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Development and evaluation of a topical formulation containing munguba butter and bacaba peel extract

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Abstract. Bacaba (*Oenocarpus bacaba* Mart) is an Amazonian palm tree that bears fruits with an intensely purple colored peel, indicating the presence of anthocyanins, a natural source of antioxidants. However, these peels are a by-product of bacaba processing, being discarded while their bioactive compounds could be used by the pharmaceutical, cosmetic, and food industries. In this sense, recently, there has been a growth in a consumer audience interested in cosmetic products that, in addition to quality, have components of natural origin and promote sustainability. Therefore, the objective was to develop an emulsified base formulation, oil-in-water type, incorporate the bacaba peel extract into it, and evaluate its antioxidant activity. Three topical formulations containing munguba butter as the internal phase (in percentages of 2, 3, and 5%) were developed, which were then subjected to preliminary and accelerated stability analyses. The bacaba peel extract was incorporated into the formulation with the highest stability (the one containing 2% of munguba butter), which had an anthocyanin content equal to 9.75 mgL^{-1} of cyanidin-3-glucoside, indicating antioxidant potential. The stability of the emulsion was maintained even after incorporation of the extract into the base formulation. The antioxidant activity of the bacaba peel extract and the extract-containing formulation was analyzed using the DPPH (2,2-DIPHENYL-1-PICRYLHYDRAZYL) radical scavenging method, obtaining EC50 values of 18.54 and 89.66 μgmL^{-1}, and the ABTS (2,2´ -azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) method, with results equal to 85.56 mM $Troloxg^{-1}$ and 55.52 mM $Troloxg^{-1}$, demonstrating good antioxidant activity in both assays. Therefore, a stable formulation with promising antioxidant activity was obtained, developed by the incorporation of bioactive compounds from the bacaba peel extract, presenting potential for use in the prevention and reduction of oxidative damage, in addition to reducing environmental impacts by using a part of the bacaba fruit that is normally discarded.
Keywords: antioxidant activity, emulsion, cosmetic stability, by-product.

Introduction

The rise of a new profile of consumers looking for quality products associated with sustainability and less environmental impacts has been boosting "green consumption", that is, the demand for products made from natural active ingredients, especially in the cosmetic and food areas (Miguel, 2011). In this sense, the Amazon forest is an important source of global biodiversity, with approximately one third of the world's biological diversity and a large number of plant species rich in bioactive compounds of interest for the production of cosmetic, pharmaceutical, and food products (Raiser et al., 2018; Torres et al., 2019; Lima et al., 2020).

Thus, *Pachira aquatica* Aublet (munguba), a fruit present in the Amazon region, stands out for its abundance of fatty acids, which makes it promising for the development of cosmetics (Raiser et al., 2018; Rodrigues et al., 2019). According to Prando et al. (2023), *Oenocarpus bacaba* Mart., an Amazonian palm tree popularly known as bacaba, also has this potential, being a natural source of antioxidant compounds.

During the processing of bacaba, its pulp is used, while its peel is commonly discarded, becoming a by-product, which, however, can be an important source of bioactive compounds (Prando et al., 2023). The bacaba peel has an intense purple

color, indicating the presence of anthocyanins, plant pigments with antioxidant properties related to the fight against and possible repair of oxidative stress, which is associated with the cell aging process and the development of several pathologies (Corrêa et al., 2019; Barros et al., 2021; Col et al., 2021; Baldissera et al., 2023).

Emulsions, dispersions in which immiscible liquids are stabilized by surfactants, occupy a prominent place in the cosmetic and pharmaceutical areas by promoting skin hydration and allowing the incorporation of actives, enhancing their topical penetration (Diavão; Gabriel, 2009; Kale; Deore, 2017; Rosário et al., 2021). Thus, oil-in-water (O/W) emulsions are more commonly used due to their "lighter" sensory characteristics and soft touch, justified by the predominance of water compared to the oil in the constitution (Coutinho; Santos, 2014).

