

Article

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Study of dissolution profiles and desintegration of capsules containing the dried hydroethanolic extract of *Calophyllum brasiliense*

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Abstract: *Calophyllum brasiliense* Cambess, Calophyllaceae, is of great interest in folk medicine and is used in the treatment of various diseases such as diabetes. Granules containing the hydroethanolic extract from the stem bark of *C. brasiliense* were obtained. The polyphenol content was standardized, and the average weight, disintegration, and the dissolution profiles of the capsules were determined after encapsulation. The capsules had an average weight of 574.5±8.0 mg. *In vitro* tests showed that the most efficient disintegration profile was in hydrochloric acid buffer (pH 1.2), with a capsule disintegration time within 9 min. The dissolution analysis showed a better uniformity of capsule content release when the test was performed in a hydrochloric acid buffer (pH 1.2), with a maximal release rate at 15 min (giving a polyphenol content of 4.38%, which corresponds to a concentration of 0.0080 mg/mL). In distilled water, the maximal release was reached at 20 min (giving a polyphenol content of 5.41%, which is equivalent to 0.0105 mg/mL). In phosphate buffer, the maximal release of capsule contents was reached at the end of the dissolution assay (30 min), with the lowest amount of released polyphenols (3.61%, which corresponds to a concentration of 0.0070 mg/mL). The encapsulated form of the hydroethanolic extract of *C. brasiliense* was shown to have the necessary traits of a desirable delivery agent, and the dissolution test was an effective analysis of this material's polyphenol release profile for the specific dosage form.

Introduction

The evolution of organic chemistry has allowed the large-scale production of synthetic compounds for therapeutic applications. Because these compounds are modularly produced by organic synthesis, molecular modifications that improve the activity and therapeutic potency of these drugs have been possible. However, interest in plant products for therapeutic applications has recently increased due to the many side effects caused by synthetic drugs, the high cost of drug therapy, the difficulty encountered by the pharmaceutical industry in developing new synthetic therapeutic agents, the high cost of research and production of new biologically active molecules, and the growing demand for medicinal plants with pharmacological activity (Capasso, 2000; Rates, 2000; Niero et al., 2003).

Medicinal plants facilitate the development and production of new drugs in a shorter period of time because researchers already have clues on the biological activities of a plant based on the history of its use by the general population prior to beginning research. (Funari & Ferro, 2005).

Calophyllum brasiliense Cambess, Calophyllaceae (Cb) is known by several common names, including "jacareúba", "guanandi", "guanandicarvalho", "cedro-do-pântano", and "landim", among others (Pereira, 1966). In folk medicine, this plant has been used for the treatment of various diseases such as bronchitis, liver and gastrointestinal disorders, pain, inflammation, diabetes, hypertension, diarrhea, rheumatism, hemorrhoids, varicose veins, and herpes (Silva et al., 2001).

Pharmacological studies on this species are

scarce; however, the extract of this plant has been shown to display antiretroviral activity (Huerta-Reyes et al., 2004), as well as activity against the *Trypanosoma cruzi* parasite (Abe et al., 2004), *Bacillus cereus*, and *Staphylococcus epidermidis* (Cottiglia et al., 2004). The extract from the stem bark has also been shown to possess gastroprotective, anti-ulcer, and cytoprotective effects (Sartori et al., 1999). In addition, anti-neoplastic activity has been reported for brasixanthones present in the stem extract (Ito et al., 2003). Furthermore, some coumarins and xanthenes of the extract have shown an inhibitory effect on the release of stomach acid (Reyes-Chilpa et al., 2006).

Herbal medicines derived from plants are produced by the appropriate technological processes using only plant material as the source for biologically active compounds. The production of herbal medicines is based on formulations developed from parts of the plant that are able to keep their phytochemical integrity during the production process (Carvalho, 2004).

Plant products are standardized based on the concentration of a single active compound. This compound, called a chemical marker, ensures the high quality of the plant material and uniformity of its biological activity. A method for ensuring uniformity is to obtain a profile of all significant known and unknown chemical compounds in the plant (Perrone et al., 2012). A method for the quantification of total polyphenol content was reported by Swain & Hillis (1959). This method is used already to tannin quantification, it is based on the reduction of phosphomolybdic tungstic acid by phenolic hydroxyl groups, producing a dark color complex in alkaline medium that absorbs in the visible spectrum ($\lambda = 620$ to 760 nm).

