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CARACTERIZAÇÃO E VEÍCULAÇÃO DE EXTRATO DA CASCA DO FRUTO DA BACABA (*Oenocarpus bacaba* Mart.) EM SISTEMAS MICROEMULSIONADOS

BRUNA MENDES CORRÊA

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Sinopse:

Estudou-se a composição da casca do fruto da bacaba (*Oenocarpus bacaba Mart*.) e a partir de sua caracterização foi desenvolvido sistemas microemulsionados contendo o extrato da casca de bacaba.

Palavras-chave:

Oenocarpus bacaba Mart., antioxidante, antocianinas, compostos fenólicos, minerais, microemulsão.

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“Na vida, não existe nada a temer, mas a entender.”

Marie Curie

Resumo

Oenocarpus bacaba Mart. é uma palmeira nativa da Amazônia com alta atividade antioxidante atribuída a seus compostos bioativos. O processamento de seu fruto (bacaba) gera a casca como resíduo, a qual possui compostos bioativos que podem colaborar para sua atividade antioxidante e prevenção de doenças, como câncer de pele, interessantes para incorporar e estabilizar em formulações no intuito de criar veículos de fármacos com benefícios. Portanto o objetivo deste trabalho foi avaliar a composição centesimal e mineral, caracterização físico-química (acidez, sólidos solúveis totais, pH, cor), fenóis totais (método de Folin-Ciocalteu), antocianinas e atividade antioxidante pelo método DPPH (2,2 difenil-1-picril hidrazil) nas cascas de bacaba, bem como incorporar seu extrato em microemulsões para avaliar sua estabilidade e potencial antioxidante. Para a composição centesimal, foi encontrado os valores de 4,87, 1,42, 29,13, 1,08 e 63,32 g 100 g^{-1} para umidade, cinzas, lipídeos, proteínas e carboidratos totais, respectivamente. Para a composição mineral, foi encontrado os teores de 582,97, 79,00, 51,79, 0,625, 37,02, 2,37 and 77,12 mg 100g^{-1} para potássio, sódio, magnésio, cobre, cálcio, manganês, e fósforo, respectivamente. A caracterização físico-química mostrou valor de pH de 5,66, acidez titulável de 0,306% de ácido cítrico, sólidos solúveis totais de 9,75° brix, e as coordenadas L* a* b* de 19,03, 8,07 e 9,25, respectivamente. Conteúdo de fenóis foi de 42, 07 mg EAG g^{-1}. A potencial antioxidante em IC_{50} foi de 1,07 mg mL^{-1} e antocianinas foi 37.31 mg 100 g $^{-1}$. Para as microemulsões foram utilizadas quantidades pré-estabelecidas de água destilada, triglicerídeo caprico/caprílico, Span 80® e Tween 80® sendo adicionados 5 % do extrato a 0.1 g mL^{-1}. Das 72 formulações testadas, oito formaram microemulsões sendo submetidas à centrifugação, caracterização físico-química (pH, condutividade elétrica e índice de refração) e estudos de estabilidade, na qual quatro mantiveram sua estabilidade física. As amostras apresentaram perfil newtoniano e viscosidade linear. O tamanho das gotículas não passou de 325 nm de diâmetro hidrodinâmico. Seu potencial antioxidante se demonstrou satisfatória. Os resultados mostraram que a casca da bacaba é uma alternativa de fonte de nutrientes e compostos bioativos, sugerindo seu uso em alimentos bem como em indústrias cosméticas, especialmente por sua atividade antioxidante e composição mineral. Em relação às microemulsões, os sistemas apresentaram estabilidade e potencial antioxidante, podendo ser utilizados para veiculação de fármacos.

Palavras Chave: *Oenocarpus bacaba* Mart., antioxidante, antocianinas, compostos bioativos, minerais, microemulsão

Abstract

Oenocarpus bacaba Mart. is a palm tree native from Amazon forest with notable antioxidant activity attributed to its bioactive compounds. Its fruit (bacaba) processing dismisses the peel, which has bioactive compounds that can collaborate to its antioxidant activity and prevent diseases, being an interesting option to incorporate in formulations in order to create drug carriers with advantages. Thus, the aim of this work was assess the centesimal and mineral composition, physicochemical characterization (acidity, total soluble solids, pH, and color), total phenolics (Folin-Ciocalteu method), anthocyanins and antioxidant activity by DPPH (2,2 difenil-1-picril hidrazil) radical scavenging method in bacaba peels. As well as incorporate its extract in microemulsions to assess its stability and antioxidant potential. For the centesimal composition, the values found were 4.87, 1.42, 29.13, 1.08 and 63.32 g 100 g^{-1} contents for moisture, ashes, lipids, proteins, and total carbohydrates, respectively. For the mineral composition, the values found were 582.97, 79.00, 51.79, 0.625, 37.02, 2.37 and 77.12 mg 100g^{-1} contents for potassium, sodium, magnesium, copper, calcium, manganese, and phosphorus, respectively. The physicochemical characterization showed values of pH of 5,66, of titratable acidity of 0,306% of citric acid, total soluble solids of 9.75 ° Brix, and coordinates L* a* e b* of 19.03, 8.07 and 9.25, respectively. Phenolic contents were quantified as 42.07 mg EAG g^{-1}. The IC_{50} for the antioxidant potential was 1.07 mg mL^{-1} and anthocyanins 37.31 mg 100 g^{-1}. For the microemulsion formulation, it was used pre-established quantities of distilled water, capric/caprylic triglycerides, Span 80® and Tween 80®, with 5 % of extract at 0.1 g mL^{-1} added. From the 72 tested formulations, eight produced microemulsions and were subjected to centrifugation, physicochemical characterization (pH, electrical conductivity and refractive index) and stability studies, with four sustaining their physical stability after the tests. All approved samples showed Newtonian profile and linear viscosity. The mean droplets size of the formulations was 308 nm. The antioxidant potential was effective. Results showed that bacaba peels are an alternate source of nutrients and bioactive compounds, suggesting their potential use in food and cosmetic industries, especially due to their antioxidant activity and mineral composition. Regarding the microemulsions, the systems presented stability and antioxidant activity, showing suitable for use as carriers for drugs, cosmetics, among others.

Key words: *Oenocarpus bacaba* Mart., antioxidant, anthocyanins, bioactive compounds, minerals, microemulsion.

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INTRODUÇÃO GERAL

O Brasil possui a maior floresta equatorial e tropical úmida da terra e não pode dispensar sua vocação para os produtos naturais, onde a região amazônica é estimada como o maior depósito de recursos genéticos do mundo, dentre os quais estão diversas espécies frutíferas e palmeiras nativas que são fundamentais na dieta e economia da população ribeirinha da região (PINTO et al., 2002; IVANI, 2010). Atualmente a indústria cosmética tem se destacado na inserção de produtos oriundos de frutos como o açaí, andiroba, buriti, castanha-do-brasil e cupuaçu por possuírem atributos associados aos seus extratos e óleos vegetais, como a potencial atividade antioxidante que buscam substituir produtos ativos sintéticos por naturais (CANÇADO & BORÉM, 2001; FERRO et al., 2006; IVANI, 2010; INFANTE, 2013).

Dentre as espécies de palmeiras e frutos disponíveis na Amazônia, a bacaba (*Oenocarpus bacaba* MART.) destaca-se como uma palmeira com grande potencial econômico, alimentar e ecológico (QUEIROZ e BIANCO, 2009). Quando maduros, seus frutos apresentam coloração roxa e formato elíptico globoso (SILVA, 2006; FINCO et al., 2012). Além do consumo de sua polpa, os frutos também são utilizados como matéria-prima para a produção de sorvetes, geléias, picolés e licores. Do endocarpo dos frutos, extrai-se um óleo com características semelhantes às do azeite de oliva (BALICK, 1986). Na medicina popular, o seu xarope é utilizado para tosse (MENDONÇA e ARAUJO, 1999). O palmito extraído da parte mais jovem de sua estirpe é muito apreciado na culinária, e a bacaba também é utilizada como planta ornamental (QUEIROZ e BIANCO, 2009). Assim, investiga-se a importância desta palmeira em diversas áreas (SILVA et al., 2009) com destaque a área alimentícia, cosmética e farmacêutica.

Abadio Finco *et al.* (2012) demonstrou em seu estudo que o fruto da bacaba apresenta propriedades antioxidantes, atribuída a seus metabólitos secundários, como os compostos fenólicos. Estes compostos estão amplamente distribuídos no reino vegetal (MALACRIDA e MOTTA, 2005) sendo encontrados em maior quantidade os flavonóides, ácidos fenólicos e taninos (AMY KING e YOUNG, 1999; CIPRIANO, 2011). Entre os flavonóides mais comuns destacam-se as antocianinas, responsáveis pela maioria das cores

em frutos, flores, folhas, raízes de plantas e caules (TEIXEIRA et al., 2008). A coloração deste pigmento oscila entre vermelho, roxo, laranja, rosa e azul, conforme as condições intrínsecas dos vegetais, como o pH (BROUILLARD, 1983; TEIXEIRA et al., 2008).

Estudos (GONDIM et al., 2005; CÓRDOVA et al., 2005; RIBEIRO et al., 2008) comprovam que cascas de frutos, muitas vezes, possuem mais nutrientes e compostos bioativos que suas polpas, como por exemplo a presença de compostos fenólicos, que são compostos bioativos responsáveis pela atividade antioxidante. Assim, observa-se a necessidade de se realizar pesquisas em relação a composição das cascas para que sejam aproveitadas, ao invés de descartadas, agregando valor econômico as mesmas (ROTTA et al., 2016).

