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Nanoencapsulation and bioaccessibility of polyphenols of aqueous extracts from *Bauhinia forficata* link

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ABSTRACT

Bauhinia forficata Link is a plant rich in polyphenols that has been used mainly for its hypoglycemic activity, which is related to its antioxidant and anti-inflammatory potential. However, the beneficial effect of these bioactive compounds is directly dependent on their bioaccessibility and bioavailability, requiring processing techniques that can improve and preserve their biological activities. This work aimed to obtain nanocapsulated extracts from the infusion (ESIN) and decoction (ESDC) of *B. forficata* Link leaves, by spray drying. The encapsulating agents used were maltodextrin and colloidal silicon dioxide. The nanocapsules were characterized by HPLC-PDA-ESI-IT-MS^n, evaluated the bioaccessibility of polyphenols after simulated digestion and their antioxidant activity. Additionally, an extensive physicochemical characterization of the nanocapsulated extracts was carried out and their stability and technological parameters were evaluated. The ESIN and ESDC extracts had yields of 57.3 % and 62.7 %, with average nanocapsules sizes of 0.202 μm and 0.179 μm, low humidity and water activity (<0.5), powder density and proper flow properties (Hausner ratio ≤ 1.25; Carr index 18–19 %). Scanning electron microscopy showed a spherical and amorphous morphology and low viscosity, which may have favored the solubility profile. The phenolic compounds of the nanocapsules degraded after 400 °C, showing high thermal stability. The infrared spectra identified the presence of maltodextrin and phenolic compounds and that there were no reactions between them. Chromatography confirmed the presence of phenolic compounds, mainly flavonols and their O-glycosylated derivatives, as well as carbohydrates, probably maltodextrin. Simulated *in vitro* digestion showed that polyphenols and flavonoids from ESIN and ESDC nanocapsules were bioaccessible after the gastric phase (49.38 % and 64.17 % of polyphenols and 64.08 % and 36.61 % of flavonoids) and duodenal (52.68 % and 79.06 % of polyphenols and 13.24 % and 139.03 % of flavoids), with a variation from 52.27 % to 70.55 % of the antioxidant activity maintained, by the ORAC method, after gastric digestion and still 25 %, after duodenal. Therefore, the nanoencapsulation of extracts of *B. forficata* is a viable option for the preservation of their bioactive compounds, making them bioaccessible and with antioxidant activity, which make them suitable for incorporation into various nutraceutical formulations, such as capsules, tablets and sachets.

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1. Introduction

Bioactive compounds are low molecular weight compounds that can be produced naturally by foods or added to improve these products, due to their functional properties (Cozzolino, 2022). Polyphenols are bioactive compounds derived from the secondary metabolism of plants and which act mainly as antioxidants, as they neutralize free radicals or their precursors, by donating an electron or hydrogen atom or by inhibiting their formation, as well as by chelation of metals and by inducing endogenous antioxidant enzymes, such as catalase, glutathione peroxidase, superoxide dismutase. Additionally, polyphenols can inhibit enzymes, such as xanthine oxidase, preventing oxidative processes, which is associated with several pathologies, such as diabetes, cardiovascular diseases, neurodegenerative disorders and cancer (Cianciosi et al., 2022; Datta et al., 2011; Perron and Brumaghim, 2009; Sasikala et al., 2018; Stagos, 2019).

Flavonoids are phenolic compounds consisting of two benzene rings, linked by a chain of three carbon atoms (C6-C3-C6), which forms a pyran ring also containing oxygen. These compounds are subclassified according to their number and arrangement of hydroxyl groups and their extent of alkylation and glycosylation, in anthocyanins, flavonols, flavanols, flavanones, flavones and isoflavones, whose daily consumption can vary from 20 mg to 500 mg in the form of red wine, apple, cocoa, garlic, tomato, tea, among others. Scientific evidence, *in vivo* and *in vitro*, has reported the promising health-promoting effects of these bioactives, due to their antioxidant and anti-inflammatory properties (Hussain et al., 2020; Kawser Hossain et al., 2016; Kumar and Pandey, 2013; Rakers et al., 2014).

Flavonoids compounds are the most abundant polyphenols in human diets and have several health benefits, being used as nutraceuticals for medicinal purposes, and some with application in dietary and herbal supplements. Therefore, a great increase in this research area has been made to obtain natural, bioaccessible and bioavailable flavonoids to exert their biological activities (Pellegrini et al., 2018; Rein et al., 2013; Wen et al., 2015). A natural source of polyphenols is the plant *Bauhinia forficata* Link, a specie native to South America, belonging to the Fabaceae family, whose leaves have been traditionally used as tea by folk medicine. Currently, the use of this plant has been encouraged by the Health Unic System (SUS), especially for its possible hypoglycemic effect (Campos et al., 2022; Franco et al., 2020; Cechinel Filho, 2009; De Moraes et al., 2010; De Souza et al., 2018; Trojan-Rodrigues et al., 2012). In addition to this effect, studies report other bioactivities such as antioxidant, anti-inflammatory, hepatoprotective, vasorelaxant, diuretic and natriuretic (Bodakhe and Ram, 2007; Cechinel-Zanchett et al., 2019; Da Cunha, et al., 2010; Damasceno et al., 2004; Damasceno et al., 2004; De Souza et al., 2017; Ecker et al., 2017; Gupta et al., 2004). These properties are attributed to the phytochemical profile of its leaves, consisting mainly of the flavonoids quercetin-3-O-rhamnosyl rutinoside, isoramnetin-3-O-rhamnosyl-rutinoside, 2-benzyltartaric acid, rutin, kaempferol-3-O-rhamnosyl rutinoside, being the kaempferol 3,7-di-O-α-L rhamnopyranoside (kaempferitrin), chemical marker for this plant species (De Sousa et al., 2004; Farag et al.; 2015; Ferreres et al., 2012; Marques et al., 2012; Pizzolatti et al., 2003).