Based on the importance of the pharmacological applications of Cb, we formulated and studied the granular of the hydroethanolic extract from the stem bark of *Calophyllum brasiliense* (HEECb) to standardize the products from this plant.

Material and Methods

Collection of plant material

The stem bark of the *Calophyllum brasiliense* Cambess, Calophyllaceae, plant was collected in the city of Ferreira Gomes (native area), Amapá, Brazil. The botanical fertilized material was identified in the herbarium of the Amapá State Research Institute (Instituto de Pesquisa Científicas e Tecnológicas do Amapá, Voucher number 0598AP).

Production of hydroethanolic extract

The HEECb were obtained by crushing and

grinding 2 kg Cb bark macerated in percolator model LM20 (Lemaq Ltda, São Paulo, Brazil) with 70% (v/v) aqueous ethanol at 45 °C for four days at a ratio of 1:8 (w/v). The extraction solution was filtered through filter paper and concentrated to dryness using a rotary evaporator model Q.218.2 (Quimis Ltda, São Paulo, Brazil) at 70 °C and 530 mmHg. The final yield was 31%.

Production of granules

Granules were obtained by manual mixture wet granulation using the following combination of extract and excipients: 21.80% cellulose Avicel® (Sigma-Aldrich Co., St. Louis, USA), 3.87% magnesium stearate Riedel-de Haën® (Sigma-Aldrich Co., St. Louis, USA), 33.35% D-lactose monohydrate Vetec® (Vetec Química Fina Ltda, Rio Janeiro, Brazil) 9.06% corn starch Duryea® (Unilever Brazil Industrial Ltda, Pernambuco, Brazil), 5.28% water and 26.64% dry HEECb.

Production of capsules

Gelatin capsules (size 1) were used to manually encapsulate the granules. To control the amount of encapsulated plant material, the average weight was determined using the following equation:

$$Tec = \frac{Mmc.Meg}{Mtg}$$

where: Tec: extract content in the capsules; Mmc: average mass of the capsule (g); Meg: mass of extract in the granules (g); Mtg: total mass of granules (g).

Average weight

The average weight was determined based on the methodology described in the Brazilian Pharmacopoeia (F. Bras. V, 2010). The weights of twenty capsules were determined.

Disintegration test

The disintegration test was performed in a capsule disintegrator model 301/3AC (Nova Ética, Ltda. São Paulo, Brazil) that contained three separate vessels. Six capsules were placed in each vessel. Distilled water (DW), hydrochloric acid buffer (HAB) pH 1.2 and phosphate buffer (PB) pH 6.8 were used as immersion liquids. The disintegration time was defined as the time required for the total disintegration of the capsules (F. Bras. V, 2010).

Dissolution test

The dissolution test was carried out in a dissolution apparatus with three vessels model 299 (Nova Ética, Ltda. São Paulo, Brazil) at a stirring speed of 75 rpm. In each vessel, 800 mL HAB pH 1.2, PB pH 6.8, or DW was used as a dissolution medium. All tanks were kept at $37 \pm 1^\circ\text{C}$. Total polyphenol contents were used as a marker for the HEECb, and assays were performed with six replicates for each dissolution medium.

The dissolution profiles were determined based on the quantification of total polyphenol content from the capsules dissolved in the dissolution vessels at 5, 10, 15, 20, and 30 min. Aliquots of 3 mL were filtered through filter paper (12.5 cm diameter, 80 g/m²), and 2.5 mL filtered samples were used to measure the amount of total polyphenols using a spectrophotometer model UVmini-1240 (Shimadzu Corporation, Kyoto, Japan) at 760 nm, based on the adapted methodology described by Sousa (2009) and Farmacopeia Brasileira (2010).

The polyphenol content was determined by triplicate based on the equation for the pyrogalllic acid standard curve at concentrations ranging from 0.01 to 0.05 mg/mL resulting from absorbance values measured after the reaction with the phosphomolybdic tungstic acid in alkaline medium (Figure 1).

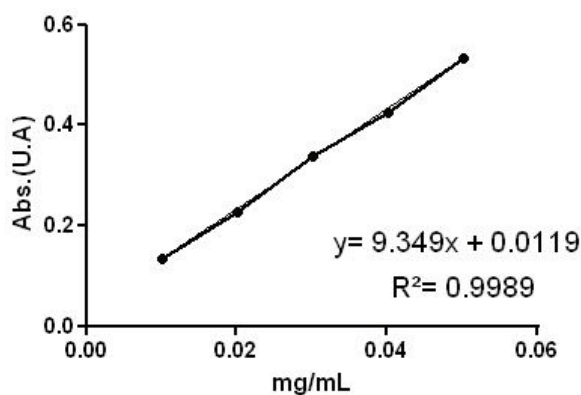


Figure 2. Pyrogalllic acid standard curve at concentrations ranging from 0.01 to 0.05 mg/mL. Pyrogalllic acid was reacted with phosphomolybdic tungstic acid in alkaline medium, and the absorbance of the product mixture was measured at 760 nm.