However, during the development of a formulation, it is essential to perform tests that ensure its quality and safety. For this, preliminary and accelerated stability analyses can be performed to stipulate parameters such as product expiration date, the need to change or adapt formulation constituents, compatibility with storage containers, among others (Brasil, 2004).

Therefore, considering the promising antioxidant potential of *Oenocarpus bacaba* Mart. peel, it was sought to develop a base emulsion of the O/W type, using munguba butter as the internal phase, and to incorporate the bacaba peel extract, a portion usually discarded from the fruit, in addition to determining its stability and antioxidant activity.

Material and Methods

Munguba butter extraction

Theextraction of munguba butter (MB) was performed according to Raiser et al. (2020), where crushed munguba seeds and hexane were combined in a 1:5 (m/v) ratio, respectively, and then subjected to the ultrasound bath (Cristofoli), at a frequency of 40 kHz, for a period of 2 hours, under heating at approximately 35 °C. After this process, the supernatant liquid was vacuum filtered and evaporated in a rotary evaporator (Fisatom 804) at an average temperature of 40 °C.

Physicochemical analysis of the munguba butter

To evaluate the quality of the extracted munguba butter, tests of peroxide index ($meqKg^{-1}$) and acidity (mg $KOHg^{-1}$ and % of oleic acid) were performed, according to the AOCs methodology (2009), both in triplicate

Bacaba extract preparation

For the production of bacaba peel, seed, and fruit extracts, the methodology of Corrêa et al. (2020) was followed, using an ultrasound bath (frequency of 40 kHz and average temperature of 35 °C) and 70% ethyl alcohol as the extraction liquid.

Determination of total anthocyanins in the bacaba fruit extracts

The determination of total anthocyanins in the extracts of the different bacaba fruit portions (peel, seed, and whole fruit) was performed according to Falcão et al. (2007), by the differential pH method by spectrophotometry in the visible region (520 and 700 nm), with the result expressed in mgL^{-1} of cyanidin-3-glucoside.

Production of base formulation

A base formulation without extract was produced to enable the incorporation of the extract in the O/W emulsion. At first, three formulations containing respectively, 2, 3, and 5% of munguba butter were produced.

Thus, the oil phase (FO), containing Polawax ® self-emulsifying wax and munguba butter, and the aqueous phase (FA), consisting of Nipagin, disodium EDTA, and distilled water, were separately heated to approximately 75°C. Then, corn starch was incorporated into the FA for structuring. After that, FA was slowly poured over FO under the stirring of a mixer, maintained for approximately 15 minutes, until complete homogenization (Diavão & Gabriel, 2009).

Upon reaching room temperature, the formulation was stored in a suitable container, and after 24 hours, the stability tests began.

Stability Assays

For the preliminary stability test, the organoleptic and physicochemical characteristics (pH, conductivity, and refractive index) of the base formulations were analyzed 24 hours after preparation. Then, the formulations were subjected to alternating 24-hour cycles at temperatures of approximately 40 °C and 5 °C for a period of 14 days, being evaluated again after that (Brasil, 2004; Brasil, 2005).

The formulations that remained stable were reprepared and submitted to the accelerated stability test. In this process, the samples were divided into 3 groups, one was kept at a temperature of 40 ± 2 °C, another at 5 ± 2 °C and the last one at 25 ± 2 °C, which were subjected to analysis every 30 days for a period of 90 days (Brasil, 2004; Torres et al., 2018).

Incorporation of bacaba extract in the base formulation

The bacaba extract was incorporated into the base formulations in different percentages (5, 2, 1, and 0.5%), by levigation with propylene glycol. The extract-containing formulations were also subjected to the stability studies as previously described.

Determination of antioxidant activity

TheAnalyses were performed with 2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) and 2,2´-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals to determine the antioxidant activity of the extracts and formulations.

