

Spray dried extract of *Calophyllum brasiliense* Cambés produces a potent hypoglycemic effect and improves total serum cholesterol and triglycerides in diabetic rats

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SUMMARY

Introduction: The species *Calophyllum brasiliense* Cambés (Calophyllaceae) is widespread throughout Central and South America. The stem bark infusion is used for lowering blood glucose. **Aim:** To optimize the spray dry extract of this plant using a D-optimal experimental design. **Materials and methods:** As factors were used the air-drying speed (3.5-4.5 m³/h), the feed flow rate of the suspension (5-11 mL/min), and the inlet air temperature (90-130 °C). The dried extract was characterized by measuring the phenolics and flavonoids content, moisture, the water activity, apparent densities, flowability, and compressibility. The antioxidant activity, the inhibitory activity of lipase and alpha-glycosidase, and the antiglycant activity of the spray dried extract (SDE) were evaluated. Subsequently, the hypoglycemic activity was evaluated in rats by monitoring the blood glucose level, triglycerides, and cholesterol. **Results:** Inlet air temperature and feed flow rate were the factors that most affected the yield and phenolic content. SDE showed a potent antioxidant effect

(IC₅₀ 1.83 µg/mL), a potent α -glycosidase (IC₅₀ 74.45 µg/mL) and pancreatic lipase (IC₅₀ 27.33 µg/mL) inhibition. A potent antiglycation effect (IC₅₀ 9.45 µg/mL) was also observed. **Conclusion:** the SDE showed a potent hypoglycemic effect at 100 mg/kg. These results suggest that SDE could activate four important pathways that can contribute to diabetes control.

Keywords: Jacareúba, diabetes, drying process, optimization, d-optimal design, *Calophyllum brasiliense*.

RESUMEN

El extracto secado por pulverización de *Calophyllum brasiliense* cambés produce un potente efecto hipoglucémico y mejora el colesterol sérico total y los triglicéridos en ratas diabéticas

Introducción: la especie *Calophyllum brasiliense* (Calophyllaceae) está muy extendida en Centro y Suramérica. La infusión del tronco reduce los niveles de glucosa en sangre. **Objetivo:** optimizar el extracto seco por aspersión (SDE) de esta planta utilizando un diseño experimental *D-óptimal*. **Materiales y métodos:** como factores se utilizaron la velocidad del gas secante (aire, 3,5-4,5 m³/h), la temperatura de entrada del aire fue 90-130 °C y la velocidad de alimentación, 5-11 mL/min. Se determinó el contenido de fenoles y flavonoides en el extracto seco, la humedad residual, la actividad del agua, las densidades aparentes, fluidez y compresibilidad. Se evaluó la actividad antioxidante e inhibidora de lipasa y alfa-glicosidasa y la actividad antiglicante. También se evaluó la actividad hipoglicemiante midiendo glucosa en sangre, triglicéridos y colesterol. **Resultados:** la temperatura del aire de entrada y la velocidad de alimentación afectaron, significativamente, el rendimiento y contenido de fenoles. El SDE mostró un potente efecto antioxidante (IC₅₀ 1,83 µg/mL), una potente inhibición de α -glicosidasa (IC₅₀ 74,45 µg/mL) y de lipasa pancreática (IC₅₀ 27,33 µg/mL). Se observó un fuerte efecto antiglicante (IC₅₀ 9,45 µg/mL). **Conclusiones:** el SDE mostró un potente efecto hipoglicemiante a 100 mg/kg. Estos resultados sugieren que el SDE podría actuar activando cuatro vías importantes para el control de la diabetes.

Palabras clave: Jacareúba, diabetes, secado, optimización, diseño d-óptimo, *Calophyllum brasiliense*.

RESUMO

O extrato seco por aspersão de *Calophyllum brasiliense* Cambés produz um potente efeito hipoglicemiante e melhora o colesterol total e triglicérides em ratos diabéticos.

Introdução: a espécie *Calophyllum brasiliense* (Calophyllaceae) é amplamente distribuída na América do Sul e Central. A infusão da casca do caule reduz os níveis de glicose no sangue. **Objetivo:** otimizar o extrato seco por pulverização (SDE) desta planta usando um planejamento experimental D-ótimo. **Materiais e métodos:** a velocidade do gás de secagem ar (3,5-4,5 m³/h), a temperatura de entrada do ar (90-130 °C) e a taxa de alimentação (5-11 mL/min) foram usados como fatores. Foi determinado o teor de fenóis e flavonóides no extrato seco, a umidade residual, a atividade de água, as densidades aparentes, a fluidez e a compressibilidade. Avaliou-se a atividade antioxidante e a atividade inibitória de lipase e alfa-glicosidase, e a atividade antiglicante do extrato seco. A atividade hipoglicêmica foi avaliada em ratos diabéticos, medindo a glicose no sangue, triglicérides e colesterol. **Resultados:** a temperatura de entrada do ar e a taxa de alimentação afetaram significativamente o desempenho e o conteúdo de fenóis. O SDE mostrou um potente efeito antioxidante (IC₅₀ 1,83 µg/mL), uma significativa inibição de α-glicosidase (IC₅₀ 74,45 µg/mL) e da lipase pancreática (IC₅₀ 27,33 µg/mL). Um forte efeito antiglicante também foi observado (IC₅₀ 9,45 µg/mL). O SDE mostrou um forte efeito hipoglicemiante à concentração de 100 mg/kg. **Conclusões:** Esses resultados sugerem que o SDE poderia atuar ativando quatro vias importantes para o controle do diabetes.

Palavras-chave: Jacareúba, diabetes, secagem, otimização, planejamento D-ótimo, *Calophyllum brasiliense*.

INTRODUCTION

The species *Calophyllum brasiliense* Cambés (Calophyllaceae) is widespread throughout Brazil, growing in all kinds of environments from humid climates such as the Amazonia and dries one like Brazilian *Cerrado* [1]. This species is popularly known as *jacareúba* [2]. The tree reaches about 40 m in height, 3 m in diameter with a thick stem bark (≈ 2 cm). The infusion of the stem bark of *Calophyllum brasiliense* Cambés (CBC) is used for treating infected wounds, stomach pains, liver disorder, inflammation, diabetes, diarrhea, and hemorrhoids [3]. Despite the great pharmacological potential of

this medicinal plant, few efforts have been made for transforming the stem bark of CBC and its extracts in standardized products that can be used, safely, in therapeutic.

The ethanolic extract of the stem bark (CBE) content phenolics and flavonoids compounds as gallic acid, quercetin, and hyperin [1]. Also content xanthonones and coumarins [4, 5]. The anti-inflammatory and hypoglycemic effect of CBE has been associated with phenolics and flavonoids [6]. The high electronic density of phenolics and flavonoids induces strong scavenging of reactive oxygen species (abundant in decompensated diabetics), contributing to the patient homeostasis [7]. On the other side, phenolics and flavonoids are strong inhibitors of enzymes associated to protein and carbohydrate metabolism [8], contributing indirectly to lowering the blood glucose of diabetics [3, 9, 10].

The stability of phenolics and flavonoids compounds in solution is affected by factors such as light, temperature, oxygen, and pH [11]. CBE and its hydroalcoholic solutions when unprotected from light under ambient conditions develop a superficial and firm film that is thicker as the concentration of solids increase. This probably occurs by the oxidative effect of light and/or oxygen on xanthonones and phenolics. For this reason, the preparation of a dried powder could be an excellent way to preserve and stabilize the actives present in the extract. Dried extracts present advantages as compared with liquid extracts such as a higher concentration, facility of storage, and transportation, and others [12].

Among the techniques for drying vegetal extracts, spray drying appears as the most promissory, allowing to prepare solid products from fruits, vegetables, and medicinal plant at a relatively low cost with additional benefices to final products, like a better stability, improved flowability and compressibility [12]. Vegetal extracts to be dried by spray drying are added with drying adjuvants, which allow obtaining powders with low moisture content, avoiding active degradation, and suitable physic mechanical properties, which is particularly important for pharmaceuticals formulations.

Metabolites present in plant extracts can activate several physiologic responses at the same time. For this reason, the use of plant extracts in diabetics arises as an excellent possibility of treatment. Thus, this work aimed the optimization of drying parameters for obtaining the spray-dried extract of *Calophyllum brasiliense* Cambés (SDE), and the evaluation, *in vitro*, various antidiabetic mechanisms (antioxidant, antiglycant, glucosidase inhibition). The hypoglycemic effect of the SDE was also evaluated in Wistar rats, in an alloxan-induced diabetic model.

MATERIALS AND METHODS

Vegetal material

The stem bark of *Calophyllum brasiliense* was collected from a population of adult trees located at 0°87'16.8"N, -51°14'62.6"W, Municipality of Ferreira Gomes, Amapá, Brazil. The species were identified, and the voucher (code 0598AP) was deposited in the Herbarium of the Institute of Studies and Research of the Amapá State (Iepa), Brazil. The drug was dried in the shadow to a constant moisture content (<14%) and milled using a knife mill, for obtaining a particle size \approx 5 mm.

