \pm 0.75\ \mu g\ ml^{-1}$), pineapple ($3293.92 \pm 9.89\ \mu g\ ml^{-1}$), graviola ($612.37 \pm 2.82\ \mu g\ ml^{-1}$), bacuri ($2506.6 \pm 4.23\ \mu g\ ml^{-1}$) and cupuaçu ($554.87 \pm 2.27\ \mu g\ ml^{-1}$). Other researches also have shown that vegetal extracts containing phenolic compounds and flavonoids have high antioxidant activity (Babbar et al., 2011; Chiari et al., 2012; Munhoz et al., 2012).

Solar protection factor (SPF) in vitro

The SPF of the *P. guava* byproduct extract was evaluated on emulsions of topic use, developed with the aim of evaluate its interaction or synergism with a chemical filter with synthetic origen, in relation to the photoprotection efficacy. The solar UV filter ethylhexyl methoxycinnamate, added to the cosmetic formulations evaluated, present the capacity to absorb solar radiation in the range of UVB (290 and 320 nm), being commonly used in photoprotector products (Silva et al., 2013).

By diffuse transmittance spectrometry, it was possible to obtain average specters in the absorbance region of the ultraviolet to the PMMA plates where reference and phytocosmetic formulations were applied. The SPF values of the two cosmetic formulations are presented in Table 3.

The phytocosmetic presented SPF higher than the reference formulation, showing an increment of 17.99% of photoprotection efficacy on the formulation. According to this result, it is possible to affirm that the extract was able to act in synergism with the chemical UV filter ethylhexyl methoxycinnamate, showing its absorption potential and capacity to increase the photoprotector efficacy in the cosmetic formulations.

The incorporation of vegetal extracts in cosmetic formulations destined to photoprotection products has high potential in the current market, since it comes against the strong industries tendency to use less raw materials with synthetic origin in their formulations, associating assets of natural origin without affecting the effectiveness of the products (De Souza et al., 2013; Polonini et al., 2014). The possibility of reusing byproduct or waste in the agroindustry shows additional advantages to this tendency, once it can contribute to the reduction of environmental impact and also increase the economic return with the development of innovative products with high added value (Melo, 2010).

Table 3
Values of SPF calculated for the cosmetic formulations.

Sample	SPF *in vitro*
Reference formulation	18.4 ± 0.7
Phytocosmetic	22.3 ± 1.1

Results expressed as mean ± standard deviation.

Conclusions

The extract from *P. guajava* byproduct standardized in ellagic acid, showed *in vitro* antioxidant activity and capacity of act in synergy with the chemical UV solar filter, demonstrating that the propriety to absorb radiation in the wavelength of the UVB specter increases the photoprotection efficacy in the cosmetic formulations and can be used to develop innovative products with efficacy and also contribute to decrease of environmental effects.

Authors' contributions

LPGM was the responsible for the liquid extract obtaining, developing of cosmetic formulations, evaluation of photoprotection efficacy *in vitro* from the cosmetic formulations and the interpretation of the obtained results. NOSG assisted in the preparation and production of the plant material, the total phenol dosing, determination of the antioxidant activity of the extract and participated in the result interpretations. MCM contributed with the analysis by HPLC. ALSD helped in the extractive method realization, formulations developing and results interpretations. This author was also responsible for the total flavonoids dosing. NLO contributed with the interpretation of the results and reviewing the final article. ECC was the coordinator of the project. All the authors read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

References

Abe, L.T., Lajolo, F.M., Genovese, M.I., 2010. Comparison of phenol content and antioxidant capacity of nuts. Cien. Tecnol. Alim. 30, 254–259.
Babbar, N., Oberoi, H.S., Uppal, D.S., Patil, R.T., 2011. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. Food Res. Int. 44, 391–396.
Blancas-Benitez, F.J., Pérez-Jiménez, J., Montalvo-González, E., González-Aguilar, G.A., Sáyago-Ayerdia, S.G., 2018. J. Funct. Foods 43, 139–145.
Carvalho, J.C.T., Gosmann, G., Schenkel, E.P., 2009. Compostos fenólicos simples e heterosídeos. In: Simões, C.M.O., Schenkel, E.P., Gosmann, G., Mello, J.C.P., Mentz, L.A., Petrovick, P.R. (Eds.), Farmacognosia: da planta ao medicamento. , 1 ed. Editora da UFRGS/Editora da UFSC, Porto Alegre/Florianópolis.
Chiari, B.G., Severi, J.A., Pauli-Credendio, P.A., Sylos, C.M., Vilegas, W., Corrêa, M.A., Isaac, V.L.B., 2012. Assessment of the chemical profile: polyphenol content andantioxidant activity in extracts of *Psidium guajava* L. fruits. Int. J. Pharm. Pharm. Sci. 4, 331–336.
Costa, S.C.C., Detoni, C.B., Branco, C.R.C., Botura, M.B., Branco, A., 2015. *In vitro* photoprotective effects of *Marcetia taxifolia* ethanolic extract and its potential for sunscreen formulations. Rev. Bras. Farmacogn. 25, 413–418.
Couto, R.O., Valgas, A.B., Bara, M.T.F., De Paula, J.R., 2009. Caracterização físico-química do pó das folhas de *Eugenia dysenterica* DC. (Myrtaceae). Rev. Eletr. Farm. 6, 59–69.
Dal'Belo, S.E., Dissertação de Doutorado 2008. Avaliação da eficácia fotoprotetora, penetração cutânea e segurança de formulações cosméticas contendo extratos