The DPPH test, a free radical captured by antioxidants, was based on the methodology of Rufino et al. (2007a), using ethanolic solutions of the samples, later diluted in methanol. A calibration curve was constructed for the bacaba extract, with a concentration range from 64 to $96\mu gmL^{-1}$, and for

the extract-containing formulation, with concentrations between 8000 and 16000 μgmL^{-1}. The results were obtained as EC50 (concentration needed to reduce 50% of the DPPH).

The methodology of Rufino et al. (2007b) was followed for the ABTS radical scavenging analysis, where the samples were diluted in ethanol in order to construct two calibration curves, one for the extract, with a concentration range from 12 to 20 μgmL^{-1}, and one for the formulation containing the bacaba extract, with concentrations ranging from 2000 to 4000 μgmL^{-1}. The antioxidant potential was calculated in mM Troloxg^{-1} of sample.

Statistical analyses

The software OriginPro (OriginLab®) was used to perform the statistical analyses. The analysis of variance ANOVA was performed, combined with Tukey test comparisons, considering a p-value greater than 0.05 as significant.

Results and discussion

The butter extracted from the munguba seed presented a yield of 25.34 ± 0.75%, a value lower than that obtained by Jorge and Luzia (2012), equal to 38.39%. This difference can be justified by climatic factors and soil conditions, as well as the stage of fruit maturation and harvesting period.

The analyses of the peroxide index and acidity as a percentage of oleic acid in munguba butter (Table 1) were also carried out to ensure its quality, an essential factor for its use as a raw material in the formulation's production.

Table 1. Physicochemical parameters of the munguba butter extracted by ultrasound

Physicochemical parameter	Result
Peroxide index (meqKg^{-1})	0.151 ± 0.00292
Acidity index (mg $KOHg^{-1}$)	2.148 ± 0.00434
Acidity expressed as a percentage of oleic acid (% oleic acid)	1.06 ± 0.0166
Refractive index	1.4689

Note: Results expressed as mean value ± relative standard deviation.

In this sense, the peroxide index of 0.151 meqKg^{-1} (Table 1) indicates that the MB obtained presented good conservation and quality, in accordance with the quality standards of ANVISA (2021), which recommends values below 15 meqKg^{-1} as ideal. Still, it presented a value lower than found by Raiser et al. (2020), 3.75 meqKg^{-1}.

For the acidity index, the found value (Table 1) was lower than that recommended by ANVISA (2021), of 4 mg $KOHg^{-1}$, and that obtained by Raiser et al. (2020), of 2.06% oleic acid. The determination of this index, according to Vieira et al. (2018), is mainly related to the degree of conservation of the butter and the vegetable raw material. Other factors that can influence are the extraction process, the climatic conditions in which the fruit was collected, and the fruit's degree of maturation. Therefore, demonstrating again the quality and good conservation state of the obtained butter, presenting few decomposition processes, which could have been intensified by heating during its extraction.

For bacaba, 3 extracts were obtained from the different portions of the fruit, in which anthocyanins were determined in order to verify which part had the highest content and, therefore, the highest antioxidant potential. The results of 0.92, 9.75, and 0.33 mgL^{-1} of cyanidin-3-glucoside for the whole fruit, peel, and seed, respectively, showed that the highest anthocyanin content was present in the peel extract. Based on the higher anthocyanin content, which provides a higher antioxidant potential, the extract produced from the bacaba peel was selected to be incorporated into the formulation-type base formulation.

The formulations were then developed from the raw materials, munguba butter, and bacaba peel extract.

Three base formulations were prepared with concentrations of munguba butter equal to 2, 3, and 5%, which were then subjected to preliminary stability. Among them, the formulations containing 3 and 5% of butter showed greater variations in their physicochemical characteristics, especially regarding conductivity, when compared to the formulation with 2% of butter. This can be justified by the great thermal stress imposed by this test, which uses intense and abrupt temperature changes.