Extract preparation

CBE was prepared by dynamic maceration. The drug (500 g) was placed in an amber bottle containing 2L of 70% hydroalcoholic solution for 72 h. The mixture was mechanically stirred for one hour, twice a day, for three days. The extractive solution was filtered using a cotton cloth and concentrated in a rotary evaporator (Ika® Werke GMBH & Co., Germany) at 40 °C, up to a final drug: solvent ratio of 2:1 (g/mL). The extract was placed into an amber bottle and stored at room temperature, protected from light.

Extract characterization

Immediately after the preparation, the appearance, aroma, and flavor of the CBE were evaluated. Total solids, density, and the refraction index were also evaluated [13]. The pH was directly measured using a pH-meter (Tecnopon®, Brazil), previously calibrated with buffer solutions (pH 4, and 7, Alphatec®, Brazil). For the development of the experimental design, the total phenolic content was determined by the Folin-Ciocalteu spectrophotometric method, using gallic acid (Sigma®, USA) as the standard [14]. The content of gallic acid in the SDE was determined by HPLC (Sigma®, USA). Flavonoids content was determined spectrophotometrically by the aluminum chloride method, using a quercetin primary standard (Sigma®, USA) [14]. All measurements were performed in triplicate and expressed as the mean with the standard deviation.

Adjuvants selection

Three experiments were made for selecting the drying aids to be used for preparing the spray-dried extract of *Calophyllum brasiliense* (SDE). The CBE (100 mL) was added with 0.25 percent (w/v) of colloidal silicon dioxide plus 5 g of microcrystalline cellulose (Avicel® PH 102), or lactose monohydrate (Sigma®, USA), or maltodextrin (Sigma®, USA). The following drying conditions were used: air drying speed 4.0 m³/h, feed flow rate 8 mL/min, an inlet air temperature of 110 °C. The adjuvants that allow

obtaining the dried product with the highest phenolics content, the highest yield, and the lowest moisture content will be selected.

Drying process

For preparing the SDE, a Mini-Spray Dryer (MSD) 1.0 (Labmaq®, Brazil) was used. A D-optimal experimental design was applied for optimizing the drying conditions (table 1). The operational parameters used as factors were air drying speed (ADS, 3.5-4.5 m³/h), the feed flow rate of the suspension (FR, 5-11 mL/min), and the inlet air temperature of the drying air (IAT, 90-130 °C). The airflow for spraying the suspension into the drying chamber (30 L/min), and the nozzle diameter (1.0 mm) were kept constant.

The suspension was stirred during the drying process using a magnetic stirrer (Fisaton®, Brazil). Phenolics content (%), process yield (%), the water activity, outlet temperature (°C), and the moisture content (%) were used as responses. The response outcomes were fitted to mathematical models (equation 1) using the Design Expert Software 12.0 (StatEase®, USA). The effect of factors for constructing the theoretical models was estimated using the Backward elimination technique. In this technique, all factor is entered into the first model equation, and each one is eliminated (one at a time) if they do not contribute (p-value >0.05) to the model fitting [15].

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{123} X_1 X_2 X_3 \quad (1)$$

Where Y are the responses and β are the coefficients representing the magnitude of the factors' effect. β_0 , intercept; $\beta_1, \beta_2, \beta_3$ are the linear effect of factors X_1, X_2, X_3 ; $\beta_{12}, \beta_{13}, \beta_{23}$ are the effect of interaction of the factors $X_1 X_2, X_1 X_3, X_2 X_3$; $\beta_{11}, \beta_{22}, \beta_{33}$, the effect of the quadratic factors X_1, X_2, X_3 ; and β_{123} are the effect of the interaction of the factors $X_1 X_2 X_3$.

The optimization of the drying process was performed using the Desirability function, a multiple response approach [15]. This function allows assigning levels of importance to responses for signaling the desirable value, to achieve the optimized response (equation 2):

$$D = (d_1 \cdot d_2 \cdot d_3 \dots d_n)^{\frac{1}{n}} = \left(\prod_{i=1}^n d_i \right)^{\frac{1}{n}} \quad (2)$$

However, the predictive power of the Desirability function (D) depends on the predictive power of the adjusted model of the individual responses. Thus, the high the good-

ness of fit (R2) and goodness prediction (Q2) of the adjusted model the higher the predictor capability of the Desirability function. Desirability represents the geometric mean of all transformed responses, being d_i the desirable ranges of the response i ; and n , the number of responses used for optimization.

Table 1. Outcomes of the D-optimal experimental design for the drying optimization showing the factors in actual and coded values.

Run	Inlet air temperature (°C)	Feed flow rate (mL/min)	Air drying speed (m ³ /min)
1	130 (+1)	5 (-1)	3.5 (-1)
2	130 (+1)	11 (+1)	3.5 (-1)
3	90 (-1)	11 (+1)	4.5 (+1)
4	130 (+1)	5 (-1)	0.5 (+1)
5	90 (-1)	5 (-1)	0.5 (+1)
6	130 (+1)	11 (+1)	0.5 (+1)
7	110 (0)	8 (0)	4 (0)
8	90 (-1)	11 (+1)	3.5 (-1)
9	110 (0)	8 (0)	4 (0)
10	90 (-1)	5 (-1)	3.5 (-1)
11	110 (0)	11 (+1)	3.5 (-1)
12	110 (0)	5 (-1)	3.5 (-1)
13	130 (+1)	8 (0)	4 (0)
14	110 (0)	11 (+1)	4 (0)
15	110 (0)	8 (0)	5 (+1)
16	90 (-1)	8 (0)	4 (0)
17	110 (0)	5 (-1)	4 (0)
18	110 (0)	8 (0)	3.5 (-1)
19	90 (-1)	5 (-1)	4.5 (+1)
20	110 (0)	8 (0)	4 (0)
21	130 (+1)	5 (-1)	0.5 (+1)
22	90 (-1)	5 (-1)	3.5 (-1)
23	110 (0)	8 (0)	4 (0)

Coded values in parenthesis: high-level (+), center (0), low-level (-1).

Water activity

The water activity of the SDE was determined using a water activity meter (Aqua Lab 4Tev[®], USA). The assay was made in triplicate, and the result was expressed as the mean \pm standard deviation.

Moisture content

Moisture content of SDE was performed immediately after the dried process using an Infrared Moisture Analyzer (Shimadzu[®], Japan). The assay was made in triplicate.

Dry extract flowability and compressibility

Carr's index (CI) was determined as Lachman and co-workers [16]. For determine the Carr's index, the bulk (D_B) and taped (D_T) density were determined using a powder flow analyzer (Micro-System[®], UK). CI was calculated as equation 3:

$$CI(\%) = \left(\frac{D_T - D_B}{D_T} \right) \cdot 100 \quad (3)$$

Flow rate (F_R) was measured by pouring a mass (m) of SDE in a glass funnel (wall of 45°) with an exit orifice of 8.0 mm and measuring the time (t) taken for the mass powder to flow through the orifice. The flow rate was calculated using equation 4:

$$F_R = \frac{m \text{ (g)}}{t \text{ (s)}} \quad (4)$$

Scanning electronic microscopy

Particle morphology was evaluated by Scanning Electronic Microscopy (SEM) (Hitachi[®] TM3030 Plus, Japan). A sample of the dry extract was spread out over the microscopy sample slide using a carbon adhesive tape and directly positioned on the microscope chamber. Microphotographs were taken using an accelerating voltage of 5.0 kV, and a magnification of 1000, and 5000x.

Particle size analyses

The Sauter Mean Diameter (D3,2) and the particle size distribution were determined by optical microscopy, using an Olympus[®] microscopy (USA). Images of the SDE (thoughtfully dispersed in a glass lamina) were taken using a Canon[®] camera (Japan). Images were analyzed using Image Pro-plus 7.0[®] Software. A total of 1500 particles measured, and the particle size and the size distribution were determined using Stat-Graphics XV.1 (Stat Easy[®], USA).

DPPH radical scavenging

The radical scavenging activity was evaluated by spectrophotometry (Shimadzu®, Japan), using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical [10, 17, 18]. For biological assays, the blank solution was prepared by dissolution of 0.25 g of colloidal silicon dioxide plus 5 g of lactose monohydrate in 100 mL of PBS buffer (pH 7.4). The mixture was sonicated for 10 min, filtered by a Millipore membrane (0.45 µm), and diluted at the same concentrations used for the SDE (2.5, 5, 10, 15, 20, 25, 50 and 100 µg/mL).