Neste contexto, o fato da casca do fruto da bacaba apresentar coloração, que varia do vermelho escuro ao violeta/azul indica a presença de antocianinas (SIMÕES, 2007), que pode colaborar para propriedades antioxidantes em formulações para pele envelhecida ou como preventivo do envelhecimento e do câncer de pele (BIAVATTI et al., 2007; ILHA et al., 2008), além de apresentar benefícios, como reduzir o risco de desenvolver algumas doenças crônicas não transmissíveis, quando ingeridas (SILVA, et al., 2004; SILVA et al., 2010). Dentre os tipos de formulações mais utilizadas estão as emulsões e atualmente as microemulsões e nanoemulsões que possuem vantagens em relação às formulações tradicionais, uma vez que são alternativas para a liberação sustentada de ativos, a solubilização de ativos lipofílicos, aumento da estabilidade física e química de moléculas lábeis, bem como a minimização de efeitos colaterais (OLIVEIRA et al., 2004; TIUMAN, 2011; LAWRENCE e REES, 2012; BONIFACIO et al., 2013)

Desta forma, o presente trabalho visou analisar a composição da casca da bacaba, obter seu extrato, e utilizar o extrato na obtenção de sistemas microemulsionados, de modo a aproveitar os recursos naturais sustentáveis da região, o que possibilita a geração de inovações nas áreas farmacêuticas e cosméticas.

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CAPÍTULO I

COMPOSIÇÃO CENTESIMAL, MINERAL E ATIVIDADE ANTIOXIDANTE DA CASCA DO FRUTO DE BACABA

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CENTESIMAL AND MINERAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE BACABA FRUIT PEEL

Abstract

Oenocarpus bacaba Mart. is a palm tree native from Amazon with highlighted antioxidant activities. Its fruit (bacaba) processing dismisses the peel, which has nutrients that can collaborate for its antioxidant activity and preventing diseases. Thus, this study assessed the centesimal and mineral composition, physicochemical characterization (acidity, total soluble solids, pH, and color), total phenolics (Folin-Ciocalteu method), anthocyanins and antioxidant activity by DPPH (2,2 difenil-1-picril hidrazil) method in bacaba peels. For the centesimal composition, it was found 4.87, 1.42, 29.13, 1.08 and 63.32 g 100 g^{-1} contents for moisture, ashes, lipids, proteins, and total carbohydrates, respectively. For the mineral composition, it was found 582.97, 79.00, 51.79, 0.625, 37.02, 2.37 and 77.12 mg $100g^{-1}$ contents for potassium, sodium, magnesium, copper, calcium, manganese, and phosphorus, respectively. The physicochemical characterization showed pH 5.66, titratable acidity 0.306% of citric acid, total soluble solids 9,75, and coordinates L* a* e b* of 19.03, 8.07 and 9.25, respectively. Phenolic contents were 42.07 mg EAG g^{-1}. The antioxidant potential IC_{50} was 1.07 mg mL^{-1} and anthocyanins 37.31 mg 100 g^{-1}. Results show that bacaba peels are an alternate source of nutrients suggesting their use in food as well cosmetic industries, especially for their antioxidant activity and mineral composition.

Key words: Anthocyanins, nutritional composition, DPPH, *Oenocarpus bacaba* Mart., total phenolic.

Resumo

Oenocarpus bacaba MART. (bacaba) é uma palmeira nativa da Amazônia que se destaca por suas propriedades antioxidantes. O processamento de seu fruto gera a casca como produto de descarte, que possui nutrientes que podem colaborar na ação antioxidante e na prevenção de doenças. Assim, o presente trabalho avaliou a composição centesimal e mineral, caracterização físico-química (acidez, sólidos solúveis totais, pH e cor), fenóis totais (método de Folin-Ciocalteu), antocianinas e potencial antioxidante pelo método do DPPH (2,2 difenil-1-picril hidrazil) em cascas de bacaba. Para a composição centesimal verificou-se teores (%) de 4.87, 1.42, 29.13, 1.08 e 63.32 para umidade, cinzas, lipídeos, proteínas e carboidratos totais, respectivamente. Para a composição mineral foi encontrado teores ($mg100g^{-1)}$ de 582.97, 79.00, 51.79, 0.625, 37.02, 2.37 e 77.12 para potássio, sódio, magnésio, cobre, cálcio, manganês e fósforo, respectivamente. A caracterização físico-química apresentou valores de pH de 5.66, acidez titulável de 0.306% de ácido cítrico, sólido solúveis totais de 9.75 e cor nas coordenadas L* a* e b* com valores de 19.03, 8.07 e 9.25 . Para o teor de fenóis foi encontrado 42.07 mg $EAG.g^{-1}$. O EC_{50} para a potencial antioxidante foi de 1.07 $mg.mL^{-1}$ e o teor de antocianinas foi de 37.31 $mg.100g^{-1}$. Os resultados mostram que as cascas de bacaba são uma fonte alternativa de nutrientes sugerindo seu aproveitamento na indústria alimentícia e cosmética, principalmente pela sua potencial atividade antioxidante e composição em minerais.

Palavras-chave: *Oenocarpus bacaba* Mart., antocianinas, fenóis totais, DPPH, minerais.

INTRODUCTION

Brazil is the third biggest country in fruit production, with great potential of expansion due to its large amount of native and exotic fruits yet economically low appreciated (PARANÁ, 2015). Bacabeira (*Oenocarpus bacaba* MART.) is a palm tree native from Amazon biome with great antioxidant properties. It produces a purple fruit (bacaba), very similar to açaí (*Euterpe oleracea* Mart.) (FINCO et al., 2012; PUERARI et al., 2015).

The economically potential use of bacaba is based on its fruit pulp as a beverage, heart of palm extraction and its edible oil, as well as the use of its leaves in handicraft, fiber and tile production, and its stem in construction industry (EMBRAPA, 2005; GUIMARÃES, 2013). Despite its local importance, low attention has been addressed to the functional and nutritional potential of bacaba (GUIMARÃES, 2013).

The fruit processing of bacaba for its pulp and edible oil extraction from the nut generates the peel as a rejected product or by-product. These tropical fruit by-products have high contents of ingredients that can be extracted and used in nutraceuticals (GORINSTEIN et al., 2011).

The development of nutraceuticals from these by-products contributes to the improvement of tropical cultures processing economy, due to the great social seek for products that offer improvements in quality of life, stimulating industries on research for new technologies that aim the reduction of economical losses and environmental impact of industry, as well to promote consumer health (MELO et al., 2011). Also, studies have proven that fruit peels can have more nutrients and bioactive compounds than their pulps (CÓRDOVA et al., 2005; GONDIM et al., 2005), as an example the phenolic compounds, a

bioactive compound often found in greater amounts in the peels than the pulps (KALT, 2000).

The antioxidant activity of bacaba is due to its secondary metabolites in your fruits and leaves (FINCO et al., 2012; LEBA et al., 2016). The purple color of its fruit peel indicates the presence of anthocyanin, a flavonoid that belongs to the phenolic class, and responsible for the major colors in flowers, fruits, leaves, stems, and roots of plants, differing among red, purple, orange, pink, and blue, depending on the intrinsic conditions of the vegetables, like pH (TEIXEIRA et al., 2008).

In this context, this work aimed to analyze the centesimal, mineral, and physicochemical composition of bacaba peel for further natural products development.

EXPERIMENTAL

Samples

The fruits of bacaba were picked in the rural area of Sinop, Mato Grosso, then taken to the Quality Control Lab at Federal University of Mato Grosso, *Campus* of Sinop. They were washed, sanitized and rinsed. Afterwards the cleaning process, fruits were submerged in ultra-pure water at 40 °C for 40 minutes in order to manually remove the peels. Subsequently, the peels were kept in oven with forced circulation of air at 40 °C for 24 hours to remove the water, then milled and stocked in freezer at -20 °C. All trials were in triplicate.

Determination of the Peel Output

For the determination of the peel output it was weighed fifty (50) fruits in analytical balance (Shimadzu®), manually peeled and after drying output was calculated.

Centesimal Composition

The moisture content was determined by drying in oven (Lucadema®) at 105 °C (IAL, 2008), the ash mineral composition by incineration in muffle (Autonics®) at 550 °C (IAL, 2008), the lipid fraction by Sohxlet method using ether as solvent (IAL, 2008). Total nitrogen (Nt) was determined by Kjeldahl method, and the crude protein by multiplying the Nt content by the conversion factor 6.25 (AOAC, 2002).The total carbohydrate content was estimated by means of the difference.

Physicochemical Characterization

The analyzed parameters were:

Total titratable acidity (ATT): determined by titration with NaOH (0.1 mol L^{-1}) using phenolphthalein as indicator and the result was expressed in percentage (%) of citric acid (m g^{-1}) (IAL, 2008).

Total soluble solids (SST): determined by straight reading of the peel (before drying) in Abbé refractometer (Polax®), previously calibrated with distilled water and the result was expressed in ^{0}Brix (IAL, 2008).

SST/ATT Ratio: it was calculated by dividing the total soluble solids by the total titratable acidity (SST/ATT) (IAL, 2008).

pH: 1 g of peel was macerated in 10 mL of water, filtered and measured in pHmeter (Tecnopon®) (IAL, 2008).

Color: it was directly analyzed the color spaces L* (brightness), a* (intensity of red versus green), and b* (intensity of yellow versus blue) in colorimeter (Kônica Minolta® - c220) at illuminant D65. The tone parameter (h*) and saturation were calculated from a*

and b* parameters by the equations: $h^*=\arctan(b^*/a^*)$, and $c^*=\sqrt{a^{*2}+b^{*2}}$ (CIPRIANO, 2011).

Determination of Total Phenolics and Antioxidant Potential by DPPH (2,2-difenil-1-picrilhidrazil) Method

For the determination of phenolics and DPPH, it was prepared a solution from the peel, 25 mL of ethanol 80% were added for each 1 g of peel. The mixture was subjected to ultrasound for 30 minutes, centrifuged for 15 minutes at 5000 rpm, and filtered. An aliquot of the filtrate (5 mL) was diluted to 50 mL in ethanol 80%, obtaining this way the extract solution for the trials.