Then, after re-preparing them, the formulations were subjected to the accelerated stability process (Tables 2, 3 and 4), in order to observe their stability over a period of 90 days.

A formulation sample containing 3% of munguba butter, kept for 90 days at a temperature of 40 °C, presented formulationing behavior, a possibly reversible but undesired process. In this case, droplets from the internal phase, the munguba butter, rise to the surface and create an oily layer, while water droplets sediment (Aulton, 2005). In addition, some formulations containing 3 and 5% of butter, kept at approximately 5 °C for 90 days, formed white granules.

Table 2. Physicochemical parametersanalyzed in the accelerated stability of the formulations containing 2% of munguba butter (MB)

Sample	Time (days)	Temperature (°C)	pH	Conductivity (μScm^{-1})	Refractive index
Formulation containing 2% of MB	01	25,0 ± 2,0	4,62 ± 0,002	68,6 ± 0,052	1,353 ± 0,001
	30	05,0 ± 2,0	4,68 ± 0,009	113,3 ± 0,036	1,349 ± 0,001
		25,0 ± 2,0	4,53 ± 0,005	76,0 ± 0,011	1,351 ± 0,001
		40,0 ± 2,0	4,40 ± 0,006	134,1 ± 0,157	1,357 ± 0,001
	60	05,0 ± 2,0	4,08 ± 0,091	119,7 ± 0,052	1,346 ± 0,001
		25,0 ± 2,0	4,65 ± 0,032	68,3 ± 0,061	1,348 ± 0,001
		40,0 ± 2,0	4,38 ± 0,009	123,6 ± 0,083	1,349 ± 0,001
	90	05,0 ± 2,0	3,99 ± 0,052	145,7 ± 0,038	1,344 ± 0,001
		25,0 ± 2,0	4,51 ± 0,041	66,7 ± 0,167	1,343 ± 0,001
		40,0 ± 2,0	4,46 ± 0,026	103,8 ± 0,158	1,351 ± 0,001

Note: Results expressed as mean value ± relative standard deviation.

Table 3. Physicochemical parametersanalyzed in the accelerated stability of the formulations containing 3% of munguba butter (MB)

Sample	Time (days)	Temperature (°C)	pH	Conductivity (μScm^{-1})	Refractive index
Formulation containing 3% of MB	01	25,0 ± 2,0	4,61 ± 0,012	127,5 ± 0,044	1,348 ± 0,004
	30	05,0 ± 2,0	4,32 ± 0,109	253,7 ± 0,022	1,359 ± 0,001
		25,0 ± 2,0	4,18 ± 0,030	89,6 ± 0,067	1,354 ± 0,002
		40,0 ± 2,0	3,88 ± 0,005	142,5 ± 0,090	1,359 ± 0,001
	60	05,0 ± 2,0	4,54 ± 0,009	155,9 ± 0,029	1,354 ± 0,003
		25,0 ± 2,0	3,99 ± 0,012	92,5 ± 0,168	1,352 ± 0,002
		40,0 ± 2,0	3,78 ± 0,014	137,5 ± 0,088	1,351 ± 0,003
	90	05,0 ± 2,0	4,29 ± 0,087	172,7 ± 0,009	1,352 ± 0,001
		25,0 ± 2,0	3,84 ± 0,013	66,4 ± 0,089	1,350 ± 0,002
		40,0 ± 2,0	3,68 ± 0,012	129,9 ± 0,327	1,359 ± 0,002

Note: Results expressed as mean value ± relative standard deviation.

