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Photoprotective and antioxidant effect of babassu mesocarp flour extracts

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ABSTRACT

In the search for new natural photoprotective agents, the mesocarp of babassu (*Attalea speciosa*) stands out as a promising candidate due to its unique chemical composition and regional significance. In this study, we investigated the *in vitro* photoprotective and antioxidant properties of babassu mesocarp flour extracts and their fractions. Antioxidant activity was evaluated using DPPH and ABTS assays. The sun protection factor (SPF) was determined through the Mansur assay, and cytotoxicity was determined in RAW cells. The samples exhibited high antioxidant activity, especially in the more polar fractions. The hydroethanolic extract had an SPF of 16.69, while the aqueous extract had an SPF of 14.83. Notably, the hydroethanolic extract exhibited no cytotoxic effects at the tested concentrations. Our findings suggest that babassu mesocarp flour is a potential source for developing photoprotective agents to shield skin from UV radiation and combat free radicals.

KEYWORDS: *Attalea speciosa*, sunscreen, sun protection factor, phenolic compound, cytotoxicity

Efeito fotoprotetor e antioxidante de extratos da farinha de mesorcarpo de babassu

RESUMO

Na busca por novos agentes fotoprotetores naturais, o mesocarpo do babassu (*Attalea speciosa*) se destaca como um candidato promissor, devido à sua composição química única e importância regional. Neste estudo, investigamos as propriedades fotoprotetoras e antioxidantes *in vitro* dos extratos da farinha de mesocarpo de babassu e suas frações. A atividade antioxidante foi avaliada usando os ensaios DPPH e ABTS. O fator de proteção solar (FPS) foi determinado através do ensaio de Mansur, e a citotoxicidade foi determinada em células RAW. As amostras apresentaram alta atividade antioxidante, especialmente nas frações mais polares. O extrato hidroetanólico teve um FPS de 16,69, enquanto o aquoso registrou um FPS de 14,83. Notavelmente, o extrato hidroetanólico não exibiu efeitos citotóxicos nas concentrações testadas. Nossos resultados sugerem que a farinha de mesocarpo de babassu é uma fonte potencial para o desenvolvimento de agentes fotoprotetores para proteger a pele da radiação UV e combater radicais livres.

PALAVRAS-CHAVE: *Attalea speciosa*, protetor solar, fator de proteção solar, compostos fenólicos, citotoxicidade

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INTRODUCTION

Ultraviolet (UV) radiation has beneficial and harmful effects on living organisms. It is vital for biological processes such as photosynthesis, vitamin D synthesis, blood pressure and circadian cycle regulation, and hormone production (Holick 2016). However, it can cause burns, DNA damage, chronic inflammation, photoaging, and skin cancer (Brenner and Hearing 2008; Chen *et al*. 2021). One strategy to avoid these harmful effects is wearing synthetic sunscreens (Autier *et al*. 2007), however, they can cause significant adverse effects, such as allergic reactions, contact dermatitis, burns, and systemic absorption (Matta *et al*. 2020; Henderson *et al*. 2022). Therefore, there is a need to develop safer and more effective sunscreens, and natural products are a promising source of active ingredients for this purpose (Decean *et al*. 2016).

Phenolic compounds are frequently associated with the photoprotective effects of natural products. These substances also have an important antioxidative effect (Nunes *et al.* 2018*;* Cefali *et al.* 2020*)* which can reduce the damage caused by UV radiation on the skin (Cefali *et al*. 2016; Chen *et al*. 2021). For example, the mesocarp hydroalcoholic extract of *Attalea speciosa* Mart ex Spreng (Arecaceae) (synonym *Orbignya phalerata* Mart.) presents an *in vitro* antioxidant effect associated with the presence of phenolic compounds (Silva *et al*. 2017*b*). This plant species, popularly known as babassu, is native and widely distributed in Amazonian transition regions in several Brazilian states, where it represents an important extractive resource (Silva *et al*. 2017b).

Several studies showed the biological activity and chemical profile of both babassu oil (Santos *et al*. 2020; Silva *et al*. 2020) and mesocarp (Silva *et al*. 2017b). However, the photoprotective activity of babassu mesocarp has not yet been prospected. In this context, we investigated the antioxidant and photoprotective effects of both hydroalcoholic and aqueous extracts from babassu mesocarp flour and its fractions. We characterized the chemical profile and evaluated the *in vitro* cytotoxic effects of the sample with the highest sun protection factor.