The quantification of total phenolics (FT) was carried according to Waterhouse (2002) methodology, with modifications. Aliquots of the extract solution were diluted in ethanol 80% and added 2.5 mL of the Folin-Ciocalteu (Vetec) solution, after 5 minutes standing it was added 2.0 mL of sodium carbonate solution 4% (mv^{-1}), standing still and protected from sun light for 1 hour. Thereafter, it was conducted the reading in spectrophotometer (PG Instruments Ltd®, T80 UV/VIS), at 750 nm wavelength.

The FT content was determined by interpolation of the samples absorbance against the calibration curve previously set with Gallic acid (20 a 180 µg mL^{-1}) as standard. The calibration curve equation was $y = 0.0005x + 0.0025$, with x as the Gallic acid concentration, and y as the absorbance at 750 nm, correlation coefficient $r = 0.997$. Results were compared to the calibration curve and expressed as mg of EAG (equivalent of Gallic acid) for g of extract.

For the antioxidant potential assay it was used the free radical DPPH (1.1-difenil-2-picrilhidrazil), according to Infante (2013) methodology, with modifications. Aliquots from the extract solution were diluted in ethanol 80%, and to 0.5 mL of every dilution it was added 3.0 mL of ethanol (99.5%) and 0.3 mL of the methanolic solution of DPPH at the concentration of 0,5 $mmolL^{-1}$. After 40 minutes standing still and protected against sun light, the absorbance of the samples were read in spectrophotometer (PG Instruments®, T80 UV/VIS) at 517 nm. According to the results it was determined the antioxidant ability of each concentration of the peel extract solution of bacaba by the equation: % DPPH inhibition = $[(A_0 - A_1) / A_0 \times 100]$, with A_0 as standard absorbance, and A_1 as sample absorbance. For the determination of IC_{50} (concentration of bacaba peel extract needed to reduce 50% of the DPPH radical) data were subjected to linear regression and the line equation was obtained (BRAND-WILLIAMS, 1994).

Anthocyanins

The extract was prepared with 1 g of bacaba peel added to 25 mL a mixture of ethanol 70% and hydrochloric acid 0,1 $molL^{-1}$ in a ratio of 3:1, this solution was submitted to ultrasound for 30 minutes. Afterwards, it was centrifuged for 15 minutes at 5000 rpm. To 5 mL of the supernatant was added a mixture of ethanol 70% and hydrochloric acid 0.1 $molL^{-1}$ (3:1) up to 50 mL.

Total anthocyanins (AT) quantification was performed according to Lee and Francis (1972) methodology, with modifications. Aliquots of the extract solution were diluted to 10.0 mL in ethanol 70% and hydrochloric acid 0.1 $molL^{-1}$ (3:1), therefore read in spectrophotometer at 535 nm. Results were expressed in milligrams of cyn-3-glu (equivalent in cyaniding-3-glycoside) per 100 g of peel and calculated by the equation:

A=a.b.c, with A as absorbance, a as coefficient of absorptivity (98.2 L cm^{-1} g^{-1}), b as light path (1 cm), and c as concentration (mg 100 g^{-1}).

Determination of Minerals

The determination of bacaba peel mineral profile was conducted after calcination of the sample in muffle (Autonics®) at 550 ^{0}C for 4 hours. Ashes were processed according to every metal determination (IAL, 2008; MAPA, 2014). Magnesium, manganese, calcium, and copper were determined by Atomic Absorption spectrophotometry (Varian®, AA140), using a gas mixture of compressed air under a pressure of 50 psi, and acetylene at 11 psi (75 KPa – 0,76 Kgf/cm²). Phosphorous content was determined by spectrophotometry in the visible region at 420 nm (Biospectro®, SP-220). On the other hand, sodium and potassium contents were determined by flame photometry (Micronal®, B462), using a mixture of air gases and butane, under a pressure of 0.8 kg cm^{-1}.

RESULTS AND DISCUSSION

Bacaba fruits used in this study had rounded edges, approximately diameter of 1.5 cm, and dark purple peels for the ripe fruits. Their mesocarp had about 1.5 mm of thickness, being white and oily, and the nuts were involved in a thin and fibrous endocarp, in agreement with data from Embrapa (2005). The fruits average weight was 1.914 g, with the peel corresponding to 14.25% of the fruit. In addition to the color of the peel (purple), which indicates the presence of anthocyanins, it was noticed an oily appearance that may become interesting to pharmaceutical, cosmetic, and food industries.

Centesimal composition of the peels (Table 1) showed high content of lipids, 29.13 g $100g^{-1}$, an outstanding value if compared with other fruit peels. Gondim et al. (2005)

obtained the highest lipid content in avocado peel, 11.04%, while fruit peels of pineapple, banana, papaya, passion fruit, melon, and tangerine did not exceed 1%. Other authors (OLIVEIRA et al., 2002; CÓRDOVA et al., 2005; MARQUES et al., 2010) researching fruit peels did not find lipid values higher than 1% as well. Thus, it was observed that the oil extracted from bacaba is not only concentrated in its nut and pulp but also in its peel. Due to the great lipid content obtained, it was performed a qualitative analyzes of the oil extracted from the peel by chromatography – mass spectrometry (Shimadzu® CG-MS QP2010), in which it was identified the presence of oleic and palmitic fatty acids, same ones found in the fruit, in agreement with Santos et al. (2017) who obtained 61.65% of oleic acid and 28.43% of palmitic acid in the fruit.

The moisture content was 4.87% performed in product after dried, since it was necessary to submerge the fruit in water at 40 °C to remove the fruit peel. Protein and ashes content were similar to other fruits as found by Gondim et al. (2005) and Marques et al. (2010).

Table 1. Results of the centesimal composition of bacaba fruit peel after dried

Parameters	**Bacaba fruit peel (g $100g^{-1}$)**
Moisture	4.87 ± 0.06
Ashes	1.42 ± 0.03
Lipids	29.13 ± 0.49
Proteins	1.08 ± 0.01
Total carbohydrates	63.32

*Average ± standard deviation of the triplicates.

Results of the physicochemical characterization of bacaba fruit peel are found in Table 2.

Table 2. Results of the physicochemical characterization of bacaba fruit peel

Parameters	**Bacaba fruit peel**
pH	5.66 ± 0.12
AAT (% of citric acid)	0.306 ± 0.00
SST (°Brix)	9.75 ± 0.15
Color (illuminant D65) L*	19.03 ± 0.07
a*	8.07 ± 0.01
b*	9.25 ± 0.39
c*	12.28
h*	48.89
SST/ATT Ratio	31.86

* Average ± standard deviation of the triplicates.

For the physicochemical characteristics there is no current legislation for the identity and marketing standards of bacaba fruit or its pulp, so it was followed the parameters set for açaí pulp (*Euterpe oleracea* Mart.), a similar fruit to bacaba and widely marketed. The Normative Instruction n° 1 issued by Ministry of Agriculture and Supplies (2002) determines that the total titratable acidity must be between 0.27% and 0.45% of citric acid and pH 4.0 to 6.2, the acidity found in bacaba peel was 0.306% of citric acid and pH 5.66, meeting the legislation standards for the açaí pulp. Other studies demonstrate that the acidity of açaí pulp may vary from 0.19% to 0.94% of citric acid, and its pH between 3.55 and 5.20, emphasizing the similarity between these two fruits (ALEXNDRE et al., 2004; NASCIMENTO et al., 2008; CIPRIANO, 2011).

The total soluble solids (SST) content is used to estimate the sugar and organic acids content of the fruit, and bacaba peel showed a SST content of 0.975 °Brix, lower than

its pulp, in which Neves et al. (2015) found 1.53 °Brix, and Canuto et al. (2010) found 2.0 °Brix. The SST/ATT ratio is considered an assessment factor of fruit taste, and in these peels it was 3.18. This value is lower than the one obtained from Brazilian grape (*Myrciaria jaboticaba* (Vell.) Berg.) (8.25), and açaí pulp (17.52) (CIPRIANO, 2011). Czelusniak et al. (2003) consider a threshold value of 20 for the SST/AAT ratio, meaning that lower values represent high acidity content, with greater relevance for industries (CIPRIANO, 2011).

In regard to the color, bacaba peel showed values of 19.03 for brightness (L*), 12.28 for color saturation (c*), and 48.89 ° for tone parameter (h*), showing that the sample had low brightness and for that it is considered to be dark (ROCHA et al., 2017); with more saturated color similar to Brazilian grape peel studied by Cipriano (2011), who found values of c* around 11.32 and tone between red (0 °) and yellow (90 °). The variation to the yellow tone can be due to residual white pulp on the peel, agreeing with Canuto et al. (2010), who found a h* of 56.0 ° for bacaba pulp.

The FT content in bacaba peel was 42.07 mg EAG g^{-1}, most likely related to the presence of anthocyanins. Finco et al. (2012) and Canuto et al. (2010) found values of 17.59 mg EAG g^{-1} and 0.3 $mmolL^{-1}$ of Gallic acid in the fruit and pulp of bacaba, respectively. Also, when compared with other fruits, the value obtained is higher, as an example, Cipriano (2011) found in Brazilian grape 2.16 mg EAG g^{-1}, and in açaí pulp 3.10 mg EAG g^{-1}. On the other hand, Infante (2013) found in peels of bacupari-mirim (*Garcinia brasiliensis* Mart.) and araçá (*Eugenia leitonii* Lerg.), 36.35 mg EAG g^{-1} and 36.08 mg EAG g^{-1}, respectively. The FT content may be related to the antioxidant activity of fruits, which is of great relevance for industry due to the improvement of health through the

prevention of diseases and delay of the ageing process, what turns bacaba into a fruit of great interest for the production of nutraceuticals, enriched foods, and cosmetics.

The DPPH test showed that it is necessary 1.07 mg mL^{-1} of bacaba peel extract to reduce DPPH in 50 %, higher value, with lower antioxidant activity than the one found by Finco et al. (2012), who obtained from the fruit a IC_{50} of 0.70 mg mL^{-1}. This variation in antioxidant activity, most of time, is due to the different experimental conditions. In addition, results showed that the peel has antioxidant activity, a promising feature for the development of products.