The polyphenol concentration was obtained as a percentage using the equation described by Sousa (2009):

$$\% Pf = \frac{x \left(\frac{\text{mg}}{\text{mL}} \right) \cdot \text{QLC} \cdot 100}{M (\text{mg})}$$

%Pf: percentage of polyphenols; x: sample concentration based on the linear regression equation; QLC: quantity of

liquid in the vessel; M: mass of extract in tablets.

Statistical analysis

Each result was analyzed by one-way ANOVA followed by a linear regression test. Comparative analysis of the dissolution profiles was performed using the Student-Newman-Keuls t-test, and results with *p* values <0.05 were considered significant. All statistical analysis was performed using GraphPad Instat[®] and Prism[®] software (version 5.03).

Results and Discussion

Combining the granules with excipients that have different physicochemical properties in a pharmaceutical formulation may affect drug dissolution and disintegration, which would in turn affect the speed of absorption and the bioavailability (Gibaldi, 1991). Therefore, the pharmacological characteristics of the excipients were considered during the granule production to present a mixture within the desired parameters for *in vitro* tests.

Some of drug formulation components, such as starch and other disintegrants favor dissolution; however, components such as magnesium stearate, which acts as a lubricant, hamper dissolution and must be added in small amounts (Gibaldi, 1991). The high content of disintegrants and diluents were used to promote the rapid bioavailability of the extracts by dissolution and to accelerate the breakdown of the granules. The small amount of magnesium stearate contributed to the controlled release of the extract to reduce the attractive forces between particles, preventing agglomeration and segregation of the material due to its small size (Kibbe, 2000; Thompson, 2006; Ansel et al., 2007).

The formulation of the granule containing the HEECb used the following excipients: cellulose, magnesium stearate, D-lactose monohydrate, and cornstarch. These excipients showed effective solubility and dissolution, favoring the bioavailability of the HEECb through gradual and controlled release in the analyzed media (Table 1, Figures 3).

Table 1. Mass and percentage composition of the final granule developed by wet granulation.

Resulting granule	Mass (g)	Percentage
Excipients	207.400	68.085%
HEECb	81.120	26.630%
Water	16.104	5.285%
Total	304.624	100%

Regarding weight variation, the capsules were within the accepted standards of the Brazilian Pharmacopoeia (F. Bras. V, 2010), which states that capsule weight can vary by up to 7.5% for capsules over 300 mg, and no more than two capsules should exceed these parameters. All of the analyzed capsules had an average weight of 574.5 ± 8.0 mg, with an acceptable range from 531.42 mg to 617.58 mg, the maximum and minimum weight values of capsules were 587 mg and 560 mg, respectively (Figure 2). Thus, none of the analyzed capsules containing HEECb granules exceeded the acceptable range. Based on the average weight, the extract content in each capsule was calculated by the previously described method to be 0.153 ± 0.008 g/capsule.

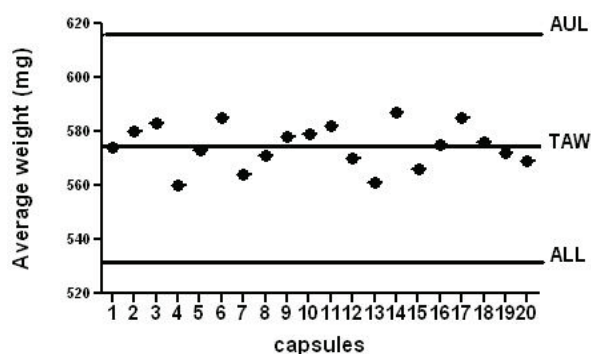


Figure 2. Determination of the weight of capsules containing HEECb granules. AUL corresponds to the acceptable upper limit of 7.5%, TAW corresponds to the theoretical average weight, and ALL corresponds to the acceptable lower limit of 7.5%.