MATERIAL AND METHODS

Babassu mesocarp flour

Babassu mesocarp flour was purchased commercially from Interstate Cooperative of Women Babassu Coconut Breakers (*Cooperativa Interestadual das Mulheres Quebradeiras de Coco Babaçu*), São Luís, Maranhão, Brazil. The cooperative is an organization of women extractive producers who collect and process babassu coconut in the Brazilian states of Pará, Maranhão, Tocantins, and Piauí (https://www.miqcb.org/). This study is registered in the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN - *Sistema Nacional de Gestão do Patrimônio Genético e Conhecimentos Tradicionais Associados*), under code A7D3957.

Extract preparation

We prepared two extracts (aqueous and hydroalcoholic) according to the methods described by Silva *et al*. (2017b), with adaptations. For each type of extract, we used 200 g of babassu mesocarp flour. To prepare the aqueous extract (AEB), we used distilled water as the solvent, and for the hydroethanolic (HEB), we used 70% hydroethanolic solution. We macerated the mesocarp flour in the respective solvents at a ratio of 1:4 (mesocarp/solvent) for 48 hours, while protecting it from light. After this period, we filtered each extract under vacuum and stored the solutions in amber bottles in a refrigerator until we could proceed with the concentration and lyophilization steps. The hydroalcoholic extract was concentrated using a rotary evaporator (Quimis, model Q34432) at a temperature of 45°C. We lyophilized the aqueous extract (Terroni, LS 3000 D) for 24 hours. After each step, we stored the extracts in a desiccator with silica. The crude aqueous extract (AEB) yield was 2.33%, and the crude hydroethanolic extract (HEB) yield was 3.76%.

Extract fractionation

To fractionate the extracts, AEB (1.2g) and HEB (4.5g) were diluted in 100 mL of methanol: water solution in a 1:1 ratio (50/50) and submitted to liquid-liquid partition using solvents of increasing polarity - hexane (Labsynth, Brazil), chloroform (Labsynth, Brazil) and ethyl acetate (Labsynth, Brazil). The resulting fractions were concentrated under vacuum at a temperature of 45ºC, to obtain the hexane (Fr-HX), chloroform (Fr-CL), ethyl acetate (Fr-EA), and hydromethanolic (Fr-HM) fractions for both AEB and HEB (Dutra *et al*. 2014).

AEB fractions yielded 56.7% for Fr-EA and 43.3% for Fr-HM. HEB fractions showed a yield of 7.8% for Fr-CL, 47.1% for Fr-EA, and 39.8% for Fr-HM. The Fr-HX of both extracts and the Fr-CL of AEB did not yield enough material.

Phenolic compound determination

To determine the concentration of total phenolic compounds (TPC), we used 0.1 mL of the extracts or fractions, previously diluted in methanol at 2.0 mg mL^{-1}. The samples were added to 0.1 mL of Folin-Ciocalteau (*Vetec Química Fina*, Brazil) and 1.0 mL of 20% sodium carbonate (*Dinâmica Química Contemporânea*, Brazil), resulting in a final volume of 4.0 mL in distilled water. The final concentration of the samples was 0.05 mg mL^{-1}. After homogenization, the dilutions were protected from light and left to react for 2 hours. The absorbance was determined at 760 nm using a UV-Vis spectrophotometer (Gehaka UV-340G). The phenolic concentration was determined using a standard curve with

gallic acid solution (Sigma-Aldrich, Brazil) in the range of 0.001 - 0.06 mg mL^{-1} (r^2 = 0.998) and expressed as gallic acid equivalent per gram of each sample (mg GAE (gallic acid equivalent g^{-1}). All assays were performed in triplicate (Silva *et al*. 2017b).