Alpha-glycosidase inhibition

The assay was made in 96-wells microplates according to Kiho and coworkers [17, 19]. The enzyme solution was prepared by dissolving 3 mg of rat intestinal acetone powder (Sigma®, USA) in 1 mL of buffer phosphate (pH 6.9). The solution was centrifuged at 5000 rpm for 15 min, filtered by a Millipore® membrane (0.45 µm), and the supernatant was used for the assay. The standard and the tested substances were prepared in concentrations of 2.5, 5, 10, 15, 20, 25, 50, and 100 µg/mL. In the microplate wells were placed 30 µL of DMSO (used as the control), the standard solution of Acarbose®, and the SDE solutions. To each well was added 170 µL of enzyme solution. Microplates were incubated protected from light, for 5 min, at 37 °C. After that, 100 µL of 4-nitrophenyl α-d-glucopyranoside was added to each well and incubated again for 20 min. The analysis of the percent of inhibition (%I) was performed in triplicate and it was determined using equation 5:

$$\%I = \left(\frac{A_{a/p}}{A_C} \right) \cdot 100 \quad (5)$$

$A_{a/p}$ = Absorbance of the Sample-Absorbance of the blank, A_C = Absorbance of the negative control-Absorbance of the Blank.

The blank solution was prepared as described above for evaluating the antioxidant effect. The IC₅₀ values were estimated by Probit analysis using the GraphPad® Software (USA), at a level of significance of p=0.05.

Pancreatic lipase inhibition

The assay was made in 96-wells microplates according to Kiho and coworkers [17, 19]. The enzyme solution was prepared by dissolving 4 mg of pancreatic lipase (type II, L3126-25G, Sigma®, USA) in 5 mL of Trisma-HCl (pH 7.6). After that, the substrate solution was prepared by dissolving 20 mg of 4-nitrophenyl palmitate (N2752-50G, Sigma®, USA) in 5 mL of acetonitrile: ethanol (1:1) solution. The standard substance,

the blank (prepared as described before), and the SDE were prepared in concentrations of 2.5, 5, 10, 15, 20, 25, 50, and 100 $\mu\text{g}/\text{mL}$. The wells were prepared as follows: 30 μL of the DMSO (Control), Orlistat[®] (Standard drug), and the SDE solutions. After that, each well was added with 250 μL of the enzymatic lipase solution, and the microplates were incubated in the dark for 5 min, at 37 °C. After that, 20 μL of substrate solution was added to each well. Microplates were incubated again for 10 min until the absorbance of the Control (at 450 nm) was equal to 1.00 ± 0.10 . The assay was performed in triplicate, and the percent of inhibition (%*I*) was calculated by using the equation 5 again. The IC_{50} was estimated by Probit analysis using the GraphPad[®] Software (USA), at a level of significance of $p=0.05$.

Antiglycation activity

Oxidative pathway

The assay was made according to Kiho and co-workers with modifications [17]. A reaction mixture was prepared to afford 10 mL of a buffer phosphate solution (0.20 M, pH 7.4) containing BSA (10 mg/mL), glyoxal (30 mM), and sodium azide (3 mM) as an antibacterial agent. For the assay, 300 μL of the reaction mixture was incubated with solutions of different concentrations of SDE (10, 25, 50, 75, 100 $\mu\text{g}/\text{mL}$, gallic acid equivalent). Quercetin was used as the standard antiglycant oxidative agent and DMSO as the negative control. As a blank, a phosphate buffer saline solution (100 μL) was used. Microplates containing substances to be evaluated were incubated at 37 °C, for 72 h, in darkness. After that, the fluorescence intensity at 370 nm (excitation) and 447 nm (emission) using a spectrofluorometer RF-1500 (Shimadzu[®], Japan). The assay was made in triplicate. Results were expressed as a percent of inhibition for estimating the IC_{50} , using the statistic package GraphPad Prism[®] 6.0, USA. Percent of inhibition was calculated as equation 6:

$$\%I = 100 - \left(\frac{F_{a/p}}{F_C} \right) \cdot 100 \quad (6)$$

$F_{a/p}$ is the blank fluorescence less the sample fluorescence, and F_C is the blank fluorescence less the control fluorescence.

Non-oxidative pathway

The assay was made in the same conditions used for the oxidative pathway but using fructose (0.1 mM) instead glyoxal. Aminoguanidine was used as the standard antiglycant drug by a non-oxidative pathway [17].

Hypoglycemic activity

Forty-nine female albino rats (10-12-week-old) were acclimatized for a week, under controlled temperature (22 ± 3 °C), relative humidity ($60 \pm 10\%$), and 12/12 h light-dark cycle. They had free access to chow and distilled water during the experiment. The assay was performed in agreement with the Ethics Committee for Use of Laboratory Animals (Ceua). Federal University of Amapá (Unifap), Macapá-AP, Brazil, protocol number 012/2017. Alloxan (100 mg/kg/every 72 h, for a week) was used by the intraperitoneal route for inducing diabetes. The blood glucose was determined using a standard glucometer. Animals with glucose level above 250 mg/dL were randomly assigned to six experimental groups (7 animals each, group II to VII):

- Group I, healthy group, treated with one mL of distilled water.
- Group II, diabetic group, received one mL of distilled water.
- Group III, diabetic group, received daily, glibenclamide 25 mg/kg.
- Group IV, diabetic group, received daily, 5 mg/kg of SDE.
- Group V, diabetic group, received daily, 25 mg/kg of SDE.
- Group VI, diabetic group, received daily, 50 mg/kg of SDE.
- Group VII, diabetic group, received daily, 100 mg/kg of SDE.

Doses of SDE were calculated as gallic acid equivalent. Treatments were administered (orally) for 25 days, using an intragastric cannula. The blood glucose was determined at days 0, 5, 10, 15, 20, and 25 using a standard glucometer (Roche®, Germany). At the same time, the food and water intake, the body weight, and the total serum cholesterol and triglycerides were determined using standard procedures of commercial kits (Sigma®, USA).

Statistical analysis

The one-way Anova followed by the Tukey's HSD test was made to evaluate statistical differences among the groups. It was considered significant statistical difference at $p < 0.05$. The analysis was made using StatGraphics® Centurion XV.1 software (USA).

RESULTS

Physic-chemical properties of CBE

The physic-chemical properties of the fresh extract of *Calophyllum brasiliense* are presented in table 2.

Table 2. Physicochemical properties of the extract of *Calophyllum brasiliense* Cambés (n=3).

Property	Value
Appearance	Brownish liquid
Aroma	Woody odor
Flavor	Astringent and bitter
Total solids (%)	2.84 ± 0.25
Density at 25°C (g/mL)	0.987 ± 0.012
pH	5.49 ± 0.02
Refraction index (n_D at 25 °C)	1.543 ± 0.025
Flavonoid (as quercetin (%))	0.65 ± 0.12
Gallic acid (%)	1.27 ± 0.10

Drying process

Table 3 presents characteristics of the dry products obtained using different combinations of drying adjuvants. The process performed with lactose monohydrate combined with colloidal silicon dioxide produced the dried extract with the higher phenolic content and yield, and the lesser moisture. Based on these results, lactose monohydrate (5%), and colloidal silicon dioxide (0.25%), were selected as drying aids for preparing the spray dried extract. The outcomes of the experimental design for the drying optimization process are presented in table 4.

Table 3. Properties of dry products obtained using different drying aids (n=3).

N.º	Drying aids (%)				Yield (%)	Phenolics (%)	Moisture (%)
	CDS	MCC	LM	MD			
1	0.25	5.00	-	-	^a 67.74±2.19	^a 5.35±0.19	^a 8.30±0.42
2	0.25	-	5.00	-	^b 75.53±3.15	^b 6.37±0.33	^b 5.62±0.38
3	0.25	-	-	5.00	^b 76.11±2.35	^c 4.86±0.72	^c 8.87±0.16

CSD: colloidal silicon dioxide; MCC: microcrystalline cellulose; MD: maltodextrin. Different letter in a column indicates statistically significant differences.

Table 4. Outcome’s matrix of the experimental design for optimizing the drying process of the hydroalcoholic extract of *Calophyllum brasiliense* (n=3).