Despite their wide distribution, the bioavailability of these compounds in the human body is low, influenced by factors such as cultivation, harvest time and position of leaves in the plant, as well as processing technologies, with consequent reduction of their antioxidant benefits (Yang et al., 2015). Therefore, the protection of these compounds with encapsulating agents becomes essential for a better absorption of these compounds by the human body (D'archivio et al., 2010; Ersus and Yurdagel, 2007; Jafari et al., 2008; Wang et al., 2017).

The most common nanoencapsulation technique for bioactive ingredients is drying by atomization, due to its economic feasibility, with lower operational costs, when compared to lyophilization and vacuum drying (Medina-Torres et al., 2016). Furthermore, it has high encapsulation efficiency, low moisture content and water activity, thus, it is effective in improving the stability and solubility of polyphenols. The improvement of these properties leads to a better conservation and an increase in the shelf life of products that apply this technique, making them more resistant to darkening and hydrolysis reactions, lipid oxidation, autooxidation and other enzymatic activities (Marques et al., 2007; Özkan and Bilek, 2014; Rocha et al, 2012).

This encapsulation process consists of the conversion of water suspensions into microparticles in powder, which are composed of a wall material and a core (encapsulated material) (Bakry et al., 2016). The wall material is a determining factor in the efficiency of nanoencapsulation and stability of the powders obtained (Kandansamy and Somasundaram, 2012). Colloidal silicon dioxide is one of the most used excipients in spraying drying using for vegetable extractive solutions, as it reduces residual humidity, as well as hygroscopicity, optimizing drying conditions. Plant extracts are normally hygroscopic and maltodextrin, which has low-cost, high-water solubility and low viscosity, promotes protection of the bioactives from oxidation, since forms a coating film minimizing the oxygen contact with encapsulated material. In addition, this ingredient is suitable for incorporation into food matrices (Amado et al., 2014; Medina-Torres et al., 2016; Parvez et al., 2022; Vasconcelos et al., 2005).

The nanoencapsulation technique has been widely used to achieve stability of polyphenols and preserve their bioactivity (Khazaei et al., 2014; Mahdavi et al., 2014), as well as the use of *B. forficata* leaves, due to their biological properties. However, most studies have focused on fresh leaves or fluid extracts, with emphasis on organic solvent extraction (Cechinel-Zanchett et al., 2018; De Souza et al., 2018), and there are still few reports on phenolic profiles and antioxidant activities of nanocapsulated dry extracts from the leaves of this plant subspecies.

Despite reports on the benefits of these compounds, their efficacy can only be confirmed by studies on their bioavailability, since this is influenced by a variety of factors, such as the structure of phytochemicals, mechanical action, changes in pH and enzymatic activity in the gastrointestinal tract. Any compound can be considered potentially effective for human health, however, to fulfill this capacity is necessary that the compound remains bioavailable after all phases involved in gastrointestinal digestion, thus the bioavailable content for absorption should be evaluated (Celep et al., 2017; Rein et al., 2013).

In vitro digestion models to simulate the medium in the gastrointestinal tract represent a simple, rapid and valid alternative for assessing bioaccessibility of bioactive compounds in a less costly and time-consuming manner than *in vivo* models, as they are easily conducted methods with reproducible results without the need of ethics committee approval, displaying correlations with *in vivo* and clinical studies (Alminger et al., 2014; Gullon et al., 2015).

In view of this fact, this work aimed to obtain nanocapsulated extracts from the infusion and decoction of *B. forficata* Link leaves, by spray drying and to perform physicochemical analyses, spectroscopy, in addition to evaluating the bioaccessibility of polyphenols through of *in vitro* digestion and its antioxidant activity, in order to identify differences between these extracts, for potential applications in nutraceutical formulations. The development of these natural products offers potential nutraceutical strategies to assist in the prevention and treatment of oxidative stress and related diseases, in addition to promoting the effective delivery of bioactive compounds to the absorption sites, being a viable and practical alternative to increase the intake of these compounds in various forms such as capsules, tablets, sachets, powders for preparing solutions, among others. Another innovative aspect of this study, in addition to the bioaccessibility of these nanocapsulated extracts, it was the fact that water was the only solvent extracting phytochemicals (green extraction), proving to be efficient, since other studies carried out with this same plant used organic solvents such as methanol and ethyl acetate, to obtain good results, has been less recommended, due to their potential toxicity.