Proanthocyanidin determination

The vanillin assay with hydrochloric acid was used to analyze the total proanthocyanidin (TPA) concentrations present in extracts and fractions. A methanolic solution of vanillin (Isofar, Brazil) at 1% and hydrochloric acid (Moderna, Brazil) at 9 mol L^{-1} were added to the samples (2.0 mg mL^{-1}) at a volume of 1.25 mL and 0.5 mL, respectively. The final concentration of the samples was 0.08 mg mL^{-1}. After the addition, the samples were incubated at 30°C for 20 minutes. The absorbance was determined at 500 nm (UV-Vis spectrophotometer - Gehaka UV-340G). The concentration was expressed as catechin equivalent per gram of each sample (mg CAT (catechin equivalent g^{-1}) using a standard curve of catechin (Sigma-Aldrich, Brazil) in methanol, within the range of 0.005-0.08 mg mL^{-1} (r^2 = 0.999). All assays were performed in triplicate (Silva *et al*. 2017b).

Antioxidant activity *in vitro*

DPPH• radical scavenging assay – To assess antioxidant activity with DPPH• radical (2,2-diphenyl-1-picrylhydrazyl), extracts and fractions of babassu mesocarp flour (2.5 to 100 µg mL^{-1}) were added to a DPPH solution (Sigma-Aldrich, Brazil) at 40 µg mL^{-1}. The mixture was incubated in a dark chamber for 30 min and the absorbances were determined at 517 nm using a UV-Vis spectrophotometer (Gehaka UV-340G). Catechin (Sigma-Aldrich, Brazil) 2 to 8 µg mL^{-1} and ascorbic acid 2.5 to 10 µg mL^{-1} (Sigma-Aldrich, Brazil) were used as positive controls. Antioxidant activity (AA) was calculated using the equation:

$$AA\ (\%) = 100 - [(A_{sample} - A_{blank}) \times 100/A_{radical}],$$

where A is the absorbance.

The results were expressed as effective concentration able to reduce 50% of free radical initial concentration (EC_{50}). All analyses were performed in triplicate (Dutra *et al*. 2014).

ABTS•+ radical formation assay – To assess the antioxidative activity by ABTS•+ (2.2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic) formation assay, a mixture containing 7 mmol L^{-1} of ABTS (Sigma-Aldrich, Brazil) and 2.45 mmol L^{-1} of sodium persulfate (Sigma-Aldrich, Brazil) was initially prepared and stored for 16 hours in a dark chamber. For the analyses, the ABTS mixture was diluted in ethanol until an absorbance of 0.700 ± 0.020 at 734 nm was obtained in a UV-Vis spectrophotometer (Gehaka UV-vis 340G). Then, 400 µL of extracts or fractions, in different concentrations (10 to 40 µg ml^{-1}), were added to a final volume of 3.0 mL of ABTS•+ radical mixture. After 6 minutes the absorbance was determined at 734 nm. Ethanol was used as a blank, and catechin and ascorbic acid (Sigma-Aldrich, Brazil) were used as positive controls. Antioxidant activity (AA) was calculated using the equation: $AA\ (\%) = 100 - [(A_{sample} - A_{blank}) \times 100/A_{radical}]$, where A is absorbance. The results were expressed as effective concentration able to reduce 50% of free radical initial concentration (EC_{50}). The analyses were performed in triplicate (Dutra *et al*. 2014).

Sun protection factor *in vitro*

The *in vitro* sun protection factor (SPF) of babassu mesocarp flour extracts and fractions was determined *in vitro* using a concentration of 200 µg mL^{-1} in methanol (Nunes *et al*. 2018). The absorbances were determined at 5 nm intervals over the range of 290-320 nm, as previously described by Mansur *et al*. (1986). The SPF was calculated using the formula:

$$SPF = CF \times \sum (290\ nm)\text{^}(320\ nm)\ EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where CF is the correction factor (constant = 10), EE is the erythermogenic effect of wavelength radiation (λ), I is the intensity of sunlight in wavelength, and Abs is the absorbance of the sample in solution, using the wavelength (λ). The EE and I constants were pre-defined and are listed in Table 1 (Mansur *et al*. 1986).

Table 1. Product of constants EE and I for *in vitro* SPF determination.

λ (nm)	EE (λ) x I (λ)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
∑	1.0000

EE = erymatogenic effect of wavelength radiation; I = sunlight intensity in wavelength; λ = wavelength (Mansur et al. 1986).

In vitro cytotoxicity assay

To evaluate the safety of the sample with the best photoprotective activity, we performed an *in vitro* cytotoxicity assay using the RAW 264.7 murine macrophage strain, which are leukocytes found in the dermis, the middle layer of skin (Kolter *et al*. 2019).