de chá verde e Ginkgo biloba. Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto.
Dalvi, L.T., Dissertação de Mestrado 2008. Mecanismos de ação de antioxidantes de origem vegetal: estudo do polifenol ácido elágico e do extrato de Caqui (*Diospyros kaki*). Brasília. Faculdade de Ciências da Saúde, Universidade de Brasília.
De Souza, F.P., Campos, G.R., Packer, J.F., 2013. Determinação da atividade fotoprotetora e antioxidante em emulsões contendo extrato de *Malpighia glabra* L – acerola. Rev. Cienc. Farm. Básica Apl. 34, 69–77.
Díaz-de-Cerio, E., Gómez-Caravaca, A.M., Verardo, V., Fernández-Gutiérrez, A., Segura-Carretero, A., 2016. Determination of guava (*Psidium guajava* L.) leaf phenolic compounds using HPLC-DAD-QTOF-MS. J. Funct. Foods 22, 376–388.
Farmacopeia Brasileira, 2010. Agência Nacional de Vigilância Sanitária, 5 ed. Anvisa, Brasília.
Flor, J., Davolos, M.R., Correa, M.A., 2007. Protetores solares. Quim. Nova 30, 153–158.
Garcia, N.O.S., Trabalho de Conclusão de Curso 2015. Avaliação da atividade antioxidante do extrato padronizado em ácido elágico obtido de resíduo agroindustrial de *Psidium guajava* L. (Myrtaceae). Faculdade de Farmácia, Universidade Federal de Goiás.
García-Niño, W.R., Zazueta, C., 2015. Ellagic acid: pharmacological activities and molecular mechanisms involved in liver protection. Pharmacol. Res. 97, 84–103.
Guaratini, T., Callejon, D.R., Pires, D.C., Lopes, J.N.C., Lima, L.M., Neto, D.G., Sustovich, C., Lopes, N.P., 2009. Fotoprotetores derivados de produtos naturais: perspectivas de mercado e interações entre o setor produtivo e centros de pesquisa. Quim. Nova 32, 717–721.
Iha, S.M., Migliato, K.F., Vellosa, J.C.R., Sacramento, L.V.S., Pietro, R.C.L.R., Isaac, V.L.B., Brunetti, I.L., Corrêa, M.A., Salgado, H.R.N., 2008. Estudo fitoquímico de goiaba (*Psidium guajava* L.) com potencial antioxidante para o desenvolvimento de formulação fitocosmética. Rev. Bras. Farmacogn. 18, 387–393.
INCA, 2010. Estimativas 2010: Incidência de Câncer no Brasil. Instituto Nacional do Câncer, Rio de Janeiro.
ISO, 2012. Determination of Sunscreen UVA Photoprotection *In Vitro*. ISO 24443. International Organization for Standardization.
Jarzycka, A., Lewinska, A., Gancarz, R., Wilk, K.A., 2013. Assessment of extracts of *Helichrysum arenarium*, *Crataegus monogyna*, *Sambucus nigra* in photoprotective UVA and UVB; photostability in cosmetic emulsions. J. Photochem. Photobiol. B 128, 50–57.
Kamath, S., Skariyachan, S., 2017. Novel insight from computational virtual screening depict the binding potential of selected phytotherapeutics against probable drug targets of *Clostridium difficile*. Interdiscip. Sci. 10, 583–604.
Kilic, I., Yeşiloğlu, Y., Bayrak, Y., 2014. Spectroscopic studies on the antioxidant activity of ellagic acid. Spectrochim. Acta A 130, 447–452.
Kong, K.W., Ismail, A., Tan, C.P., Rajab, N.F., 2010. Optimization of oven drying conditions for lycopene content and lipophilic antioxidant capacity in a by-product of the pink guava puree industry using response surface methodology. Food Sci. Technol. 43, 729–735.
Leong, L.P., Shui, G., 2002. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem. 76, 69–75.
Liu, Y., Liu, Y., Liu, Y., Liu, H., Shang, Y., 2018. Evaluating effects of ellagic acid on the quality of kumquat fruits during storage. SCI Hortic. (Amst.) 227, 244–254.
MAPA, 2007. Comunicado Técnico On Line 127, 2007. Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. Ministério da Agricultura, Pecuária e Abastecimento. EMBRAPA, Fortaleza.