Run	Phenolic Content (%)	Process Yield (%)	Water activity	Moisture Content (%)	Outlet Temperature (°C)
1	5.61 ± 0.23	66.84 ± 2.33	0.47 ± 0.07	5.72 ± 0.42	105.00 ± 1.25
2	5.93 ± 0.19	66.21 ± 3.11	0.47 ± 0.07	4.86 ± 0.12	93.00 ± 1.33
3	5.27 ± 0.22	77.97 ± 0.98	0.58 ± 0.09	5.18 ± 0.24	63.60 ± 1.42
4	5.42 ± 0.28	57.38 ± 1.55	0.43 ± 0.08	5.21 ± 0.29	112.70 ± 1.88
5	5.42 ± 0.20	71.48 ± 2.22	0.44 ± 0.09	4.95 ± 0.22	78.70 ± 1.95
6	4.84 ± 0.17	77.71 ± 3.47	0.51 ± 0.08	6.87 ± 0.31	100.00 ± 1.22
7	5.17 ± 0.19	70.63 ± 2.10	0.57 ± 0.07	5.78 ± 0.27	85.60 ± 1.37
8	6.13 ± 0.31	71.79 ± 2.99	0.49 ± 0.08	6.13 ± 0.41	64.00 ± 1.38
9	5.71 ± 0.23	74.74 ± 2.46	0.43 ± 0.09	6.75 ± 0.40	82.00 ± 1.76
10	4.97 ± 0.17	74.87 ± 2.45	0.57 ± 0.06	5.91 ± 0.09	73.00 ± 1.19
11	7.89 ± 0.21	70.31 ± 0.75	0.40 ± 0.09	5.03 ± 0.19	87.30 ± 1.99
12	6.06 ± 0.19	68.44 ± 1.21	0.68 ± 0.05	5.61 ± 0.23	90.67 ± 1.28
13	6.26 ± 0.23	73.87 ± 4.51	0.38 ± 0.09	5.57 ± 0.26	107.67 ± 1.72
14	6.22 ± 0.22	74.66 ± 1.77	0.62 ± 0.08	5.27 ± 0.29	88.33 ± 1.55
15	6.97 ± 0.19	64.57 ± 2.85	0.53 ± 0.05	7.29 ± 0.38	91.00 ± 1.63
16	5.38 ± 0.22	58.47 ± 0.88	0.53 ± 0.03	6.58 ± 0.33	70.70 ± 2.51
17	6.33 ± 0.10	70.01 ± 1.71	0.41 ± 0.03	6.64 ± 0.22	91.00 ± 1.79
18	7.17 ± 0.18	73.74 ± 2.08	0.57 ± 0.08	6.54 ± 0.28	83.00 ± 1.82
19	5.62 ± 0.11	72.98 ± 3.55	0.43 ± 0.06	5.12 ± 0.15	70.50 ± 1.80
20	5.26 ± 0.22	72.13 ± 2.82	0.44 ± 0.09	5.80 ± 0.19	87.20 ± 1.49
21	5.75 ± 0.29	58.88 ± 1.69	0.44 ± 0.09	5.34 ± 0.25	105.36 ± 1.65
22	5.21 ± 0.20	73.37 ± 2.31	0.56 ± 0.09	5.72 ± 0.27	72.25 ± 2.23
23	6.01 ± 0.16	73.24 ± 3.21	0.44 ± 0.09	6.35 ± 0.30	90.25 ± 1.28

Inlet air temperature and the feed flow rate were the factors that most affected phenolic content (figure 1A). The highest phenolic content was reached at 110 °C (Central level of the inlet air temperature). The increase in the feed flow rate of the suspension improved the phenolic content being higher at 11 mL/min. The air-drying speed practically no affects the phenolics content. The data of this response was transformed into the inverse square root ($1/\sqrt{}$) for fitting a quadratic model that showed a non-significative Lack of fit (table 5), adjusted R2 of 0.8237, and Q2 of 0.7888. figure 1B represents

the combined effect of factors on phenolic content, showing the large curvature of the response surface, due to the quadratic effect of the factors and their interactions (table 5).

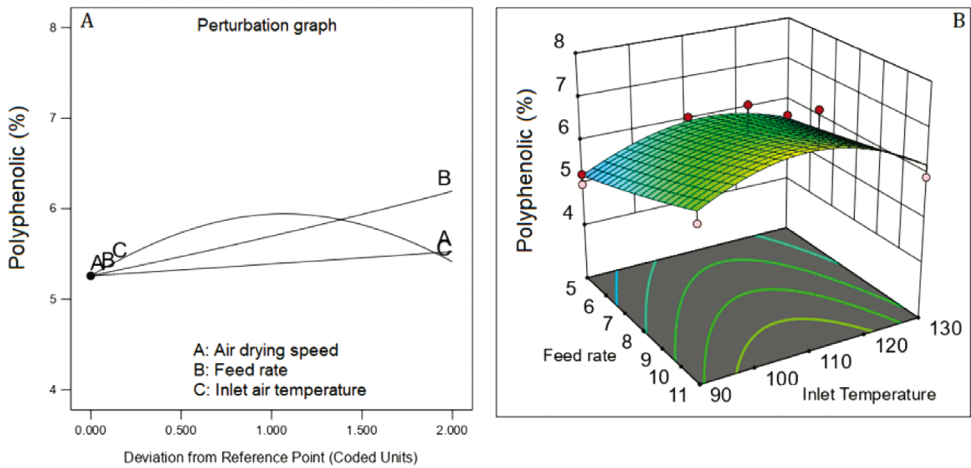


Figure 1. Effect of feed flow rate and inlet air temperature on phenolics content. Air drying speed at $4 \text{ m}^3/\text{h}$.

Table 5 also presents the effect of factors and their interactions on SDE properties. Mathematical transformations were made (when possible) for fitting the response outcome to theoretical mathematical models.

The feed flow rate of the suspension was the factor that most affected the performance of the drying process (figure 2A). The highest yield was achieved at a feed flow rate of $8 \text{ mL}/\text{min}$ (center level), being minor at the lower ($5 \text{ mL}/\text{min}$) and higher ($11 \text{ mL}/\text{min}$) levels. The increase of inlet air temperature (line C, figure 2A) and the air-drying speed (line A, figure 2A) worsened the yield. The outcome of yield was fitted to a reduced cubic model with an adjusted R^2 of 0.8887 and Q^2 of 0.7973. Figure 2B shows the large curvature of the response surface caused by the effect of factors and their interaction (table 5). The interactions feed flow rate: air-drying speed and feed flow rate: inlet air temperature improved the yield. However, the interaction air-drying speed: inlet air temperature diminished the yield (coefficients with negative signs, table 5).

Table 5. Effect of factors and interactions and their contribution to fitting the theoretical models of responses.

Factor	Total phenolics (%)		Process yield (%)		Water activity -		Moisture content (%)		Outlet temperature (°C)	
	Coefficient	P _{value}	Coefficient	P _{value}	Coefficient	P _{value}	Coefficient	P _{value}	Coefficient	P _{value}
Intercept	0.403	0.017	70.57	0.001	0.4672	0.027	0.3985	0.009	86.42	0.0000
ADS	0.003	0.008	-4.580	0.030	-0.0100	0.010	-0.0170	0.010	-	-
FR	0.003	0.005	2.940	0.001	-0.0300	0.034	-0.0030	0.005	-32.21	0.0000
IAT	0.009	0.043	7.700	0.001	-0.0400	0.034	-0.0010	0.043	-8.83	0.0009
ADS ²			-1.570	0.031		0.045			-	-
FR ²			1.610	0.022		0.214	0.0260	0.003	-	-
IAT ²	0.023	0.014	-	-		0.125			-	-
ADS*FR	0.014	0.020	3.280	0.001	-0.0800	0.008	-0.0200	0.030	-	-
ADS*IAT			-0.150	0.009			-0.0150	0.012	-	-
FR*IAT			1.980	0.035					-	-
ADS*FR*IAT			1.480	0.010					-	-
Theoretical models										
Transformation	1/√		None		1/√		1/√		None	
Type of model	Quadratic		Cubic		2FI		Quadratic		Linear	
R ²	0.8237		0.8887		0.8391		0.3662		0.9525	
Q ²	0.7888		0.7973		0.7145		0.1105		0.9416	
LoF (p _{value})	0.0690		0.0553		0.1680		0.8090		0.8563	

2FI: two factors interaction; LoF: lack of fit test; R²: goodness of fit; Q²: goodness of prediction; ns: no significant; Significance at p <0.05.

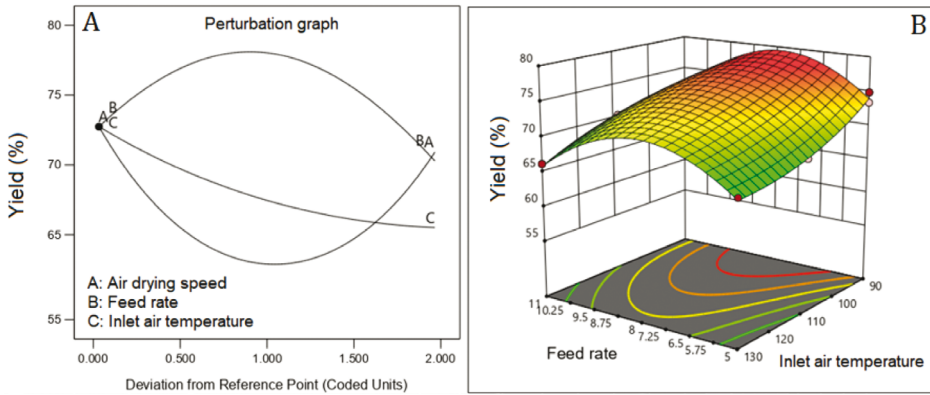


Figure 2. Effect of feed flow rate and inlet air temperature on the yield. Air drying speed at 4 m³/h.