RAW 264.7 cells were added into 96-well plates at a concentration of 1 x 10^6 cells mL^{-1} in a final volume of 100 µL $well^{-1}$ of RPMI medium (Sigma-Aldrich, UK), supplemented with 10% fetal bovine serum (Gibco, United Kingdom), 100 U mL^{-1} penicillin (Sigma-Aldrich, Brazil), 100 µg mL^{-1} streptomycin (Sigma-Aldrich, Brazil), 2.5 µg mL^{-1} amphotericin (Gibco, Israel), 2 mM glutamine (Gibco, Brazil), and 1 mM sodium pyruvate (Sigma-Aldrich, Brazil) to facilitate spreading and adhesion. The cells were then incubated at 37 °C, 5% CO_2, and 95% humidity for 12 hours. Afterwards the cells were treated with the selected sample in serial concentrations at a 1:4 ratio (500 to 1.95 µg mL^{-1})

for 48 hours, following the same conditions as previously described. For the blank, samples at different concentrations or only culture medium were used, whereas RAW 264.7 cells in supplemented RPMI medium were used for the negative control. To evaluate cell viability, 10 µL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) (Sigma-Aldrich, Brazil) was added to 5 mg mL^{-1} $well^{-1}$. The MTT assay relies on a colorimetric reaction that converts MTT, a water-soluble yellow compound, into insoluble and purple formazan crystals. This reaction only occurs due to the activity of mitochondrial enzyme succinate tetrazolium reductase, which is only active in living cells (Mosmann 1983).

After three hours of incubation, without light, we discarded the supernatant and added 100 µL of dimethyl sulfoxide (DMSO) (Synth, Brazil) to dissolve the formazan crystals. The absorbance was determined at 550 nm in a microplate reader (Biotek, USA) to determine cell viability activity. Cell viability was calculated using the equation:

$$\text{Viability (\%)} = [(A_{sample} - A_{blank}) \times 100 / (A_{negative\ control} - A_{blank})]$$

where A = absorbance.

We performed analyzes in triplicate (Oliveira-Neto *et al*. 2022).

Analysis by HPLC-MS

The most active sample was analyzed using a high-performance liquid chromatograph (LC-20A, Shimadzu) equipped with an autoinjector (SIL 30AC, Shimadzu) and a reverse-phase C-18 analytical column (250 x 4.6 mm, 5 µm, Phenomenex Luna, CA, USA), coupled to a mass spectrometer (Amazon Speed ETD, Bruker, MA, USA). Initially, the sample was diluted in HPLC-grade methanol (5 mg mL^{-1}) and filtered with a 0.22 µm PTFE® filter. The mobile phase was composed of ultrapure water with 0.1% formic acid (A) and methanol (B), using the following gradient: 1 minute, 5% B; 20 minutes, 30% B; 25 minutes, 50% B; 40 minutes, 80% B; 50 minutes, 100% B, with a flow rate of 1 mL min^{-1}. The ionization conditions used a capillary voltage of 4.5 kV at 325°C, in negative mode, with a scan range of 100 to 2,000 *m*/z. Identification was performed based on the molecular ions and their fragmentation, which were compared with literature data (Dutra *et al*. 2014).

Statistical analysis

Linear regression was used to determine the concentrations of TPC, TPA, and EC_{50} for DPPH and ABTS assays. Pearson's correlation was used to analyze the relation between these parameters and SPF. Shapiro-Wilk test was used to assess the normality of variable distributions. To compare the concentrations of TPC, TPA, EC_{50} for DPPH and ABTS assays, FPS values, and cytotoxicity between the treatments, we used a one-way analysis of variance (ANOVA) followed by a Tukey test. P-values ≤ 0.05 were considered significant. Data were analyzed using either GraphPad Prism 9.0 software or Microsoft Excel 365.

RESULTS

Phenolic compounds and proanthocyanidins

HEB had a significantly higher concentration of both TPC and TPA, than AEB (Table 2). Furthermore, AEB partitioning led to a significant increase in TPC in Fr-EA. In contrast, for HEB, the maximum TPC was found in Fr-HM. TPA followed the same pattern, with Fr-EA in AEB and Fr-HM in HEB. Interestingly, HEB's Fr-CL had the lowest concentration of both TPC and proanthocyanidins.