Table 4. Physicochemical parameters analyzed in the accelerated stability of the formulations containing 5% of munguba butter (MB)

Sample	Time (days)	Temperature (°C)	pH	Conductivity (μScm^{-1})	Refractive index
Formulation containing 5% of MB	01	25,0 ± 2,0	4,76 ± 0,005	140,6 ± 0,062	1,345 ± 0,002
	30	05,0 ± 2,0	4,71 ± 0,021	320,0 ± 0,019	1,359 ± 0,001
		25,0 ± 2,0	4,22 ± 0,005	117,3 ± 0,079	1,355 ± 0,001
		40,0 ± 2,0	4,07 ± 0,021	142,5 ± 0,090	1,358 ± 0,001
	60	05,0 ± 2,0	4,54 ± 0,008	177,1 ± 0,148	1,353 ± 0,001
		25,0 ± 2,0	4,12 ± 0,006	93,9 ± 0,018	1,352 ± 0,001
		40,0 ± 2,0	3,77 ± 0,043	195,4 ± 0,044	1,351 ± 0,005
	90	05,0 ± 2,0	4,57 ± 0,005	206,6 ± 0,079	1,358 ± 0,002
		25,0 ± 2,0	3,85 ± 0,010	82,9 ± 0,045	1,357 ± 0,001
		40,0 ± 2,0	3,66 ± 0,038	148,5 ± 0,102	1,350 ± 0,006

Note: Results expressed as mean value ± relative standard deviation.

The formulation containing 2% of munguba butter showed no sign of instability during the accelerated stability tests, maintaining its physicochemical and organoleptic properties. Therefore, it was found that the higher butter content in the formulations (3 and 5%) affects the stability of the emulsions, probably due to the predominance of saturated fatty acids, demonstrating that lower contents should be used for better viability, thus agreeing with Raiser et al. (2018).

After establishing the ideal concentration of munguba butter for the base emulsion, the bacaba peel extract was incorporated by preparing the base formulation containing 2% of munguba butter and adding the bacaba peel extract at concentrations of 5, 2, 1, and 0.5%.

The 5% of bacaba peel extract incorporated did not allow an adequate homogenization, and the presence of lumps of the extract in the formulation was evident. For the percentages of 2 and 1%, there was total incorporation of the extract, however, when submitting the formulations to preliminary stability, the coalescence process was noted for both percentages.

The phenomenon of coalescence is caused by internal phase droplet fusions, generating larger ones, which may evolve to a complete phase inversion (Aulton, 2005). This process is indicative

of instability for emulsions and was possibly caused by the thermal stress the formulations went through.

When 0.5% of the bacaba peel extract was incorporated, there was complete incorporation into the base formulation, and there were no instabilities during the preliminary stability test, maintaining its physicochemical and organoleptic characteristics throughout the analysis. Therefore, the formulation containing 2% of munguba butter and 0.5% of bacaba peel extract was prepared again and submitted to the accelerated stability test (Table 5).

Table 5. Physicochemical parameters analyzed in the accelerated stability of the formulation containing 2% of munguba butter plus 0.5% of bacaba extract

Sample	Time (days)	Temperature (°C)	pH	Conductivity (μScm^{-1})	Refractive index
Formulation containing 0.5% of bacaba extract	01	25.0 ± 2.0	4.78 ± 0.004	123.2 ± 0.019	1.353 ± 0.001
	30	05.0 ± 2.0	4.73 ± 0.002	276.6 ± 0.063	1.350 ± 0.001
		25.0 ± 2.0	4.66 ± 0.004	201.7 ± 0.062	1.352 ± 0.001
		40.0 ± 2.0	4.55 ± 0.001	227.3 ± 0.042	1.353 ± 0.001
	60	05.0 ± 2.0	4.46 ± 0.045	124.0 ± 0.075	1.346 ± 0.001
		25.0 ± 2.0	4.65 ± 0.005	140.0 ± 0.017	1.351 ± 0.001
		40.0 ± 2.0	4.60 ± 0.021	178.8 ± 0.039	1.350 ± 0.001
	90	05.0 ± 2.0	4.43 ± 0.007	248.0 ± 0.077	1.346 ± 0.001
		25.0 ± 2.0	4.61 ± 0.002	95.8 ± 0.168	1.349 ± 0.001
		40.0 ± 2.0	4.65 ± 0.049	138.8 ± 0.035	1.352 ± 0.002

Note: Results expressed as mean value ± relative standard deviation.