The high FT and anthocyanin contents, as well the antioxidant activity of bacaba peel agree with Finco et al. (2012), who found similar contents for FT and anthocyanins in bacaba fruit, and associated these high contents with its great antioxidant activity. In regard to the anthocyanins, previous studies showed that the fruits *Oenocarpus bataua* Mart. and *Oenocarpus bacaba* Mart. have high content of anthocyanins (FINCO et al., 2012; REZAIRE et al., 2014), collaborating for their antioxidant activity. Tauchen et al. (2016) correlated this antioxidant potential of anthocyanins from *Oenocarpus bataua* in the antiproliferative activity of cancer cells.

The peel of bacaba fruit was dark purple, indicating the presence of anthocyanins. The value of total anthocyanins (equivalent in cyanidin-3-glycoside) found in the peels, 37.31 mg of cyn-3-glu 100 g^{-1}, was similar to Finco et al. (2012), who obtained 34.69 mg of cyn-3-glu 100 g^{-1} from the fruit. In comparison with açaí pulp, Cipriano (2011) found 74.30 mg $100g^{-1}$ of total anthocyanins, greater than the value found in bacaba peel, although the fruit segment as well as the extracting technique were different.

Anthocyanins are found in several fruits, such as cherry, açaí, plum, strawberry, apple, and grape, however not yet widely marketed due to its tough extraction and low

stability. In industry there is an interest about its use as dyeing agent, since the synthetic ones may harm consumer's health somehow, therefore stands the importance of verifying the composition and antioxidant activity of bacaba peel for its further use (CIPRIANO, 2011).

The minerals content: sodium, potassium, calcium, magnesium, copper, manganese, and phosphorous of the bacaba fruit peel are found in Table 3.

Table 3. Results of the minerals content of bacaba fruit peel

Minerals	**Bacaba fruit peel (mg $100g^{-1}$)**	**DRI (mg per day)**	**% DV (100 g portion)**	**% DV (15 g portion)**
Sodium	79.00 ± 0.39	2400.0	3.29	0.49
Potassium	582.97 ± 2.28	2500.0	23.32	3.50
Calcium	37.02 ± 1.38	1000.0	3.70	0.55
Magnesium	51.79 ± 0.04	260.0	19.91	2.99
Copper	0.625 ± 0.03	0.9	69.44	10.42
Manganese	2.37 ± 0.04	2.3	103.04	15.47
Phosphorous	77.12 ± 0.11	700.0	11.01	1.65

* Average ± standard deviation of the triplicates. Daily Recommended Index (DRI) and Daily Value (DV).

Regarding the mineral composition of bacaba fruit peel (Table 3), we found that potassium is the most abundant, being important to the neuromuscular activity regulation, such as weakness, fatigue, and cramps, followed by sodium, important in controlling absorption and transportation of nutrients such as chlorine, amino acids, water, and glucose. Besides, phosphorous, a mineral responsible for the maintenance of pH and nucleotide

synthesis, presented a content close to sodium, followed by magnesium, a mineral involved in bone formation and with enzymes in the metabolism of cholesterol, amino acid, and carbohydrate. Beyond these, it was found calcium, essential mineral for the blood coagulation, neural transmission, teeth and bones formation, and muscular contraction, crucial to the organism (DOUGLAS, 2001). In comparison to açaí, Menezes et al. (2008) found in its lyophilized pulp higher concentrations of potassium, calcium, magnesium, manganese, and copper. Moreover, sodium and phosphorous were the minerals with higher concentrations in bacaba peel. Regarding other fruits, Marques et al. (2010) found lower contents of sodium, potassium, phosphorous, magnesium, and manganese in mango peel, while Gondim et al. (2005) found lower contents of copper, sodium, potassium, and magnesium in avocado, pineapple, banana, papaya, passion fruit, and melon peel than the contents found in bacaba peel.

Considering the daily intake recommendation for adults (BRASIL, 2005), the different mineral elements present in a 15 g (1 tablespoon) portion of bacaba fruit shell (Table 3) serve from 0.49 to 15.47% of the daily value.

The Brazilian legislation (BRASIL, 1998) determines that food to be considered sources vitamins and mineral needs at least a daily value (DV) of 15 % in relation at daily recommended index (DRI) of reference per 100 g of solid food, and to be considered rich food in minerals and vitamins, the food needs have a DV of 30 % in relation of DRI of reference per 100 g of solid food. Thus according with Table 3, the peel of bacaba can be considered a potassium and magnesium source, and rich in copper and manganese (GRANATO et al., 2009).

Regarding potentially toxic micronutrients, the copper content (6.25 mg kg-1) did not exceed the permitted limit (10 mg kg-1 for fresh or industrialized fruits) by the current

legislation (BRASIL, 1998), which establishes maximum limits of tolerance for inorganic contaminants in food.

Thereby, bacaba fruit peel showed to be a valuable source of oil, anthocyanins, and minerals, able to be repurposed for the production of dye agents, nutraceuticals, and cosmetics.

CONCLUSION

Bacaba fruit peel showed to be a source of nutrients and have a good antioxidant potential, claiming the importance of its repurpose instead of disposed as residue. Besides, taking sustainable advantage of these natural sources might contribute to the community development, not to mention the creation of new products.

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CAPÍTULO II

DEVELOPMENT , CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF BACABA SHELL EXTRACT MICRO EMULSION SYSTEMS

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DEVELOPMENT, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF BACABA SHELL EXTRACT MICRO EMULSION SYSTEMS

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Abstract

Bacabeira (*Oenocarpus bacaba* MART.), a palm tree native from Amazon, has flavonoids found in its fruit shell with antioxidant activity, interesting to incorporate and stabilize in formulations in order to create drug vehicles with advantages. Thus, the aim of this work was to incorporate bacaba shell extract in microemulsions to assess its stability and antioxidant potential. All formulations contained pre-established quantities of distilled water, capric/caprylic triglycerides, Span 80® and Tween 80®, with 5 % of extract at 0.1 g mL^{-1} added. From the 72 tested formulations, eight produced micro emulsions and were subjected to centrifugation, physicochemical characterization (pH, electrical conductivity and refractive index) and stability studies, with four sustaining their physical stability. All samples showed Newtonian profile and linear viscosity. The droplets size did not exceed 325 nm of hydrodynamic diameter. The antioxidant potential was satisfactory. Therefore, the systems presented stability and antioxidant activity, suitable for their use as drug vehicles.

Key Words: Antioxidant Activity, Bacaba, Stability Study, Micro emulsion.

Resumo

A bacabeira (*Oenocarpus bacaba* MART.), palmeira nativa da Amazônia, possui flavonóides encontrados na casca de seu fruto com propriedades antioxidantes, interessantes para incorporar e estabilizar em formulações no intuito de criar veículos de fármacos com benefícios. Portanto, objetivo deste trabalho foi incorporar extrato de casca de bacaba em microemulsões para avaliar sua estabilidade e potencial antioxidante. As formulações contêm quantidades pré-estabelecidas de água destilada, triglicerídeo caprico/caprílico, Span 80® e Tween 80® sendo adicionados 5 % do extrato a 0.1 g mL^{-1}. Das 72 formulações testadas, oito formaram microemulsões sendo submetidas à centrifugação, caracterização físico-química (pH, condutividade elétrica e índice de refração) e estudos de estabilidade, na qual quatro mantiveram sua estabilidade física. As amostras apresentaram perfil newtoniano e viscosidade linear. O tamanho das gotículas não passou de 325 nm de diâmetro hidrodinâmico. Seu potencial antioxidante se demonstrou satisfatória. Portanto, os sistemas apresentaram estabilidade e potencial antioxidante, podendo ser utilizados para veiculação de fármacos.

Palavras Chave: Atividade Antioxidante, Bacaba, Estudo de Estabilidade, Microemulsão.

INTRODUCTION

Brazil has a notable biodiversity, with new species discovered every year [1]. The Amazon region is highlighted due its important scenario for bio prospecting, once the last decade was marked by the consciousness of the importance of natural sources and their use, and it was noticed an increase in the number of native plants in that region used as food, in the traditional medicine, in search for new drugs, in the cosmetic industry, to obtain biofuel, and others [2,3]. In this context, stand out the fruits, although accessible for the regional consumption, their commercial use is least or absent. Nutritional and health attributes of many of these fruits are unexplored and their scientific research is yet insufficient [4].

In the fruit abundance around Amazon region there is bacabeira (*Oenocarpus bacaba* Mart.) that produces an eatable purple fruit, called bacaba, which is locally consumed as natural juice or processed as beverages, jams and ice creams [5]. It was attributed to its fruit shell antioxidant activity due to the presence of phenolic compounds, such as anthocyanin, a flavonoid that acts as chromophore agent in leaves, flowers, fruits and stems, varying among purple, red, pink, orange and blue, what indicates to be responsible for its purple color [5-7].

Antioxidant compounds extracted from plants, as anthocyanins, are of great interest to protect the skin from damages caused by excessive solar radiation, such as premature aging and skin cancer, besides decreasing the risk of developing some non-communicable chronic diseases, when ingested [8-11]. Thereby, the nutritional, pharmaceutical and cosmetic potential regarding this fruit become interesting due to its popular use.

The seek for new vehicles, in a sense of establishing alternatives for the delivery of active ingredients, solubilization of lipophilic active ingredients, as well the increase of physical and chemical stability of labile molecules and minimizing side effects have been increasing in order to overcome difficulties of administration of bioactive molecules and to create a vehicle for pharmaceutical formulations [12,13]. In this context, studies [11,14,15] have been emerging in an attempt to carry plant extracts in micro emulsions to incorporate and stabilize antioxidants, as the phenolic compounds, for topic application.

Micro emulsions are characterized as oil and water dispersions, stabilized by surfactants and co-surfactants that form an interfacial film, they are thermodynamically stable, macroscopically homogeneous and translucent [16-18]. Their advantages as systems for topical drug administration are: high stability, higher drug solubility, easy manufacture and improvement of percutaneous penetration of molecules [11,19].