Antioxidant activity

HEB exhibited significantly higher DPPH activity, while the choice of solvent did not notably influence the ABTS activity (Table 3). Among the AEB' fractions, Fr-EA showed a significantly lower EC_{50} value for DPPH scavenging. In contrast, within HEB, both Fr-EA and Fr-HM demonstrated enhanced reducing activity, with no significant difference between them. From the AEB' fractions, Fr-EA and Fr-HM displayed reduced EC_{50} activity in targeting ABTS radicals, again with no statistically significant difference between

Table 2. Total phenolic compounds and proanthocyanidins in babassu mesocarp flour extracts and fractions.

Sample	TPC (mg GAE g^{-1})		TPA (mg CAT g^{-1})	
	AEB	HEB	AEB	HEB
Extract	427.88 ± 19.62^{a}	604.05 ± 33.47^{d}	83.23 ± 0.66^{a}	194.88 ± 8.1^{6}c
Fr-HX	nd	nd	nd	nd
Fr-CL	nd	139.06 ± 3.37^{e}	nd	29.70 ± 1.26^{d}
Fr-EA	648.19 ± 10.1^{b,d}	536.50 ± 35.98^{c}	134.22 ± 1.72^{b}	228.32 ± 24.23^{c}
Fr-HM	529.01 ± 6.62^{c}	686.18 ± 18.84^{b}	74.33±0.96^{a}	336.14 ± 31.71^{e}

Values represent the mean of triplicate measurements ± standard deviation. TPC = total phenolic compounds; GAE = gallic acid equivalents; TPA = proanthocyanidins; CAT = catechin; nd = not detected; AEB = aqueous extract of babassu; HEB = hydroethanolic extract babassu; Fr-HX = hexane fraction; Fr-CL = chloroform fraction; Fr-EA = ethyl acetate fraction; Fr-HM = hydromethanolic fraction. Values with different letters in the two columns of the same parameter indicate a significant difference (one-way ANOVA, Tukey test) at $p \leq 0.05$.

Table 3. DPPH and $ABTS^{+}$ antioxidant activity of babassu mesocarp flour extracts and its fractions.

Sample	DPPH (EC_{50} µg mL^{-1})		ABTS (EC_{50} µg mL^{-1})	
	AEB	HEB	AEB	HEB
Extract	9.26 ± 0.59^{a}	5.16 ± 0.19^{b,e}	6.60 ± 0.32^{a,c,d}	4.79 ± 0.08^{a,c,e}
Fr-HX	nd	nd	nd	nd
Fr-CL	nd	95.56 ± 0.95^{d}	nd	22.87 ± 3.16^{b}
Fr-EA	6.39 ± 0.10^{b}	6.12 ± 0.15^{b}	4.19 ± 0.15^{a,c,e}	4.71 ± 0.23^{a,c,e}
Fr-HM	11.49 ± 0.46^{c}	5.23 ± 0.23^{b,e}	4.07 ± 0.13^{a,c,e}	7.19 ± 0.45^{a,d}
CAT	4.55 ± 0.04^{e,f}		2.34 ± 0.20^{d}	
AA	5.28 ± 0.55^{b,f}		2.85 ± 0.02^{d}	

Values represent the mean of triplicate measurements ± standard deviation. EC = half-maximal effective concentration; nd = not detected; AEB = aqueous extract of babassu; HEB = hydroethanolic extract babassu; Fr-HX = hexane fraction; Fr-CL = chloroform fraction; Fr-EA = ethyl acetate fraction; Fr-HM = hydromethanolic fraction; CAT = catechin; AA = ascorbic acid. Values with different letters in the two columns of the same parameter indicate a significant difference (one-way ANOVA, Tukey test) at $p \leq 0.05$.

them. Within the HEB fraction set, Fr-EA displayed superior antioxidant activity, whereas Fr-CL presented poorer reducing activity. The positive controls surpassed AEB in the DPPH assay but not HEB. Moreover, these controls exhibited greater antioxidant prowess than both the extracts and fractions in the ABTS assay.