Water activity and moisture content

Water activity and the moisture content showed inverse correlation with the three factors (figure 4A and 5A). The inverse square root of both responses fitted quadratic models. The fitted model for water activity showed good predictability (table 5) with a difference of 1.246 between R² (0.8391) and Q² (0.7145). Contrarily, the model fitted for moisture content showed low values of R² (0.3662) and Q² (0.1105), which means low predictability. The response surface graph of water activity (figure 3B) shows a decreasing curvature produced by the interaction between the air-drying speed and the feed flow rate (coefficient with a negative sign, table 5). Figure 4B presents the sharp curvature of the moisture content produced by the quadratic effect of feed flow rate (a positive sign of the coefficient FR², table 5), which was maximum at 8 mL/min (center level).

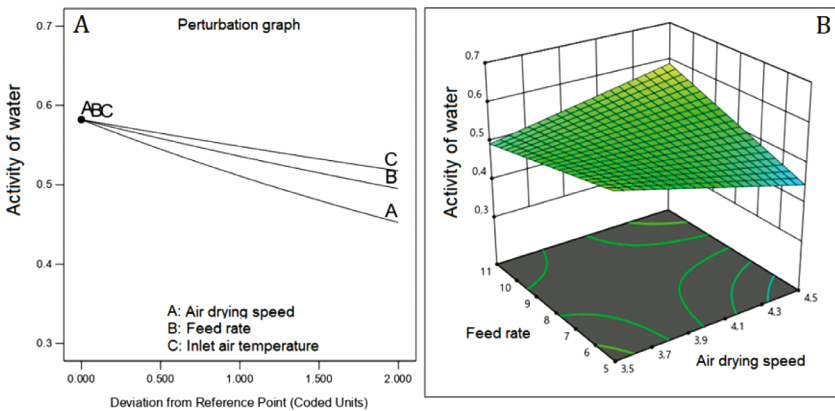


Figure 3. Effect of feed flow rate and air-drying speed on water activity. Inlet air temperature at 110 °C.

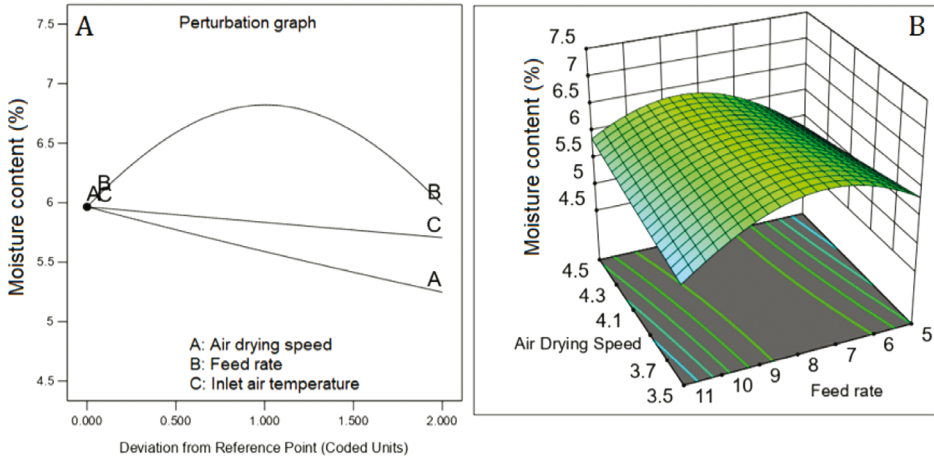


Figure 4. Effect of feed flow rate and the air-drying speed on moisture content. Inlet air temperature at 110 °C.

The outlet air temperature diminished as the feed flow rate increased, showing an inverse correlation (line B, figure 5A). The outlet air temperature raised with the increase of inlet air temperature. By their side, as the air-drying speed increased, the outlet temperature decreased, showing an inverse correlation. This response fits a linear model with an adjusted R2 of 0.9525, Q2 of 0.9416, and a non-significant Lack of fit (table 5). Figure 5B shows a sharp decrease in the response surface of the outlet air temperature caused by the linear effect of the factors, but mainly, by the effect of the feed flow rate.

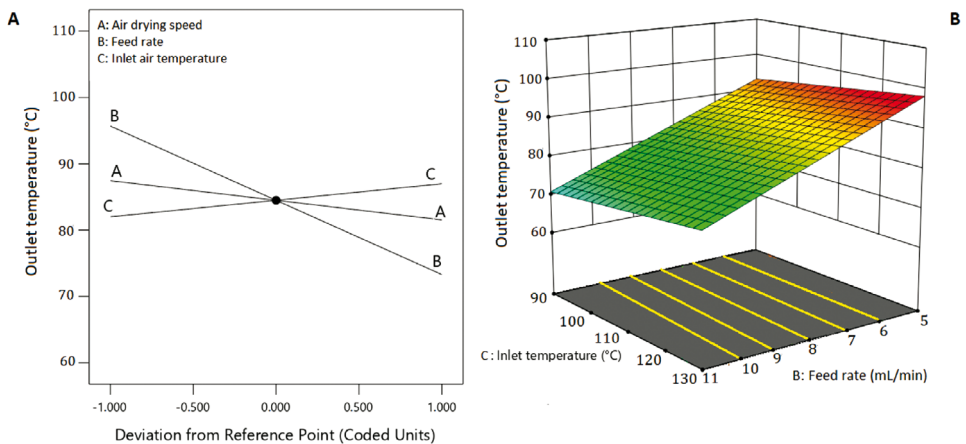


Figure 5. Effect of inlet air temperature and the feed flow rate of the suspension on the outlet temperature. Air drying speed at 4 m³/h.

Multiple response analyses

The optimization of the drying conditions was made by using the Desirability function. The objective was to obtain a dried extract with the high phenolics content, with an excellent product recovery (yield), a low moisture content, and a low activity of water. The prediction showed a Desirability value of 0.731. The predicted value for each parameter is presented in figure 6.

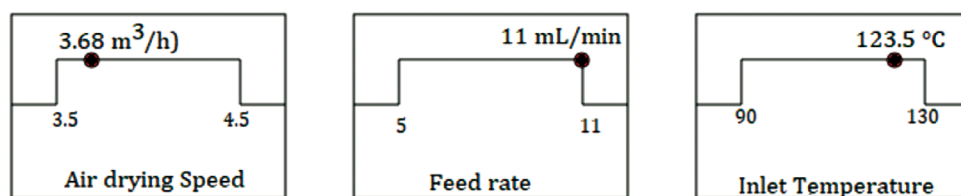


Figure 6. Values of the factors predicted by the desirability function for optimizing the spray drying process.

Three experiments were made using for drying the predicted conditions: Air Drying Speed 3.68 m³/h, Feed flow rate 11 mL/min, and Inlet air temperature 123.5 °C. Table 6 presents a comparison between the predicted and experimental results at $p < 0.05$. In all cases, the t-test suggests that there were no statistically significant differences between the experimental and the predicted values.

Table 6. Statistical comparison of Predicted vs. Observed results for the drying optimization process.

Response	Predicted value	Observed value	t- test, p-value
Yield (%)	74.91 ± 2.60	72.75 ± 1.60	0.0729, 0.9454
Phenolic content (%)	6.48 ± 0.62	6.97 ± 0.05	1.3645, 0.2441
Water activity	0.47 ± 0.06	0.41 ± 0.01	1.6617, 0.1719
Moisture content (%)	5.23 ± 1.85	5.73 ± 0.35	0.3171, 0.2641
Outlet temperature	87.25 ± 3.25	84.46 ± 4.25	1.3658, 0.2214

n=3, significant at $p < 0.05$.

Physic mechanical properties

The optimized SDE exhibited a tap density of 0.470 ± 0.017 g/mL, bulk density 0.367 ± 0.012 g/mL, Carr's index $21.99 \pm 1.06\%$, and a flow rate 2.95 ± 0.17 g. s⁻¹ (using an exit orifice of 8 mm).

Particle morphology and size

The grains morphology of the optimized SDE of *Calophyllum brasiliense* was evaluated by SEM. Microphotographs obtained at 1000 (figure 7A) and 5000x (figure 7B) of magnification can be observed the presence of shape-rounded particles of diverse sizes, with clusters of particles of minor sizes. The SDE showed a normal distribution of particle size (figure 8) with a Sauter mean diameter ($D_{3,2}$) of 61.23 μm . The data analyses for normality showed standardized skewness of 0.87 and kurtosis of -0.59.