Therefore, the use of micro emulsions, as topical vehicle for cosmetic formulations containing antioxidant, may be an alternative for skin protection against excessive solar radiation. Thus, this work aimed to carry bacaba shell extract containing anthocyanins in micro emulsions in order to assess its antioxidant potential.

MATERIAL AND METHODS

Bacaba Shell Extract

All fruits were picked in the rural area of Sinop – MT, they were washed, sanitized and rinsed, and therefore they were submerged in ultra pure water at 40 °C for 40 minutes in order to manually remove the shells, which were further kept in oven with forced

circulation of air at 40 °C for 24 hours to remove the water. Furthermore, the shells were milled and stocked in freezer at -20 °C.

The bacaba shell extracts were prepared with two different solvents: ethanol 70 % (v/v) and propylene glycol. The extraction was done using 1 g of shell for every 25 mL of solvent (1:25) and left standing still for 24 hours in the fridge. Afterwards, the extracts were filtered and stocked in the refrigerator.

System Composition

For the development of the formulations it was used: distilled water, capric/caprylic triglyceride (TCC, Henrifarma®, Brazil), sorbitan monooleate – Span 80® (SP, Sigma-Aldrich®, Brazil), polysorbate 80 – Tween 80® (TW, Synth®, Brazil) and bacaba shell extract at 0.1 g mL^{-1}.

Development of the Micro Emulsion Systems

All formulations were prepared using pre-established amounts of distilled water, TCC, SP and TW, with concentrations ranging from 10 to 80 %. Afterwards, 5 % of bacaba shell extract was added to all formulations.

After 72 hours of preparation at 25 °C, all formulations were visually classified as micro emulsion (ME), liquid emulsion (LE), gel emulsion (GE) and phase separation (PS). With this data it was built a pseudo-ternary phase diagram through SigmaPlot version 8.0 software.

From the pseudo-ternary phase diagram it was determined the amount of TCC, SP and TW that formed the micro emulsion (ME) area, object of this study.

Physicochemical Characterization

All formulations that formed ME were preselected and subjected to physicochemical characterization after 24 h of their preparation. Aliquots were centrifuged at 3600 rpm with Quimis® centrifuge (Brazil) at room temperature, and then assessed their pH, electric conductivity and refractive index [13,17].

Stability Study

Due to evaluate the preliminary stability, all samples were exposed to alternate cycles of temperature, at 5 ± 1 °C and 40 ± 1 °C, every 24 h and after 14 days of cycles it was identified the thermally stable systems. The accelerated stability trials were performed with the most stable formulations from the preliminary stability study, being exposed to different temperatures (5 ± 1 °C, 25 ± 1 °C and 40 ± 1 °C) for 90 days, having their physicochemical properties evaluated every 30 days. All trials were in triplicate.

Rheological Characterization

The rheological characterization was performed by Modular Compact Rheometer – MCR 102 (Anton Paar®, Germany) with 600 μL of each ME placed on the reading surface. During the readings, TruGap™ supported at 0,099 mm continually controlled the space measurement. T-Ready™ conquered the accurate temperature control, and the measurement cell was a Toolmaster ™ CP 50. All data and graphs were managed by SigmaPlot 8.0 software. Established parameters, fundamentals of shearing stress control (τ) with 0-5 Pa for the ascending curve and 5-0 Pa for the descending curve were used to build the flow and viscosity curves. It was performed 75 readings per test, at 25 °C and isothermal conditions.

Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) technic was used to evaluate the particles average size of the ME formulations, with a colloidal suspension of the samples prepared from the formulation dilution in deionized water. The readings were obtained by Zetasizer Nano ZS (Malvern®, United Kingdom) at 632.8 nm.

Determination of the Antioxidant Potential by DPPH (2,2-diphenyl-1-picrylhydrazyl) Method

The antioxidant activity of the micro emulsion systems was performed through the DPPH (2,2-diphenyl-1-pricylhydrazyl) free radical method with reading in quartz (1 cm) in spectrophotometer (PG Instruments Ltd, T80 UV/VIS). The samples were serially diluted (10 – 50 $mgmL^{-1}$) in methanol for the test. The DPPH radical inhibition percentage was calculated by the equation: % DPPH inhibition = $[(A_0 – A_1)/A_0 \times 100]$, with A_0 as standard absorbance and A_1 as sample absorbance. For the determination of the ME concentration needed to reduce in 50 % the DPPH (IC_{50}) radical, data were assembled in linear regression to obtain the line equation [20.21].

RESULTS AND DISCUSSION

For the development of the systems, it was initially calculated the ideal surfactant proportion, according to the Hydrophilic Lipophilic Balance (HLB) of the formulation's oily phase, the TCC presents HLB of 10.8, with SP/TW of 3.93:6.07 as the adequate proportion of these surfactants. Once established surfactants, TCC, aqueous phase and bacaba shell extract at 5 % (in alcohol 70 % and propylene glycol), a total of 36 formulations were prepared for each solvent used in the extracts.

The pseudo-ternary phase diagrams used to classify macroscopically the formulations are in Figure 1. From the proportions given by the diagrams it was obtained a total of 72 formulations with conflicting balance characteristics.

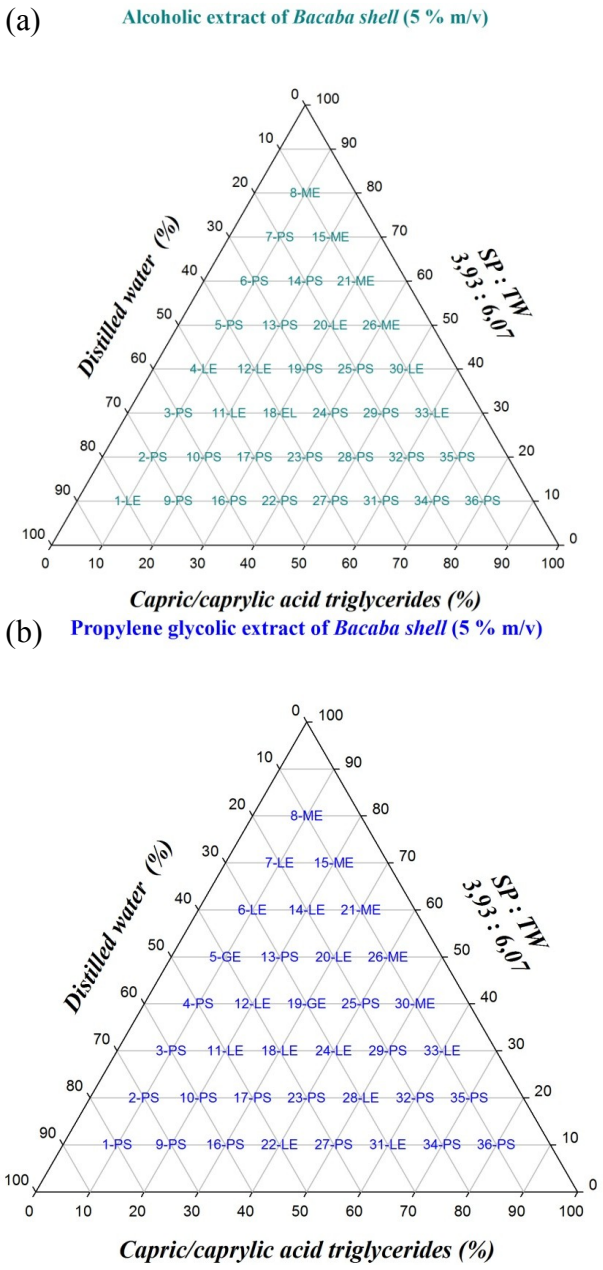

Figure 1. Pseudo-ternary diagrams of the 72 formulations in pre-established proportions of TCC, distilled water, surfactants and ethanolic extract 70 % (a), and propylene glycolic

extract (b). Micro emsulsion (ME), liquid emulsion (LE), gel emulsion (GE) and phase separation (PS).

From the pseudo-ternary diagrams it was possible to observe the ME domain area, with identification of homogeneous and translucent systems with surfactant mixture concentrations over 40 % (Figure 1). From the 36 formulations with extract prepared in ethanol 70 %, four showed ME features, being called 8MA, 15MA, 21MA and 26MA (Figure 1(a)). The same occurred with the 36 formulations prepared with extract in propylene glycol, with the formulations named 8MP, 15MP, 21MP and 30MP (Figure 1(b)).

All nine formulations selected according to the ME area from the pseudo-ternary diagrams were subjected to centrifugation. The ME 8MA, 15MA, 21MA, 26MA, 8MP, 15MP, 21MP and 30MP showed macroscopic stability, remaining translucent and homogeneous, being considered normal (N) and 26MP showed phase separation. The results of the preliminary stability trials of the formulations in the beginning and at the end of 14 days are in Table 1.

Table 1. Physicochemical parameters of the preliminary stability study of the formulations

Preliminary Stability		
Formulation	Centrifugation	
	Time (days)	
	0	14
8 MA	N	N
15 MA	N	N

21 MA	N	PS
26 MA	N	PS
8 MP	N	N
15 MP	N	N
21 MP	N	PS
26 MP	PS	-
30 MP	N	PS

Formulations	pH	
	Time (days)	
	0	14
8 MA	6.94 ± 0.05	6.66 ± 0.08
15 MA	6.89 ± 0.17	6.73 ± 0.10
21 MA	6.83 ± 0.13	-
26 MA	6.89 ± 0.10	-
8MP	6.92 ± 0.04	6.64 ± 0.12
15MP	6.82 ± 0.10	6.78 ± 0.08
21 MP	6.78 ± 0.12	-
30MP	6.74 ± 0.04	-

Formulation	Conductivity (μScm^{-1})	
	Time (days)	
	0	14
8 MA	7.18 ± 1.47	5.80 ± 0.04
15 MA	4.12 ± 0.10	2.52 ± 0.73
21 MA	5.99 ± 0.66	-
26 MA	10.98 ± 1.41	-
8 MP	4.61 ± 0.70	3.92 ± 0.51
15 MP	1.83 ± 0.57	2.03 ± 0.13
21 MP	3.59 ± 0.17	-
30 MP	4.31 ± 0.03	-

*Normal (N) and phase separation (PS).