Mansur, M.C.P.P.R., Leitão, S.G., Cerqueira-Coutinho, C., Vermelho, A.B., Silva, R.S., Presgravec, O.A.F., Leitão, A.A.C., Leitão, G.G., Ricci-Júniora, E., Santos, E.P., 2016. *In vitro* and *in vivo* evaluation of efficacy and safety of photoprotective formulations containing antioxidant extracts. Rev. Bras. Farmacogn. 26, 251–258.
Melo, P.S., Dissertação de Mestrado 2010. Composição química e atividade biológica de resíduos agroindustriais. Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba.
Mole, S.A., Waterman, P.G., 1987. A critical analysis techniques for measuring tannins in ecological studies. Oecologia 72, 137–147.
Munhoz, V.M., Lonni, A.A.S.G., De Mello, J.C.P., Lopes, G.C., 2012. Avaliação do fator de proteção solar em fotoprotetores acrescidos com extratos da flora brasileira ricos em substâncias fenólicas. Rev. Cienc. Farm. Básica Apl. 33, 225–232.
Nascimento, C.S., Nunes, L.C.C., De Lima, A.A.N., Júnior, S.G., Neto, P.J.R., 2009. Incremento do FPS em formulação de protetor solar utilizando extratos de própolis verde e vermelha. Rev. Bras. Farm. 90, 334–339.
Oliveira, T.L.S., Alves, S.F., De Morais, S.R., Oliveira, E.M.S., Freitas, M.T., 2012. Quality control of *Psidium guajava* L. cv. 'Pedro Sato'. J. Pharm. Res. 4, 2338–2343.
Polonini, H.C., Brandão, M.A.F., Raposo, N.R.B., 2014. A natural broad-spectrum sunscreen formulated from the dried extract of Brazilian *Lippia sericea* as a single UV filter. RSC Adv. 4, 62566–62575.
Polonini, H.C., Raposo, N.R.B., Brandão, M.A.F., 2011. Fotoprotetores naturais como instrumento de ação primária na prevenção do câncer de pele. Revista APS 14, 216–223.
Rolim, A., Maciel, C.P.M., Kaneko, T.M., Consiglieri, V.O., Salgado-Santos, I.M.N., Velasco, M.V.R., 2005. Validation assay for total flavonoids, as rutin equivalents, from *Trichilia catigua* Adr. Juss (Meliaceae) and *Ptychopetalum olacoides* Bentham (Olacaceae) commercial extract. J. AOAC Int. 88, 1015–1019.
Silva, D.G., Sarruf, F.D., De Oliveira, L.C.D., Arêas, E.P.G., Kaneko, T.M., Consiglieri, V.O., Velasco, M.V.R., Baby, A.R., 2013. Influence of particle size on appearance and *in vitro* efficacy of sunscreens. Braz. J. Pharm. Sci. 49, 251–261.
Silva, R.V., Costa, S.C.C., Branco, C.R.C., Branco, A., 2016. *In vitro* photoprotective activity of the *Spondias purpurea* L. peel crude extract and its incorporation in a pharmaceutical formulation. Ind. Crop. Prod. 83, 509–514.
Sousa, M.S.B., Vieira, L.M., Da Silva, M.J.M., Lima, A., 2011a. Caracterização nutricional e compostos antioxidantes em resíduos de polpas de frutas tropicais. Cienc. Agrotec. 35, 554–559.
Sousa, M.S.B., Vieira, L.M., Lima, A., 2011b. Fenólicos totais e capacidade antioxidante in vitro de resíduos de polpas de frutas tropicais. Braz. J. Food Technol. 14, 202–210.
Verotta, L., Panzella, L., Antenucci, S., Calvenzani, V., Tomay, F., Petroni, K., Caneva, E., Napolitano, A., 2018. Fermented pomegranate wastes as sustainable source of ellagic acid: antioxidant properties, anti-inflammatory action, and controlled release under simulated digestion conditions. Food Chem. 246, 129–136.
Vinayagam, R., Jayachandran, M., Chung, S.S.M., Xu, B., 2018. Guava leaf inhibits hepatic gluconeogenesis and increases glycogen synthesis via AMPK/ACC signaling pathways in streptozotocin-induced diabetic rats. Biomed. Pharmacother. 103, 1012–1017.
Violante, I.M.P., Souza, I.M., Venturini, C.L., Ramalho, A.F.S., Santos, R.A.N., Ferrari, M., 2009. Avaliação *in vitro* da atividade fotoprotetora de extratos vegetais do cerrado de Mato Grosso. Rev. Bras. Farmacogn. 19, 452–457.