Photoprotective effect

The HEB showed better photoprotective activity compared to the AEB and the fractions. The extracts and the fractions showed significantly higher SPF values when compared to catechin alone, but some of them were lower than those found in the positive control, octyl methoxycinnamate (Table 4). The correlation coefficients (r) of FPS with TPC, TPA, DPPH and ABTS were -0.37, 0.14, -0.43, and 0.20, respectively, but all correlations were statistically non-significant.

Cytotoxic assay

In all concentrations tested, viability was greater than 80%, when compared to untreated cells, indicating that HEB does not exhibit significant cytotoxic activity at these concentrations (Figure 1).

Extract chemical composition

The LC-MS analysis of the hydroalcoholic extract allowed the identification of seven compounds, including three proanthocyanidins of type A (dimer and trimer) and type B (dimer), as well as the monomer (epi)catechin (flavan-3-ols), which is the basic unit of proanthocyanidins. In addition, three flavonols were identified, with quercetin as the aglycone, a glycosylated compound (quercetin-glicoside), and another with a methyl group (isorhamnetin) (Table 5; Figure 2).

DISCUSSION

The present study shows unprecedented findings on the photoprotective activity of babassu mesocarp flour extracts. Plant extracts can protect the skin from UV rays through various mechanisms (Dunaway *et al*. 2018; Chen *et al*. 2021). Our results showed that babassu mesocarp flour extracts have significant UV ray-absorbing capacity and high antioxidant activity. Phenolic compounds are generally credited as photoprotective players in plant extracts (He *et al*. 2021), and babassu mesocarp flour is rich in these compounds (Barroqueiro *et al*. 2016; Silva *et al*. 2017*b*). It is reasonable to propose that they are associated with the activities described here.

The chemical analysis of the babassu mesocarp flour extract revealed high levels of total phenolic compounds and proanthocyanidins, especially in the hydroethanolic extract. Our findings also support previous research indicating that ethanol is more efficient than water in extracting phenolic compounds (Tan *et al*. 2013; Ye *et al*. 2015). However, the effectiveness of a solvent largely depends on the type and concentration of phenolic substances in the plant material. Ethanol, having intermediate polarity, can extract a diverse range of substances (Ye *et al*. 2015).

The partition of babassu mesocarp flour extract using ethyl acetate and hydromethanolic solution, which are medium and high-polarity solvents respectively, increased the

Table 4. *In vitro* sun protection factor of babassu mesocarp flour extracts and its fractions.

Sample	AEB	HEB
Extract	14.83 ± 0.09[a]	16.59 ± 0.04[c]
Fr-HX	nd	nd
Fr-CL	nd	3.43 ± 0.31[d]
Fr-EA	4.99 ± 0.47[b]	8.39 ± 0.53[e]
Fr-HM	4.42 ± 0.20[b,d]	6.20 ± 0.32[f]
CAT	1.03 ± 0.03[g]	
OMC	23.70 ± 0.69[h]	

Values represent the mean of triplicate measurements ± standard deviation. AEB = aqueous extract of babassu; HEB = hydroethanolic extract babassu; Fr-HX = hexane fraction; Fr-CL = chloroform fraction; Fr-EA = ethyl acetate fraction; Fr-HM = hydromethanolic fraction; CAT = catechin; OMC = octyl methoxycinnamate. Values with different letters indicate a significant difference (one-way ANOVA, Tukey test) at $p \leq 0.05$.

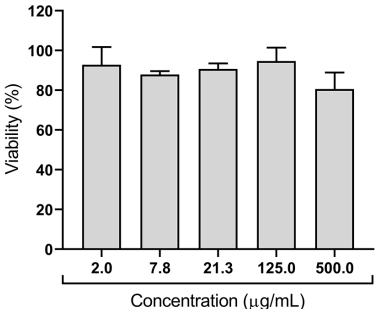

Figure 1. Effect of the hydroalcoholic extract of babassu mesocarp flour against RAW 264.7 macrophage viability. The cells were treated with serial concentrations of hydroalcoholic extract for 48 hours and cytotoxicity was measured by the MTT assay. Data represent means ± SD of sextuplicate cultures.

Table 5. Chemical constituents found in babassu mesocarp flour hydroethanolic extract.