After 14 days of the preliminary stability study, the samples 21MA, 26MA, 21MP and 30MP showed visual heterogeneity, being withdrawn from the stability study. The other formulations presented stable pH, with very less variation after the test cycles. The electrical conductivity was sustained along the study, showing that all ME formulations were oil in water systems (O/W), once the value found exceeded the conductivity of the water (>1.3 μScm^{-1}).

Formulations 8MA, 15MA, 8MP and 15MP were subjected to accelerated stability study and results are in Table 2.

Table 2. Physicochemical parameters during the accelerated stability study

Formulation	**pH**			
	Time (days)			
	0	30	60	90
8MA	7.04 ± 0.01	6.90 ± 0.12	6.93 ± 0.20	6.90 ± 0.11
15MA	7.09 ± 0.09	6.98 ± 0.06	6.99 ± 0.08	6.83 ± 0.04
8MP	7.05 ± 0.09	6.92 ± 0.05	6.90 ± 0.19	6.78 ± 0.25
15MP	7.03 ± 0.03	6.83 ± 0.17	6.92 ± 0.21	6.99 ± 0.52
Formulation	**Conductivity (μScm^{-1})**			
	Time (days)			
	0	30	60	90
8MA	5.80 ± 1.05	4.79 ± 1.10	3.99 ± 1.25	2.81 ± 0.02
15MA	5.58 ± 1.19	4.26 ± 1.86	4.06 ± 2.04	2.89 ± 1,24
8MP	2.84 ± 1.10	2.42 ± 0.57	4.07 ± 0.56	3.16 ± 0.27
15MP	2.78 ± 0.25	2.04 ± 0.28	3.27 ± 0.71	1.96 ± 0.34

Formulation	Refractive Index			
	Time (days)			
	0	30	60	90
8MA	1.45 ± 0.001	1.45 ± 0.001	1.45 ± 0.001	1.45 ± 0.005
15MA	1.45 ± 0.001	1.45 ± 0.002	1.45 ± 0.002	1.45 ± 0.003
8MP	1.45 ± 0.001	1.45 ± 0.001	1.45 ± 0.002	1.45 ± 0.001
15MP	1.45 ± 0.001	1.45 ± 0.001	1.45 ± 0.002	1.45 ± 0.002

At the end of the accelerated stability study the ME formulations showed themselves stable, with slight pH variation, kept between 6.78 and 7.09, suggesting no decomposition reactions. The conductivity of samples 8MA and 15MA decreased, most likely due to the evaporation of interstitial water [22], although there was no phase inversion. Samples 8MP and 15 MP showed mild conductivity variation, maintaining stable. Concerning the refractive index, there was no change during the test, remaining 1.45 for all formulations, supporting the stability of the systems. Therefore, in spite of the low variation of conductivity in some samples, the systems were stable, homogeneous and translucent throughout the stability study.

For the rheological characterization, flow and viscosity curves were used as a function of the shear rate (τ) (Figure 2). According to the flow curves (Figure 2(a)), it was possible to determine the Newtonian behavior of the formulations, due to the fact that the curves began at the origin and exhibited linear ascending and descending behavior. In addition, the viscosity curves (Figure 2 (b)) showed no variation as the shear rate increased, with values between 0.15 and 0.30 Pas, confirming the Newtonian behavior of the systems.

The tendency of the systems to Newtonian profile, sustained viscosity, even with shear rate increased, and the stability studies indicate a physical stability of the ME formulations [13]. This behavior is in agreement with other authors [13,23,24].

Lately, ME systems have become a practical platform of drug delivery in improving the target specificity, its therapeutic activity and reducing toxicity, highlighting the great potential of ME as a vehicle for a variety of drugs due to its several polarity domains [17]. That way, obtaining systems with physical stability for their use as drug vehicles becomes of great importance in developing new products that may contribute with controlled and/or sustained release of active ingredients.

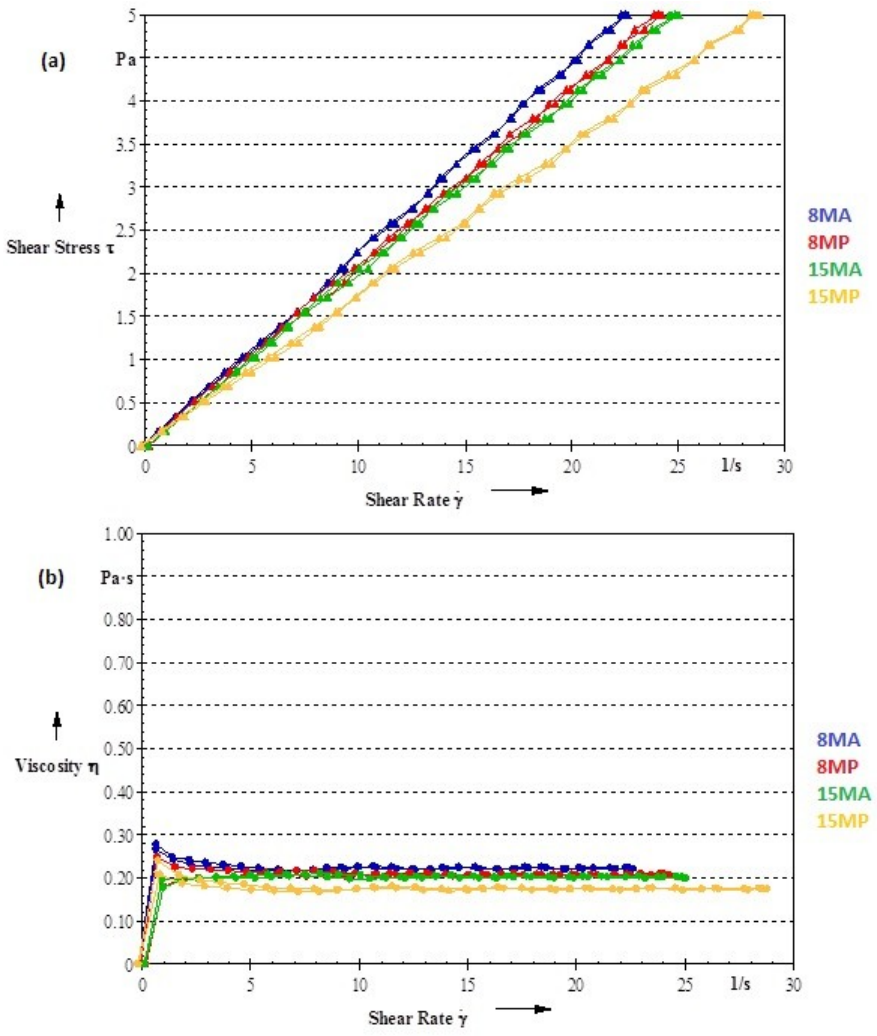

Figure 2. Flow (a) and Viscosity (b) curves of bacaba shell extract micro emulsions (8MA, 15MA, 8MP and 15MP)

The droplet size and the distribution of the particle size of the ME internal phase were determined by dynamic light scattering (DLS) [25], and the droplets hydrodynamic diameter (HD) of formulations 8MA, 15MA, 8MP and 15MP were respectively 324.73; 300.07; 287.63 and 322.20 nm (Figure 3), and showed themselves independent of the constituents proportion, except formulation 8MP ($p = 0.0053$). The polydispersivity index

(PDI) of the formulations was 0.35 + 0,01 (p = 0.1526), indicating homogeneity of the formulations' droplets.

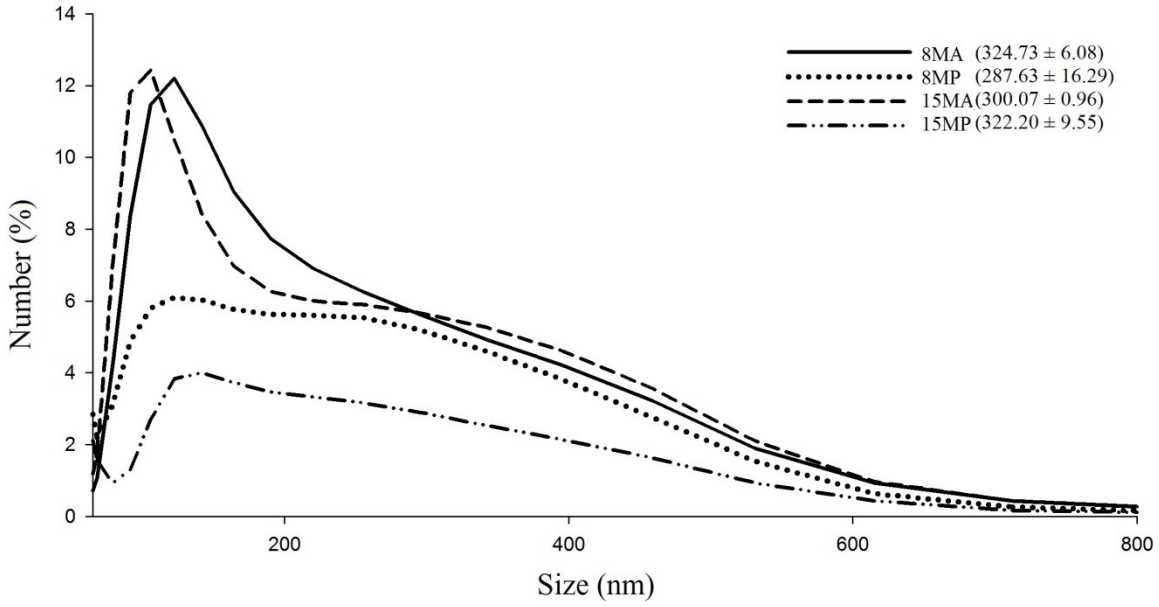

Figure 3. Hydrodynamic diameters of bacaba shell extract ME.