Disintegration is a very important step for the drug bioavailability and absorption. In order for the capsules to disintegrate efficiently, the active compound must be solubilized and totally disaggregated, consisting only of small particles (Ansel et al., 2007). The disintegration test of the capsules showed the rapid disintegration of the encapsulated granules, and the capsules were more efficiently disintegrated in the HAB (pH 1.2), with total disintegration occurring by 9 min. The disintegration in DW and in (pH 6.8) were also rapid, but they were still slower than in the HAB, with total disintegration occurring by 13 and 18 min, respectively (Table 2).

Table 2. Disintegration time (in min) of the capsules in different disintegration media (n = 6).

Dissolution media	Disintegration (min)
Distilled water	13
Hydrochloric acid buffer (pH 1.2)	9
Phosphate buffer (pH 6.8)	18

The absorption of drugs delivered in a solid dosage form in physiological media depends on the dissociation of the active substance contained in the drug formulation. The release of the drug from its solid form involves three basic stages: disintegration, disaggregation, and dissolution (Adams et al., 2001). *In vitro* dissolution is very important to evaluate for drug performance, and these results can be correlated with the results of *in vivo* experiments (Amidon et al., 1995). Therefore, it is essential to control the experimental conditions to ensure reliability and reproducibility of the test.

The dissolution test showed that the capsules performed best in HAB (pH 1.2), with a maximal release reached at 15 min and an HEECb polyphenol content of 4.38% (0.0080 mg/mL) at the end of dissolution assay (Figure 3). In DW, the maximal release occurred at 20 min and became stable thereafter. However, the polyphenol content in DW reached values above the polyphenol content in HAB (5.41%, which is equivalent to 0.0105 mg/mL) (Figure 3). PB (pH 6.8) showed the lowest amount of dissolution, reaching a maximal release at the end of the analysis (at 30 min) and having a low polyphenol content (3.61%, which corresponds to concentration of 0.0070 mg/mL) (Figure 3).

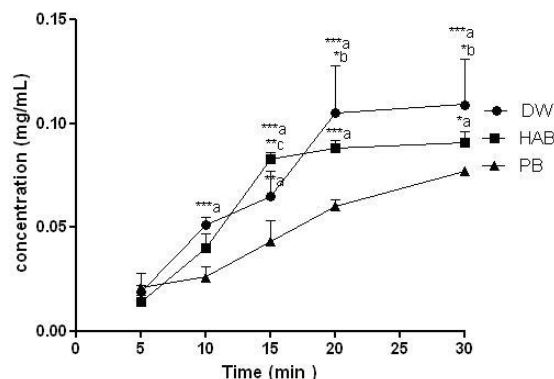


Figure 3. Dissolution time profile of the capsules (n=6) in distilled water (DW), hydrochloric acid buffer (HAB, pH 1.2), and phosphate buffer (PB, pH 6.8). Samples were reacted with phosphomolybdic tungstic acid in alkaline medium and analyzed at 760 nm. Comparison analysis of the dissolution profiles performed using Student-Newman-Keuls t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (a) compared to PB, (b) compared to HAB, (c) compared to DW.

Each of the different dissolution media presented significant amounts of dissolved HEECb polyphenols after 10 min. DW medium showed the best dissolution profile. However, HAB showed a higher amount of HEECb polyphenols at 15 min compared to DW and PB ($p < 0.05$). At 20 min, PB was the most inefficient dissolution medium, and at the end of the dissolution assay, DW and HAB showed higher

maximal release values of HEECb polyphenols. PB showed an ineffective dissolution profile, with a small increase at 30 min (Figure 3).

Based on the times observed in the dissolution assay, DW showed the best ability to release polyphenols. DW was the most suitable medium for polyphenol extraction due to the variation of phenolic compounds' solubility according to the polarity of the solvent used and the degree of polymerization of these compounds. The solvents commonly used for this purpose are methanol, ethanol, acetone, water, and propanol, among others (Nacz, 2004). Considering the release level of total polyphenols in HAB, which showed a significant difference compared to DW, HAB was the medium that showed the best uniform release of the HEECb marker, regardless of dissolution time. However, both DW and HAB demonstrated effective dissolution rates that may correlate with their *in vivo* performances.

Conclusion

Calophyllum brasiliense was effectively extracted by hydroethanolic, and the efficiency of granule formulation was demonstrated by the dissolution profile of the polyphenols used here as a phytochemical marker. The marker was best released in hydrochloric acid buffer and distilled water, based on the average weight and the results of the disintegration tests, the developed capsule was within the standards recommended by the Brazilian Pharmacopoeia (F. Bras. V, 2010).

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