The pH varied between 4.55 and 4.82 in the evaluated temperatures, which, according to Alves et al. (2016), makes them suitable for topical use, as they have a pH compatible with that of the skin, between 4 and 6.5. In addition, it was observed that at the temperature of 25°C, there was no significant variation in pH ($p < 0.05$), which indicates there were few or minimal decomposition processes in the samples, emphasizing their stability.

For the refractive index, there was also no significant variation, demonstrating the stability of the formulation, since changes in the values of this parameter can indicate phase separation in the emulsion.

However, there was a significant variation ($p > 0.05$) in the electrical conductivity between different temperatures and at the same temperature over the 90 days, which may be related to the loss of water from the external phase of the formulation. Thus, this parameter was useful to classify the emulsion as the O/W type, as the measurements showed values higher than the electrical conductivity of water, which is 1.3 μScm^{-1} (Torres et al., 2019).

After developing a stable emulsion-type formulation, containing munguba butter and bacaba peel extract, the antioxidant activity of the bacaba peel extract and the formulation was determined.

The bacaba peel extract presented an EC50 of 18.54 μgmL^{-1}, a value close to that observed by Prando et al. (2023), who obtained a value of 15.60 mgL^{-1}. For the formulation, an EC50 of 89.66 μgmL^{-1} was obtained, which verifies the formulation's antioxidant action.

When compared to a nanoemulsion-type formulation containing 0.5% of the same extract prepared by Prando et al. (2023), with an EC50 value equal to 8.14 mgL^{-1}, it was observed that the antioxidant potential obtained in the O/W emulsion was lower. This is probably due to the difference in the diameter of the internal droplets between the formulations, which is smaller in nanoemulsions (up to 100 nm), favoring the interaction with the extract and increasing the antioxidant activity of the formulation (Prando et al., 2023).

Another factor that may have influenced this difference is the base formulation, since Prando et al. (2023) used baru oil (*Dipteryx alata* Vogel), rich in unsaturated fatty acids, tocopherols, and phytosterols, with high antioxidant activity, unlike munguba butter, rich in saturated fatty acids (Moraes et al., 2018; Raiser et al., 2020). This fact may have favored synergy between the components of the formulation, resulting in greater antioxidant activity.

The antioxidant activity by the ABTS method showed values of 85.56 mM $Troloxg^{-1}$ for the extract and 55.52 mM $Troloxg^{-1}$ for the formulation containing munguba butter and 0.5% of bacaba extract, corroborating the values obtained by the DPPH method and confirming the antioxidant potential of the extract and the formulation.

When comparing the results of the ABTS test with those obtained for the nanoemulsion developed by Prando et al. (2023), the nanoemulsion system has a higher antioxidant activity (643.69 mM $Troloxg^{-1}$ for the extract and 933.88 mM $Troloxg^{-1}$ for the nanoemulsion).

In this sense, the advantage of developing an O/W emulsion-type formulation obtained by the conventional method is the fact it is an inexpensive and easy-to-prepare method, which generates a moisturizing emulsion with a pleasing texture to the touch. Additionally, the incorporation of the bacaba peel extract added an important antioxidant action to the formulation, and can be used to prevent skin damage caused by oxidative stress, such as cell aging (Corrêa et al., 2020; Prando et al., 2023). In addition to promoting the reduction of environmental

impacts by using a by-product of the bacaba fruit processing, a portion usually discarded.

Conclusion

The developed formulation showed good stability and promising antioxidant activity, having the potential to be used in the prevention and reduction of oxidative processes through the biological activity of the bacaba peel extract. Simultaneously, this formulation becomes a sustainable alternative by promoting the use of normally discarded portions of the fruit, which results in a reduction of environmental impacts.

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