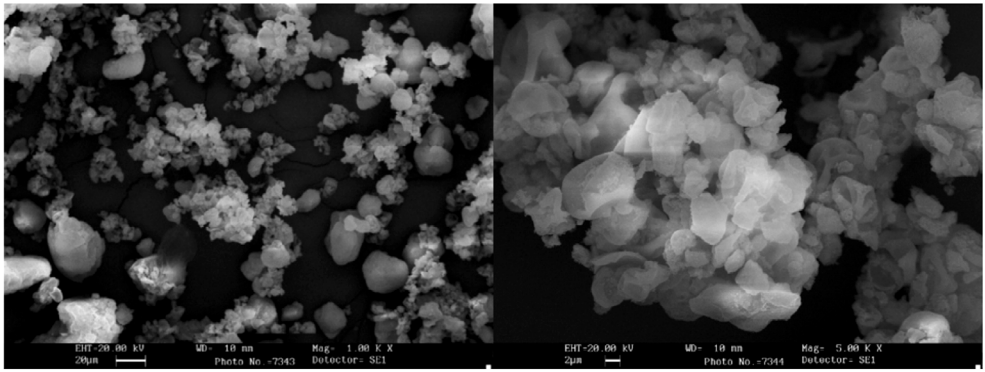


Figure 7. Scanning electronic microphotographs of the optimized spray dry extract of *Calophyllum brasiliense* Cambés.

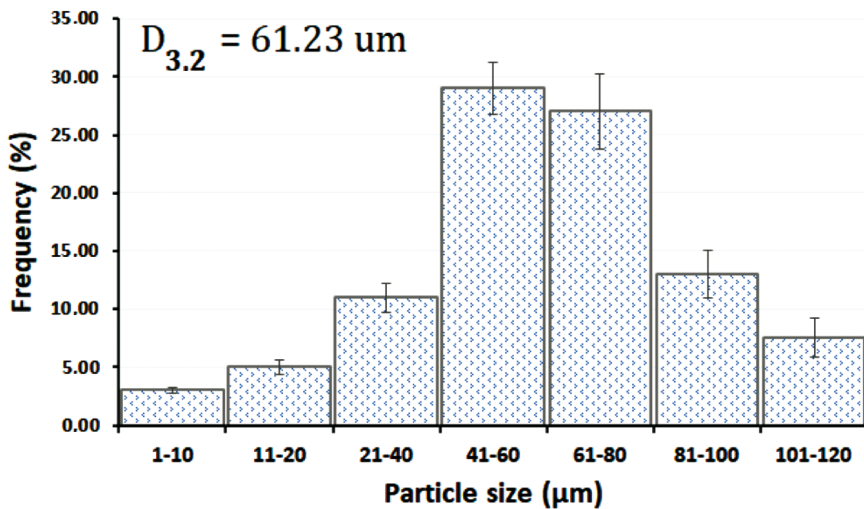


Figure 8. Particle size distribution of the spray dried extract of *Calophyllum brasiliense* Cambés.

Flavonoids and gallic acid content

The spray dried extract of *Calophyllum brasiliense* Cambés content $9.5 \pm 0.52\%$ of gallic acid and $4.85 \pm 0.81\%$ of flavonoids as quercetin.

Antioxidant activity

The antioxidant effect of the SDE and the capacity to inhibit α -glycosidase and pancreatic lipase is presented in table 7. The blank solution showed neither antioxidant activity nor α -glycosidase and pancreatic lipase inhibitory effect. The antioxidant effect of the SDE ($IC_{50} 1.83 \pm 0.90 \mu\text{g/mL}$) was not statistically different from the gallic acid ($IC_{50} 2.87 \pm 0.95$), the standard antioxidant compound ($t=148.56$; $p=0.1478$).

Table 7. Antioxidant effect and enzymatic inhibition capacity of the spray dried extract.

Substance	IC_{50} ($\mu\text{g/mL}$)		
	DPPH	α -glycosidase	Lipase
Blank	Φ	Φ	Φ
Dry extract	1.83 ± 0.90^a	74.45 ± 1.45^a	27.33 ± 2.70^a
Gallic acid*	2.87 ± 0.95^a	-	-
Acarbose**	-	65.30 ± 0.60^b	-
Orlistat**	-	-	0.29 ± 0.01^b

Control. Φ : do not inhibit. Different letters in a column denote statistically significant differences.

Lipase and α -glycosidase inhibition

The t-test showed a statistically significant difference ($t=6.24$; $p=0.0234$) between the inhibitory effect of α -glycosidase produced by SDE ($IC_{50} 74.45 \pm 1.45$) and Acarbose® ($IC_{50} 65.30 \pm 0.60$) (table 7). On the other side, SDE exhibited a lipase inhibitor effect ($IC_{50} 27.33 \mu\text{g/mL}$) less potent and statistically different ($t=15.15$; $p=0.0001$) of Orlistat® ($IC_{50} 0.29 \mu\text{g/mL}$).

Antiglycation effect

The potential of SDE to inhibit the end-glycation products (AGEs) formation by both the oxidative and the non-oxidative pathway was evaluated (table 8) using bovine serum albumin (BSA) as model protein. By the oxidative way, SDE showed an IC_{50} of $9.85 \mu\text{g/mL}$, while by the non-oxidative pathway exhibited an IC_{50} of $27.55 \mu\text{g/mL}$. In both cases, the effect of the SDE was stronger than the controls (quercetin by the oxidative pathway and aminoguanidine by the non-oxidative one).

Table 8. Antiglycation effect of the spray dried extract of *Calophyllum brasiliense* Cambés.

Test Substance	Oxidative Pathway		Non-Oxidative Pathway	
	Inhibition (%) (at 100 µg/mL)	IC ₅₀ (µg/mL)	Inhibition (%) (at 100 µg/mL)	IC ₅₀ (µg/mL)
Spray dried extract	99.25 ± 3.25 ^a	9.85 ± 1.25 ^a	98.55 ± 5.78 ^a	20.77 ± 3.27 ^a
Quercetin	86.55 ± 4.18 ^b	36.25 ± 2.92 ^b	-	-
Aminoguanidine	-	-	80.75 ± 6.24 ^b	27.35 ± 4.71 ^b

Different letter in the column indicates statistically significant differences at $p < 0.05$.

Hypoglycemic activity

The blood glucose level of animals of the group I kept between 95-105 mg/dL (figure 9) during the experiment, however, in diabetic animals without treatment (group II) it increased to 335 mg/dL. The blood glucose of the animals treated with glibenclamide 25 mg/kg (group III) was reduced to normal levels in 10 days. All groups treated with the SDE (groups IV to VII) reduced the glucose levels. SDE at 5 mg/kg (groups IV) and 25 mg/kg (group V) was not sufficient to reduce the blood glucose of animals to normal levels. However, doses of 50 mg/kg (group IV) and 100 mg/kg (group V) reduced blood glucose to normal levels in 15 days. No significant statistical difference was observed between the effect of glibenclamide (25 mg/kg) and the SDE at 50 and 100 mg/kg ($p < 0.05$).

Throughout the experiment, the animals of all groups drank water and ingested food normally, except the animal of the group II, which reduced the food consumption and increased the intake of water (table 9). The animals' body weight of all groups, at the day 0, did not present statistically significant differences, however, the day 25, animals of the group II (diabetics without treatment) exhibited bodyweight higher and statistically different than the rest of the groups ($p < 0.05$).

During the experiment, the total serum cholesterol of the group I kept between 91 and 93 mg/dL, differently from group II where increased to 162.35 mg/dL at the end of the experiment. (table 9). In the group III the serum cholesterol increased to 152.83 mg/dL, the day 25. In groups IV and V the serum cholesterol increased slightly above of the superior normal limit of 130 mg/dL [20], however, group VI and group VII showed a strong diminution of total serum cholesterol, reaching (the day 25) values statistically equal to the Group I (normal control).

Serum triglyceride of the group I showed normal values along the experiment (51-54 mg/dL). The group II exhibited elevated values of serum triglycerides along the time reaching 244.15 mg/mL, at the day 25. In the group III it was observed an increase of

the serum triglycerides up to 249.35 mg/dL (table 9), however, the groups IV to VII diminished the serum triglycerides to normal values after at the day 25 (group IV) and at the day 15 the groups V, VI and VII. Notice that, the day 25, the serum triglyceride of the group I and VII were not statistically different ($p > 0.05$).