The droplets size was larger than ME from other studies [13,18] that did not exceed 150 nm, however their HD maintained a nanometric scale, indicating that the systems were thermodynamically stable and were formed spontaneously [26].

Short chain alcohols are usually added to several micro emulsion systems as co-surfactants, with butanol as one of the best for ME formulations [27,28]. In the developed systems it was not used a specific co-surfactant, however the extracts were prepared in alcohol, contributing to the ME formation by interacting with the surfactants and improving the interfacial film flexibility.

The results of antioxidant activity trials were expressed in IC_{50}, and ME 8MA, 15MA, 8MP and 15MP presented 62.12, 63.26, 137.76 and 118.60 $mgmL^{-1}$, respectively. Therefore, it is clear that systems with ethanol 70 % extract showed a better antioxidant activity, with lower amount of ME needed to reduce DPPH in 50 % than the systems containing propylene glycol extract. Ethanol in combination with water, due to their non-polar characteristic, are the solvents of choice for anthocyanin extraction [29], what may explain why ME with ethanol 70 % extract showed better antioxidant activity than ME with propylene glycol extract, in other words, ethanol 70 % extract was able to extract, incorporate and stabilize larger amount of antioxidants than propylene glycol extract.

The development of stable systems to incorporate and deliver drugs offers advantages, given that it allows active ingredients incorporation and cooperates preventing diseases for their antioxidant activities [8,9,11,30]. Its thermodynamic stability offers advantages over the unstable dispersions, such as suspensions and emulsions, having a way longer lifetime. Therefore, the development of micro emulsion systems with potential antioxidant activity becomes a good choice for drugs and/or cosmetics incorporation in pharmaceutical and cosmetic industries.

CONCLUSION

The development of micro emulsion systems with bacaba shell extract showed potential antioxidant activity, which combined with their physical and thermodynamic stabilities may turn them into drug or cosmetic active ingredients carrier. It was also observed that systems developed in ethanol 70 % showed higher antioxidant activity

attributed to the greater affinity of anthocyanin to ethanol during its extraction, being the best option for the development of cosmetics and medicines.

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ANEXO I – Normas Para a Submissão Na Bioscience Journal

AUTHOR GUIDELINES

Dear authors,

The Bioscience Journal informs that the next period for submissions will happen from 16/04 to 16/05.

For more information, please visit:

http://www.seer.ufu.br/index.php/biosciencejournal/about/submissions#onlineSubmissions

The essay must strive for clarity, brevity and conciseness. The text should be typed in Times New Roman, size 11, simple space and with a margin of at least2 cm. All lines must be numbered. Papers should be submitted without the identification of the authors. The authors' names, title and address of work must be presented to metadata submission and in the cover letter. Figures and tables must be inserted in the text, as close as possible to where cited.

The article will be sent to three (03) reviewers in the area in question, in the shortest possible time, without identifying the authors and will be considered as approved upon 02 favorable opinions.

Only papers written in English will be accepted.

The journal reserves the right to make changes as to rules, spelling and grammar in the original, in order to maintain the standard patterns of the language, while respecting the style of the authors. The final proofs will be sent to the authors, together with the payment slip for publication.

Papers which are published become the property of the Bioscience Journal, having their reprint, in whole or in part, subject to the express permission of the journal Editor. The original source of publication must be assigned.

No reprints will be provided. The articles will be available for printing in PDF format on the journal website.

A publication fee will be charged to the amount of R$ 40.00 (forty reais) per published page of the approved papers to national authors and $ 30 (thirty US dollars) for foreign authors. (Form of payment will be informed later).

Once the article has been reviewed and approved, the journal will categorize the contributions according to the following categories:

1. Original Articles - Articles that present a contribution which is entirely new to knowledge and allow other researchers, based on the written text, to judge the conclusions,

check the accuracy of the analyzes and deductions of the author and repeat the investigation if they so wish. The articles must contain: Title, Summary (200 to 400 words), Keywords, Introduction, Material and Methods, Results, Discussion (or Results and Discussion) and Conclusion (optional), Acknowledgements (if applicable). They must also contain: Title, Abstract (200 to 400 words) and key words in Portuguese and References. The papers must not exceed 20 pages (including text, references, figures, and annexes).

2. Review Articles - Articles that present comprehensive and updated review of a subject of interest from the scientific community and which offer significant contribution to the area of knowledge under discussion. The articles must contain: Title, Summary (200 to 400 words), Keywords, Introduction, Development, Conclusion, Acknowledgements (if applicable). They must also contain: Title, Abstract (200 to 400 words) and keywords in Portuguese and References. The papers must not exceed 30 pages (including text, references, figures and any annexes). In this paper/work category only contributions made at the invitation of the editors (General or Associate) will be accepted for submission.

3. Case report (s) - Predominantly clinical articles, of high relevance and which are current, with original reports from clinical and basic areas. The articles must contain: Title, Summary (200 to 400 words), Keywords, Introduction, Case Report, Discussion, Conclusion (optional) and Acknowledgements (if necessary). They must also contain: Title, Abstract (200 to 400 words) and Keywords in Portuguese and References. The papers must not exceed 10 pages (including text, references, figures and any annexes).

4. Communication – Non original article, demonstrating the experience of a group or a service, preferably covering teaching, research, health policy and professional practice. Or an article to report the results (partial or not) of work that offers relevant information to scientific knowledge, but which does not allow for firm conclusions. It must contain: Title, Summary (200 to 400 words), Keywords, Introduction, Contents and Acknowledgements (if necessary). It must also contain: Title, Abstract (200 to 400 words) and Keywords in Portuguese and References. The papers must not exceed 10 pages, including attachments.

Presentation of Papers

Format: All papers/collaborations must be submitted through the Electronic System for Journal Publishing - SEER,
Address: http://www.seer.ufu.br/index.php/biosciencejournal/about/submissions#onlineSubmissions

The text must be saved in RTF (Rich Text Format) extension or Microsoft Word (2003) format. The metadata must be filled out with the Paper/Work title, name (s) of author (s), last academic degree, work institution, postal address, telephone, fax and email.

The text will be cordially written with intercalation of tables and figures, already inserted in the text, with the minimum amount required for its understanding.

As a measure of secrecy the body of the paper must not include the authors' names, which must be sent separately, with personal data (title, mailing address, email address and institution to which he/she is connected).

Paper title: The title must be brief and sufficiently specific and descriptive, containing the keywords that represent the contents of the text separated by colon, both accompanied by their translation into Portuguese.

Abstract: An informative summary must be prepared with about 200 to 400 words, including objective, method, results, conclusion, accompanied by its translation into Portuguese. Both must have 800 words at most.

Keywords: The keywords must not repeat words in the title, the scientific name of the species studied must be included. Words should be separated by a colon and begin with a capital letter. Authors must submit 3-6 terms, taking into consideration that a term may be composed of two or more words.

Acknowledgements: Acknowledgements as to help received in the preparation of the paper must be mentioned at the end of the article, before the references.

Notes: The notes contained in the article must be indicated with an asterisk immediately after the sentence to which they refer. The notes must be at the bottom of the corresponding page. Exceptionally, numbers may be adopted for the notes together with asterisks on the same page. In which case, the notes with asterisks precede the notes with numbers, regardless of the order of these notes in the text.

Appendices: Appendices can be used in the case of extensive lists, statistics and other supporting elements.

Figures and Tables: Clear photos (black and white or in color), graphs and tables in black and white (strictly essential for clarity of the text) will be accepted, and must be marked in the text by their order number, in the places where they must be inserted. If the illustrations submitted have already been published, mention the source. (See rules for preparation of figures, in the next section).

Manuscripts, even if they present scientific relevance and are methodologically correct, may be refused if they are not properly organization and if they are outside the norms of the Bioscience Journal.

GUIDELINES FOR THE PREPARATION OF FIGURES

1. Figures may be made in software depending on the authors' preference (Excel, Sigma Plot, etc.) They must be inserted and sent in TIFF or JPG format with a minimum resolution of 300 dpi.

2. The figures must have a maximum width of8.0 cmor16.0 cm.

3. The titles and the x and y axes scale must be in Times New Roman size 11. The axel lines and other lines (e.g., regression curves) must have a thickness of 0.3mm. All information contained inside the figure (e.g., equations, captions) must be in Times New Roman size 10 or at least 8. Right hand and top edges in graphs are not necessary.

4. All figures must be conveniently inserted into the text after being mentioned, consecutively and in Arabic numerals. The figures should be inserted in the text by means of the "Insert → Image/Figure→ File" command.

5. Figures may be made up of multiple graphs, both horizontal and vertical, respecting the maximum width of 16.0cm and 8.0cm, respectively. When dealing with figures of multiple graphs, the same must be identified by letters (A, B, C, D) in capital letters in brackets, source Times New Roman size 11. Papers that have been consulted and cited in the text are the responsibility of the author.

Information coming from personal communication, papers in progress and unpublished papers must not be included in the reference list, but indicated in a footnote on the page in which they are cited.

References: NBR 6023/2002. The accuracy and appropriateness of the references to papers that have been consulted and cited in the text are the responsibility of the author. Information coming from personal communication, papers in progress and unpublished papers must not be included in the reference list, but indicated in a footnote on the page where they are cited.

The references included at the end of each article must be written on separate pages from the main text, in alphabetical order according to the ABNT NBR - 6023, August 2002 norms. All authors must be mentioned in the list of references at the end of the article. The use of the expression et al is not allowed.

Observe the reference examples below:

The Book as a whole:

GRAZIANI, Mario. Cirurgia buco-maxilo-facial. 6. ed. Rio de Janeiro: Guanabara Koogan, 1976. 676 p.

Book chapter without proper authorship:

PERRINS, C. M. Social systems. In: _____. Avian ecology.Glasgow: Blackie, 1983. chapter. 2, p. 7-32.

Book chapter with proper authorship:

GETTY, R. The Gross and microscopic ocurrence and distribution of spontaneous atherosclerosis in the arteries of swine. In: ROBERT JUNIOR.; A., ATRAUSS, R. (Ed.). Comparative atherosclerosis.New York: Harper & Row, 1965. p. 11-20.