Peak	Rt (min)	[M - H]$^-$ (m/z)	MS^2 ion fragments					Proposed substance
1	10.2	577.23	559.15	451,04	425.03	407.05	288.79	B-type procyanidin dimer
2	11.3	288.89	288.78	244.80				(epi) Catechin
3	13.0	863.40	736.28	711.23	573.20	575.16	451.05	A-type procyanidin trimer
4	15.5	575.18	558.20	449.07	423.03	284.83		A-type procyanidin dimer
5	17.4	463.12	300.82	150.47	178.49			Quercetin-glucoside
6	25.0	300.86	300.80	150.40				Quercetin
7	27.1	314.92	314.88	300.84				Isorhamnetin

RT = retention time; [M-H]$^-$ = negative mode ionization.

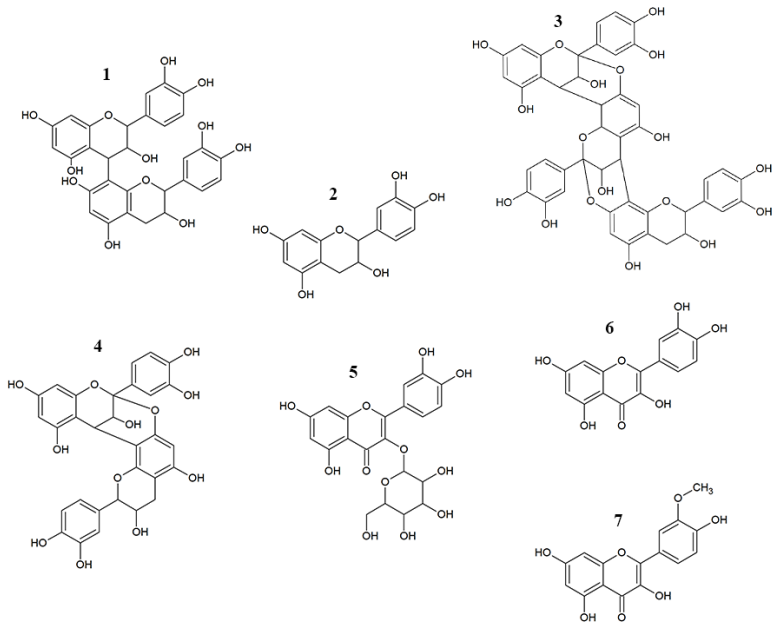

Figure 2. Chemical structure of compounds identified in babassu mesocarp flour hydroethanolic extract by LC-MS. B-type procyanidin dimer (1); (epi) catechin (2); A-type procyanidin trimer (3); A-type procyanidin dimer (4); quercetin-glucoside (5); quercetin (6); isorhamnetin (7).

concentration of phenolic compounds and proanthocyanidins compared to the extracts. Instead, non-polar solvents such as dichloromethane failed to solubilize significant amounts of these compounds, a finding similarly described by Silva *et al*. (2017b). This is because hydrogen bond formation and the capacity to break plant cell membranes play pivotal roles in the solubilization of these compounds (Robards 2003). Less polar solvents like acetone are more efficient in extracting proanthocyanidins and tannins (Mané *et al*. 2007), whereas flavonoids, catechols, and tannins are more soluble in ethanol (Spigno *et al*. 2007). Phenolic acids and catechins, are best extracted using high-polarity solvents like methanol (Chirinos *et al*. 2007). Consequently, no single solvent can extract all phenols present in plant material.

Reflecting the levels of phenolic compounds and proanthocyanidins, the hydroethanolic extract and its more polar fractions showed more significant antioxidant activity as measured by DPPH and ABTS. These assays evaluate the extracts and fractions' ability to neutralize free radicals through electron transfer from active substances (Dudonné *et al*. 2009). Solar radiation generates free radicals in the skin, either directly as reactive oxygen species or through oxidative process induction in cells (Dunaway *et al*. 2018). Plant extracts with antioxidant properties, such as HEB, can be used as photoprotective agents to reduce ROS formation and minimize oxidative damage to the skin (He *et al*. 2021). They can also reduce melanin polymerization, which occurs in response to oxidative stress caused by UV radiation, thus decreasing its photoprotective effect (Chen *et al*. 2021).