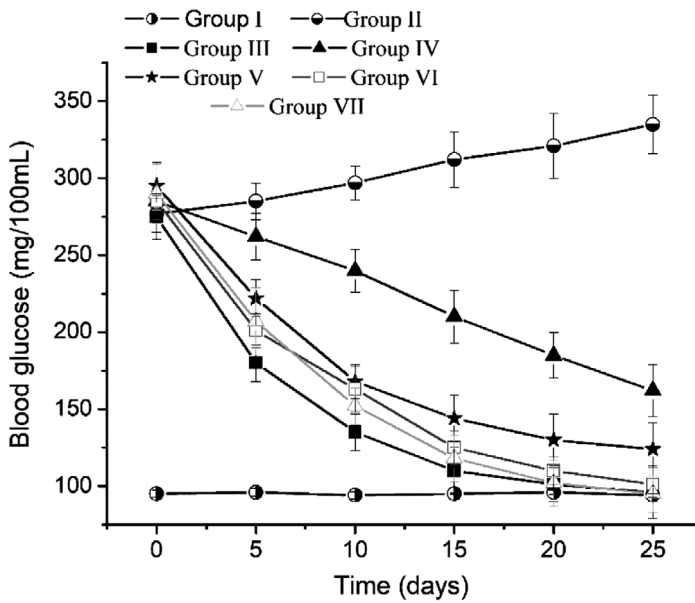


Figure 9. Hypoglycemic effect of the spray dry extract of *Calophyllum brasiliense* Cambés in Alloxan induced-diabetic rats. Group: I, healthy group, treated with one mL of distilled water; II, diabetic group, received one mL of distilled water; III, diabetic group, received daily, glibenclamide 25 mg/kg; groups IV-VII, diabetic groups treated daily with 5, 25, 50 and 100 mg/kg of SDE, respectively.

DISCUSSION

CBE appears as a brownish liquid, having a strong woody aroma with a bitter and astringent flavor. The content of phenolics ($1.27 \pm 0.10\%$, expressed as gallic acid) and flavonoids ($0.65 \pm 0.12\%$, as quercetin) provided the woody aroma and the astringent taste to the extract. The extraction method used here differs from that proposed by Carvalho and coworkers whose made a hot extraction at 45°C for 96 h, using a drug/solvent ratio of 1:8 (v/v) [6]. Our methodology is simpler than that, exhausting the drug in just 3 days at room temperature, producing an extract with higher solids content (2.87%).

Table 9. Intake of food and water, body weight, and total serum cholesterol and triglycerides of experimental groups during the evaluation of the hypoglycemic effect of the spray dried extract of *Calophyllum brasiliense*.

Parameter	Day	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Water intake (mL)	25	32.55 ± 4.56 ^a	38.25 ± 3.27 ^b	30.45 ± 2.58 ^a	33.25 ± 4.10 ^a	32.58 ± 3.91 ^a	32.72 ± 5.16 ^a	31.29 ± 4.22 ^a
		23.50 ± 2.35 ^a	15.33 ± 3.37 ^b	24.11 ± 4.63 ^a	23.42 ± 3.33 ^a	24.58 ± 4.60 ^a	23.77 ± 3.19 ^a	25.88 ± 3.01 ^a
Food intake (g)	0	155.05 ± 6.51 ^a	158.55 ± 6.55 ^a	157.21 ± 4.99 ^a	159.28 ± 7.39 ^a	159.81 ± 4.70 ^a	156.99 ± 6.19 ^a	158.77 ± 7.21 ^a
		167.33 ± 7.58 ^a	178.82 ± 5.11 ^a	169.96 ± 6.01 ^a	168.28 ± 5.10 ^a	167.45 ± 6.60 ^a	167.97 ± 5.31 ^a	165.55 ± 4.55 ^a
Body weight (g)	25	175.35 ± 5.11 ^a	192.40 ± 6.88 ^b	177.25 ± 8.22 ^a	175.85 ± 4.69 ^a	176.83 ± 4.09 ^a	174.22 ± 6.70 ^a	172.10 ± 5.58 ^a
		91.33 ± 2.25	138.45 ± 3.36 ^a	138.91 ± 3.82 ^a	142.44 ± 4.55 ^a	142.48 ± 4.02 ^a	141.37 ± 2.74 ^a	144.70 ± 3.77 ^a
Cholesterol (mg/dL)	15	92.57 ± 2.85	148.41 ± 5.66 ^a	145.86 ± 4.02 ^a	139.41 ± 2.32 ^a	136.47 ± 3.33 ^a	133.66 ± 3.28 ^a	102.06 ± 4.20 ^a
		90.21 ± 2.16 ^a	162.35 ± 4.80 ^b	152.83 ± 3.05 ^c	131.53 ± 4.17 ^c	128.55 ± 3.57 ^d	128.41 ± 5.11 ^c	92.00 ± 4.01 ^c
Triglycerides (mg/dL)	0	52.25 ± 1.26 ^a	192.12 ± 3.00 ^a	193.25 ± 2.25 ^a	195.12 ± 3.17 ^a	185.08 ± 4.27 ^a	196.32 ± 3.37 ^a	192.02 ± 3.19 ^a
		53.15 ± 1.26 ^a	214.11 ± 3.17 ^a	209.33 ± 3.55 ^a	164.02 ± 2.87 ^a	130.22 ± 4.27 ^a	116.32 ± 3.01 ^a	74.00 ± 2.08 ^a
	25	51.37 ± 2.09 ^a	244.15 ± 4.99 ^b	249.35 ± 3.57 ^c	111.28 ± 4.18 ^d	99.82 ± 3.93 ^a	65.49 ± 3.39 ^a	52.30 ± 3.01 ^a

Group: I: healthy group, treated with one mL of distilled water; II: diabetic group, received one mL of distilled water; III: diabetic group, received daily, glibenclamide 25 mg/kg; groups IV-VII: diabetic groups treated daily with 1, 10, 100 and 1000 mg/kg of SDE, respectively. Normal values: serum total cholesterol 80-130 mg/dL; serum triglycerides 43-110 mg/dL [20].

Throughout the investigation for obtaining pharmaceutical products based on plant extracts is crucial to establish physic-chemical parameters (properties) for controlling the quality and warrantee the product reproducibility. The content of phenolics, flavonoids, total solids, and other properties like density (0.787 g/mL, at 25 °C), refractive index (1.543), and pH (5.49) could be used, preliminarily, for controlling the quality of CBE. Up to our knowledge, there are no technological studies about the preparation and physic-chemical characterization of the extract of *Calophyllum brasiliense* Cambés.

Botanical extracts submitted to spray drying processes, generally produce sticky materials that adhere to the wall chamber, diminishing the product recovery, hampering the yield, and having unacceptable technological properties [21]. To avoid these problems, solid excipients known as drying aids (adjuvants) are using. An appropriate selection of the drying aids is crucial for obtaining vegetal dried extracts with suitable physic-mechanical properties [22]. In this work, the effect of different combinations of drying aids in the dried extract properties was evaluated, for selecting those providing the high recovery of the dried material (yield), the high content of phenolics, and the minor moisture content (table 3).

The higher phenolic content, and higher yield as well as the minor moisture content, was obtained using lactose monohydrate (5.0%) and colloidal silicon dioxide (0.25%). The drying with maltodextrin or microcrystalline cellulose, instead lactose, had minors yield and produced extracts with higher moisture content. This result contradicts diverse works where the use of microcrystalline cellulose as drying aids for vegetal extracts produced excellent materials. However, there is not a rule, and the drying adjuvants need to be evaluated on a case-by-case basis.

A drying method is as good as its capability to preserve the drug. The critical response in the CBE drying process is the phenolic content. The amount of phenolics in the SDE is a measure of preservation of the actives during the drying process. The antioxidant, hepatoprotective, and hypoglycemic effect of CBE are associated with phenolics and flavonoids content. The inlet air temperature was the factor that most affected the phenolics content. This response was negatively correlated with the air inlet temperature. On the contrary, Phenolics content was positively correlated with the feed flow rate and was practically no affected by the air-drying speed. The higher amount of suspension in the drying chamber, due to the higher feed rate, increases the amount of heat interchanged, diminishing the chamber temperature avoiding the phenolics' degradation. This result agrees with Kha and coworkers [23], which observed that temperatures above 110 °C produced a significant loss of phenolics with consequent loss of the antioxidant activity. The fitted model for phenolics (quadratic model) showed excellent agreement (difference of 0.0349) between the goodness of fit (R2)

and goodness of prediction (Q_2). Difference minor than 0.3 between these values suggest good model predictability.

In spray drying processes, the product recovery - yield - depends not only on the drying parameters but on the characteristics of the product to be dry and the use of drying aids, among other factors. Usually, the drying of plant extract produces sticky materials and low processes yield. Drying aids are used for obtaining a high product recovery, free-flowing materials, and for improving the extract solubility, and stability. The feed flow rate of the suspension was the factor that most affected the yield. The yield was inversely correlated with inlet air temperature and the air-drying speed. This result could be explained because of the higher the inlet air temperature, the higher the heat transfer with a high loss of heat to the environment. On the other side, the increase in feed flow rate could result in a higher yield, probably, due to a decrease in the average drying temperature [24]. In both cases, the product recovery increases, improving the yielding. The yield data fitted a reduced cubic model showing high values of R^2 and Q_2 and a difference of 0.0914 between these values, suggesting good model predictability [15].