Monographs, dissertations and theses:

CORRALES, Edith Alba Lua Segovia. Verificação dos efeitos genotóxicos dos agentes antineoplásicos citrato de tamoxifen e paclitaxel. 1997.84 f. Dissertação (Mestrado em

Genética e Bioquímica) – Curso de Pós-Graduação em Genética e Bioquímica, Universidade Federal de Uberlândia, Uberlândia, 1997.

Papers presented at events: Conferences, Seminars, Meetings ...

NOVIS, Jorge Augusto. Extensão das ações de saúde na área rural. In: CONFERÊNCIA NACIONAL DE SAÚDE, 7., 1980, Brasília. Anais... Brasília: Centro de Documentação do Ministério da Saúde, 1980. p. 37-43.

Journal articles:

COHEN, B. I.; CONDOS, S.; DEUTSCH, A. S.; MUSIKANT, B. L. La fuerza de fractura de tres tipos de materiales para el muñon en combinacion com tres espigas endodontiacales distintas. R. Cent. C. Biomed. Univ. Fed. Uberlândia, Uberlândia, v. 13, n. 1, p. 69-76, dez. 1997.

Observation: As for the title of the journals, a single standard must be adopted. In the list of references all titles of journals must be presented abbreviated or in full, and in bold.

Note: As for electronic documents, the normal reference must be made, with information as to the description on the medium or support being added at the end.

Example :

Chapter of book with proper authorship available on CD - ROM :

FAUSTO, A. I. da F.; CERVINI, R. (Org.). O trabalho e a rua. In: BIBLIOTECA nacional dos direitos da criança. Porto Alegre: Associação dos Juizes do Rio Grande do Sul, 1995. 1 CD-ROM.

Periodical article in electronic media:

ROCHA-BARREIRA, C. A. Caracterização da gônada e ciclo reprodutivo da Collisella subrugosa (Gastropoda: Acmaeidae) no Nordeste do Brasil. Brazilian Journal of Biology, São Carlos, v. 62, n. 4b, nov. 2002. Disponível em: Acesso em: 20 abr. 2003.

Recommendations: It is recommended that the ABNT rules concerning submission of articles in periodicals (NBR 6023/2002), presentation of citations in documents (NBR 10.520/2002), presentation of original papers (NBR 12256), norm for dating (NBR 5892), progressive numbering of the sections of a document (6024/2003) and abstracts (NBR 6028 /2003), as well as the norm for IBGE tabular presentation, be observed.

Transfer of Copyright:

All persons listed as authors must sign the Transfer of Copyright:

“I declare that, in the case of acceptance of the article, the Bioscience Journal shall be the owner of the copyrights relating to same, which will become the sole property of the Journal, prohibiting any reproduction, in whole or in part, in any other place or means of

publication, printed or electronic, without the prior and required authorization being requested, and if obtained, will include an appropriate acknowledgment to the Journal".

Signature (s) of author (s) Date ___ / ___ / ____

Opinions expressed by authors are their exclusive responsibility.

Statement of Responsibility:

All persons listed as authors must sign the responsibility statement in the following terms:

I certify that I participated in the conception of the paper to take public my responsibility for its content, not omitting any affiliations or financial agreements between authors and companies that may be interested in publishing this article;

- I certify that the manuscript is original and that the paper, in part or in whole, or any other paper with substantially similar content of my authorship, was not sent to another journal and will not be sent, while its publication is being considered by the Bioscience Journal, be it in printed or electronic format.

Address for submission of papers:

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SUBMISSION PREPARATION CHECKLIST

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

1. Only papers written in English will be accepted. The contribution is original and unpublished and is not being evaluated for publication by any other journal, failing that, justify in "Comments to the Editor".
2. The files for submission are in Microsoft Word (2003), RTF or WordPerfect format.
3. The text is simple-spaced, using a 11-point font; uses italics, rather than underlining (except with URL addresses), with figures and tables inserted in the text and not at the end.
4. The identification of authorship of this paper was removed from the file (Word 2003) and the option Properties in Word, thus ensuring the confidentiality of the journal. The text meets the formatting standards of the journal cited in "Guidelines for authors" in the "About" section
5. At the moment of online submission, the main author should send a letter signed by all authors, requesting the submission of the article and possible publication, exclusively by this journal. The letter should be scanned and transferred in "additional documents".
6. All "URL" addresses in the text (e.g.: http://pkp.ubc.ca) are active.
7. The article is being submitted correctly to the corresponding section according to its reference area.

8. Manuscripts, even those presenting scientific relevance and being methodologically correct, may be refused if presented in a disorganized manner and outside the norms of the Bioscience Journal. Well written manuscripts and those presented in accordance with the standards are reviewed faster and also require less effort from reviewers.
9. A publication fee will be charged to the amount of R$ 40.00 (forty reais) per published page, for the approved papers. (Form of payment will be informed later).
10. All of the items above are basic requirements for the submission of an article and, if not according to the standards of the journal, or if the metadata are not filled out correctly, that particular article WILL NOT be considered for review.

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ANEXO II – Normas Para Submissão Na Revista Latin American Journal of Pharmacy

Manuscripts submitted to **Latin American Journal of Pharmacy** are only accepted on the understanding that they are subject to editorial review and that they have not been, and will not be, published in whole or in part in any other journal.

Papers must be written in English. If English is not authors' native language, the manuscript should be checked by someone proficient in the language before submission. Manuscripts in which English is difficult to understand may be returned to the author for revision before scientific review.

Types of Contribution

Original articles should contain material that has not been previously published elsewhere, except in a preliminary form. These papers should not exceed 5000 words including tables, references and legends of tables and figures. Short Communications are research papers constituting a concise but complete description of a limited investigation, which will not be included in a later paper. They should be as completely documented as a regular paper and should not occupy more than 2,500 words including tables, references and legends of tables and figures. Reviews and mini-reviews will be exceptionally accepted in areas of topical interest and will normally emphasize literature published over the previous five years. Letters to the Editor are published from time to time on subjects of topical interest.

Manuscript Preparation

Manuscripts must be neatly typed (size page A4), double-spaced throughout, including figures and tables, with at least 2 cm margins on all sides. The Editor reserves the right to adjust style to certain standards of uniformity. Every page of the manuscript must be numbered at the right top, preceded by the name of the author to whom the correspondence should be sent. The usage of italics should be limited to scientific names of organisms. A cover letter is not required, but if included it should be placed at the beginning of the manuscript.

Manuscripts in general should be organized in the following order:

- Title: should be clear, concise, and unambiguously reflect the paper's contents.

- Name(s) of author(s): first name, initial(s) of the middle name(s), and family name of each author. The corresponding author should be identified with an asterisk (*).

- Affiliations: include the name of department (if any), institution, city and state or country where the work was done, indicating which authors are associated with which affiliation.

- E-mail address of the corresponding author, as all correspondence, including proofs, should be sent only to him.

- Summary: not exceeding 150 words, reporting concisely on the major findings. Many abstracting services use abstracts without modification, so this section should be comprehensible in its own right.

- Key Words: at least three and not more than six in alphabetical order will be listed.

- Introduction: briefly review important prior publications and state the reasons for the investigation being reported.

- Materials and methods: description of methods, equipment and techniques (including statistical treatments used in the research).

- Results: efforts should be made to avoid jargon, to spell out all non-standard abbreviations the first time they are mentioned and to present the contents of the study as clearly and concisely as possible.

- Discussion (may be combined with the Results section).

- Conclusions (at the author's discretion): must not reiterate any discussion or introductory comments, they must be genuine conclusions drawn from the results of the study.

- Acknowledgements and any additional information concerning research grants, etc.

References: will be numerated correlatively as they are cited in the text and listed separately under the title “References” (please use a hanging indent: second and subsequent lines indented). The style used for citation of articles in journals (1), monographs (2), chapters in books (3), and internet references (4) which must be strictly observed, is given in the following examples:

(1) Medeiros R., G.F. Passos, C.E. Vitor J. Koepp, T.L. Mazzuco, L.F. Pianowski, M.M . Campos & J.B. Calixto (2007) *Brit. J. Pharmacol*. 151: 618-27.

- Journal names should be abbreviated according to ISI style (you are invited to consult the sites http://www.efm.leeds.ac.uk/~mark/ISIabbr/A_abrvjt.html or http://images.isiknowledge.com/WOK46/help/WOS/L_abrvjt.html)

(2) Vogel, W.H., B.A. Scholkens, J. Sandow & G. Muller (2002*). "Drug discovery and evaluation, Pharmacological assay"*, Second Edition, Spinger-Verlag, Berlin Heidelberg, New York, pp. 906-44.

(3) Aristide, V. & J.W. Martin (2005) "*Doxorubicin*", in "Analytical profiles of drug substances" (F. Klaus, ed.), Academic Press, New York, pp. 245-74.

(4) Duke, J.A. *"Medical Botany. Module 8: Amazonian (Iberoamerican)"*. Available at (http://www.ars-grin.gov/duke/syllabus/module8.htm).

For 2-6 authors all authors are to be listed, with "&" separating the last two authors; for more than six authors, use the first six authors followed by et al. For three or more authors use *et al*. in the text.

- Tables and Figures: will be numbered using Arabic numerals in the order they appear in the text. Letters and symbols included into the figures should be made in a suitable size, since figures are usually reduced to half a column wide size (7.5 cm). Photographs, charts and diagrams are all to be referred to as "Figures". They should accompany the manuscript. All illustrations should be clearly marked with the figure number. All figures are to have a self-explanatory caption. Captions of Tables and Figures should be supplied on a separate sheet.

Page charges

- Page charges are required for publication in Latin American Journal of Pharmacy. These cover some of the costs of publication and allow us to more fairly share charges between researchers and libraries. Please be aware that the journal is published by the College of Pharmacists of Buenos Aires Province, Argentina, which does not receive direct funding from any external agencies. Support from page charges allows lower subscription prices and thus a greater circulation for the journal.

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