B-type proanthocyanidins dimers, trimers, and tetramers were reported in the babassu mesocarp flour hydroethanolic extract by Silva *et al*. (2017b). In this study, we identified, for the first time, A-type proanthocyanidin dimers and trimers, which have an additional ether linkage (Silva *et al*. 2017a). The A-type dimer exhibited the molecular ion with *m/z* 575, with fragment ions of *m/z* 449 and *m/z* 423 after the loss of 126 Da and 152 Da, respectively. The trimer (*m/z* 863) showed a mass difference of 288 Da compared to the dimer, indicating the loss of one unit of epi(catechin). The fragment ion of *m/z* 711 was formed after the loss of 152 Da, due to the retro Diels-Alder rearrangement of the epi(catechin) unit. Furthermore, the fragment ions of *m/z* 573 and 451 suggest that both compounds are A-type proanthocyanidins. The main fragmentation mechanisms for proanthocyanidin identification were heterocyclic ring B cleavage with loss of 126 Da and retro Diels-Alder rearrangement with loss of 152 Da (Sarnoski *et al*. 2012; Silva *et al*. 2017*a*). In addition, three flavonols were identified for the first time. The UV-Vis absorptions of the sample agree with the mass spectra, due to the characteristic absorptions of flavan-3-ols (269-279 nm) and flavonols (300-380 nm) (Villiers *et al*. 2016).

The desired photoprotective effect of a substance or extract is its ability to absorb or repel UV radiation, measured by its SPF (Ebrahimzadeh *et al*. 2014; He *et al*. 2021). Similar to antioxidant activity, the hydroethanolic extract exhibited a more intense photoprotective activity than the aqueous extract, with both showing SPF values following the standards of the Brazilian National Health Regulatory Agency (ANVISA) and the US Food and Drug Administration (FDA) (ANVISA 2012; FDA 2018). However, partition reduced the SPF by 2 to 5 times compared to the extracts, suggesting that

substances of different polarities may be acting synergistically to enhance the photoprotective effect.

Recent studies indicate a correlation between SPF and the content of total phenolic compounds but not with antioxidant activity (Ebrahimzadeh *et al*. 2014; Hashemi *et al*. 2021). However, the SPF of the babassu mesocarp flour extracts did not correlate with either parameter, which may be owed to that not all phenolic compounds can absorb UV radiation (Kostyuk *et al*. 2018). The chemical structure and polarity of these compounds can influence this effect. For a compound to have a good SPF, it must preferably absorb 290 to 320 nm (Nunes *et al*. 2018), which is not evident in some phenolic substances (Kostyuk *et al*. 2018). Furthermore, we found that the catechin, the monomer of the identified proanthocyanidins, has no SPF. Therefore, despite the high content of phenolic compounds in babassu mesocarp flour extracts and fractions, it is reasonable to assume that they are not primarily responsible for the photoprotective effect. Likewise, antioxidant activity and SPF are not correlated as they operate through distinct mechanisms. Specifically, antioxidant agents act by neutralizing free radicals (Gulcin 2020), whereas SPF measures a substance's capacity to absorb UV radiation (Henderson *et al*. 2022).

Finally, we demonstrated that HEB has no cytotoxicity *in vitro* on macrophage immune system cells in the skin (Kolter *et al*. 2019), even at high concentrations. Other studies have also shown low toxicity of babassu mesocarp for several organs and cells (Barroqueiro *et al*. 2016) including fibroblasts, lymphocytes and peripheral blood mononuclear cells (Leal *et al*. 2018). This feature is desirable since current photoprotectors have several adverse effects, such as skin sensitivity, contact dermatitis, allergic reactions, and systemic absorption (Matta *et al*. 2020; Henderson *et al*. 2022).

CONCLUSIONS

Our study reveals novel insights into the biological attributes of babassu mesocarp flour extracts, highlighting their pronounced capacity to absorb UVB rays and their potent antioxidant activity, especially in the hydroethanolic extract. Despite the high concentrations of phenolic compounds and proanthocyanidins in the extract, there is no direct correlation with its photoprotective activity. This indicates that other constituents within the extracts, including the identified flavonols, might be contributing to the photoprotective effects, suggesting potential synergistic interactions among various compounds. Given these properties, babassu mesocarp flour extracts emerge as promising alternatives to synthetic sunscreens, providing UV protection and reducing oxidative skin damage with potentially lower toxicity.

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DATA AVAILABILITY

The data that support the findings of this study are available, upon reasonable request, from the corresponding author, Aramys Silva Reis.