Water activity and the moisture content are critical properties of pharmaceutical products. Low moisture content and low water activity are necessary for obtaining suitable physic-mechanical properties [25]. Water activity below 0.60 impairs microbial proliferation improving product stability [26]. The water activity and moisture content are associated and depend on the evaporation rate of liquid from the particle, which is governed by the quantity of heat interchanged during the process [16, 25], among other thermodynamical factors. The moisture content of SDE in all runs kept below 7.29%, and the water activity remained below 0.62. These values are suitable for maintaining the microbiological stability of solid pharmaceutical products. As was expected, the higher the inlet air temperature and air-drying speed, the lower the moisture content and water activity due to the increase of the water evaporation rate and the decrease of the resident time of the extract in the drying chamber [21, 27]. In the same way, the combined effect of feed flow rate and air-drying speed reduce the moisture content and water activity (coefficient with a negative sign, table 5).

The outlet temperature depends on factors like the inlet air temperature, and the heat transferred from the drying gas to the solid material during the evaporation process [27]. In this work, the outlet air temperature was inversely correlated to feed flow rate and the air-drying speed (figure 5A, table 5). This result agrees with Quek *et al.* [28], which reported that the increase of feed flow rate diminished the outlet air temperature and raised the amount of material adhered to the wall chamber. The outlet air temperature fitted a linear model with high values of R^2 and Q_2 (0.9525 and 0.9416,

respectively). Figure 5B exhibits the linear response surface of these responses due to the positive effect of the factors (table 5). The higher the outlet temperature, the minor the moisture content, and water activity [27]. However, with the increase in outlet air temperature, a diminution of the phenolic content was observed. This result agrees with the work of Cortés and coworkers during the production of the spray-dried extract of *B. pilosa* [2], which informed the same behavior for outlet temperature.

The process optimization ensures the reproducibility of drying processes and the obtainment of dried materials of high quality. The responses used for optimization were phenolics content, yield, outlet temperature, and the water activity, due to the high predictability of the fitted models [15]. The optimization aimed to establish processing parameters (values of factors), for producing an SDE with a high content of phenolics, high yielding, and low moisture and water activity. The desirability function had a value of 0.731. The closer to one the desirability value the higher probability to achieve the desired goals [26]. All the responses showed no statistical differences between the observed and predicted values (table 6). This result allows validating the spray-dryer performance using the operational conditions predicted by the optimization tool.

The physic mechanical properties and particle shape and size of the optimized SDE were evaluated. Particle shape and particle size are properties to be considered in solid pharmaceutical materials. SDE exhibits rounded particles (figure 7A) with a Sauter mean diameter of 61.23 μm . The particle size distribution showed normality with kurtosis and skewness between ± 2 [15]. Particles of minor sizes probably formed due to a low number of drying aids in their formation were observed. The trend to form clusters (figure 7B) could result from a high viscosity and surface tension of the liquid suspension, and probably, by a faster grain consolidation [27]. The rounded particles presents in SDE, with a normal size distribution, and a low moisture content of SDE could explain the good flowability (2.95 g/s; orifice 8 mm) and compressibility (Carr' index 21.99%) of this product [15]. Size distribution, particle shape, and moisture are crucial properties for storing and handling solid materials, especially during the implementation of pharmaceutical operations as mixing and compression [15, 29]. The optimization of the drying process and the use of drying aids allows obtaining an SDE with suitable physic-mechanical properties for use in solid forms, like granules, capsules, and tablets.

The use of botanical extracts is as an excellent way for treating multicausal disease like diabetes. Besides the multiple mechanisms acting together, plant extracts produce few adverse effects and are well-accepted by patients. One of the mains uses of the extract of CBC is for reducing blood glucose of diabetic patients [30, 31]. However, are lacking studies evaluating possible hypoglycemic mechanisms of CBC extracts. In this context, the antioxidant activity of SDE and its capability to inhibit α -glycosidase

were evaluated. In both assays, the Blank solution does not show inhibitory activity, indicating that the effect produced by the SDE is produced by compounds present on it. SDE showed an excellent antioxidant effect, that was not statistically different from the gallic acid ($t=1.56$; $p=0.1478$, at $p < 0.05$). In the same way, SDE inhibited α -glycosidase at IC_{50} of $74.45 \mu\text{g/mL}$. The effect was slightly minor and statistically different than Acarbose[®] ($IC_{50}= 65.30 \mu\text{g/mL}$). Phenols and flavonoids are OH-rich compounds that could be bonding to carbohydrates impairing the normal enzymatic function. On the other side, they are responsible for the strong scavenging of free radicals, producing a potent antioxidant effect [7]. Alpha-glycosidases hydrolyze complex carbohydrates for forming simple carbohydrates [31]. The inhibition of α -glycosidase avoids the sharp increase of the postprandial carbohydrates level in blood, improving the glycemic control [31]. SDE also inhibited pancreatic lipase showing an IC_{50} of $67.49 \mu\text{g/mL}$ but was less potent than Orlistat[®] ($IC_{50} 0.29 \mu\text{g/mL}$). In obese diabetics, the use of lipase inhibitors could be an effective way of lowering fat absorption, reducing the risks associated with type II diabetes. Drugs α -glycosidase and pancreatic lipase inhibitors are scarce. Botanical extracts with both effects are still more reduced remarking the potential of this extract as a therapeutic agent.

The SDE showed a potent antiglycation effect with IC_{50} $9.85 \mu\text{g/mL}$ by the oxidative pathway and $20.77 \mu\text{g/mL}$ by the non-oxidative one. By both pathways, the effect was more potent than the control quercetin $36.25 \mu\text{g/mL}$ (oxidative way), and amino-guanidine $27.35 \mu\text{g/mL}$ (non-oxidative way). Blood carbohydrates are augmented in uncompensated diabetics. Advanced glycation end products (AGEs) are produced by the reaction of lipids and/or proteins with reduced sugars. They are the main cause of degenerative diseases associated to diabetes like atherosclerosis, chronic kidney disease, and retinopathy [32]. Although the antiglycation effect of plants extract has not been established, some studies suggest that may act hindering the formation of AGEs by preventing further oxidation of Amadori products [33]. This effect can be potentiated by the strong antioxidant effect produced by the SDE, in which phenols and flavonoids plays a special role. In this sense, must be take on account the strong antiglycation effect of the SDE by the oxidative pathway with IC_{50} $9.85 \mu\text{g/mL}$.

In this work, the hypoglycemic effect of SDE in diabetic rats was evaluated using four doses (5, 25, 50, and 100 mg/kg of body weight). Animals of all groups consumed food and drank water normally, except in the group II, which consumed a smaller amount of food that was statistically different from the rest of the groups. This probably happened by the uncontrolled increase of carbohydrates in blood as the animals get worse because they were not treated. The body weight increased in all groups, however, in the group II was higher and statistically difference from the rest of the groups.

Low doses of the SDE (5, and 25 mg/kg) were not effective for reducing the blood glucose of the diabetic rats (groups IV and V), however, doses of 50 and 100 mg/kg produced a potent blood sugar reduction, that was not statistically different from the effect of Glibenclamide 25 mg/kg (group III), onward the day 15 of the experiment. The potent hypoglycemic effect of the SDE could be occurs via inhibition of α -glycosidase, and due to the strong antioxidant effect of this extract.

The SDE lowered total serum cholesterol and triglyceride levels of diabetic animals, being the effect more potent at 100 mg/kg (group VII). The reduction of carbohydrates blood levels onward the day 15 allow improves cholesterol and triglycerides levels. This result agrees with that reported by Carvalho and co-workers who prepared granules for using a liquid extract of CBC [6]. On the other side, the antioxidant effect of the SDE and the inhibition of pancreatic lipase produced by this extract can actively contribute for reducing serum triglycerides.

The spray dried extract of CBC produced a potent hypoglycemic effect in diabetics animal, however, despite the animals has consumed a normal food, after the day 15 the blood glucose level, total serum cholesterol and triglyceride were normal, allowing that the animals maintaining a normal live eating food and drinking water normally. These results must be associated to the effect of the SDE, which could have controlled the diabetes symptoms by three different mechanisms: via antioxidant, via α -glycosidase and lipase inhibition, and via antiglycation. SDE of *Calophyllum brasiliense* Cambés arise as a promissory standardized product, which could be used properly in diabetic/obese patients.

CONCLUSIONS

There are evidence suggesting the use of plant products as an excellent therapeutic strategy for treating multicausal diseases like diabetes. The diversity of compounds presents in the extracts produces a multi-target effect synergizing the beneficial response of patients. The SDE optimized and characterized in this work exhibited excellent hypoglycemic effect in vivo, probably mediated by the potent antioxidant and antiglycation activity, and the powerful capability for inhibiting α -glycosidase. At the same time, SDE at 100 mg/kg reduces the serum cholesterol and triglycerides, probably mediated by the inhibition of pancreatic lipase. The SDE of *Calophyllum brasiliense* Cambés arise as a promissory hypoglycemic product due to the capacity to activate different mechanisms associated to diabetes control. However, other studies must be done for evaluating the real utility of SDE in diabetics.

DISCLOSURE STATEMENT

The authors declare that there are not conflicts of interest.

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