2. Materials and methods

2.1. Sample

The leaves of *Bauhinia forficata* Link were obtained in Teresina, Piaui, Brazil (5°03′7.2″ S 42°77′26.4″ O), during the month of January 2018. Exsiccates of the plant material is deposited at Graziela Barroso Herbarium, Federal University of Piaui (UFPI), under voucher number 31423, with registration at SISGEN AE1E536.

2.2. Chemicals

The chemicals maltodextrin, colloidal silicon dioxide, (S)-(-)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), Folin-Ciocalteu, gallic acid, aluminum trichloride ($AlCl_3$), 2, 4, 6 - tripyridyl −1, 3, 5 - triazine (TPTZ), calcium chloride ($CaCl_2$), α-amylase, pepsin, hydrochloric acid (HCl), pancreatin, bile, sodium hydroxide (NaOH), protease, Viscozyme (arabinase, cellulase, β-glucanase, hemicellulase and xylanase) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA) and HPLC reagents were purchased from Merck KGaA (Darmstadt, Germany).

2.3. Preparation of extracts and nanoencapsulation

The leaves of *B. forficata* were dried in an industrial oven at a temperature of 40 °C for 52 h and then pulverized. The aqueous extracts obtained were prepared in the proportion of 10 % (w/v), according to Menezes et al. (2007), since the teas, obtained by infusion or decoction, are used in the control of diabetes, for this plant species (Silva-López and Santos, 2015). The two forms of extraction were analysed to evidence physical–chemical differences between them.

The nanoencapsulation of the aqueous extracts, by infusion and decoction, was performed using the spray drying technique, in a BUCHI® Mini Spray Dryer B-290. For the preparation of the solution, the aqueous extracts of the infusion and the decoction were mixed with the encapsulating agents in the proportions of 70 % of vegetal material and 30 % of encapsulating agents (w/w). The encapsulating agents used were maltodextrin and colloidal silicon dioxide, being 15 % of each, as recommended by Rowe et al. (2009) and verified by Rolim et al. (2013), who obtained greater encapsulation and protection of the bioactive constituents of extracts of *B. forficata*, in these proportions. After homogenization of the encapsulating agents with the extracts, in a magnetic stirrer, the solution was submitted to the Spray Dryer device under the following conditions: inlet temperature 110 °C, flow rate 5.4 mL/min, injection 15 %, injection pressure 0.9 bar and 70 % spray. Finally, the nanocapsulated extracts were named dry extract of the infusion (ESIN) and dry extract of the decoction (ESDC). The drying process performance (%DS) was determined according to equation (1):

$$\%DS = \frac{[Mass\ of\ \ microcapsules\ obtained\ after\ drying]}{[(Inicial\ mass\ of\ wall\ material + fluid\ extract)\ ] \times 100} \quad (1)$$

2.4. Physical-chemical analyses

The moisture content and water activity were verified according to methods described by the Association of Official Analytical Chemists (AOAC, 2012). The density was determined in an automatic compactor (Copley IV 2000® Densimeter), subjected to 500, 750 and 1250 compactions and the analysis parameters were 300 beats/minute, according to Schüssele and Bauer-Brandl (2003). The relationship between the mass of the samples and the volume occupied by the powder before and after compaction determined the bulk (db) and compacted (dc) densities. The evaluation of the dust compaction was performed using the Hausner ratio (Hr) and Carr index (%IC), using equations (2) and (3):

$$Hr = \frac{CompactedDensity}{ApparentDensity} \quad (2)$$

$$(\%IC) = \frac{(dc - db)}{dc} \times 100 \quad (3)$$

The study of microstructure and evaluation of the morphology and surface of ESDC and ESIN dry extracts were performed in the scanning electron microscope (SEM) with field emission cannon, FEI®, model Quanta FEG 250, with acceleration voltage from 1 to 30 kV, equipped with EDS of SDD (Silicon drift detectors), Ametek®, model HX-1001, Apollo X-SDD detector. For the micrographs, the samples were dispersed and fixed on aluminum substrate (stub) using double-sided carbon adhesive tape coated with gold in a metallizer, Quorum®, model Q150R, for 30 s, at 20 mA, by plasma generated in argon atmosphere.

The granulometry of the dry extracts was performed using standardized and superimposed sieves in decreasing order of pore size (20, 40, 60, 80 and 120 mesh), mounted on a magnetic vibration base (Bertel® Tamisador). The determination of the average particle size of ESDC and ESIN powder extract was performed according to the method in the Brazilian Pharmacopoeia (Brasil, 2010).

The rheological behavior was determined according to Medina-Torres et al. (2016), by means of a *Brookfield* R/S plus SST 2000 model concentric cylinder rheometer of the *Searle* type. The measurements were made at a temperature of 25 °C, which was adjusted by means of a thermostatic bath coupled to the equipment, which provided the shear stress and strain rate data using RHEO V 2.8 software. Rheological analysis was obtained with a deformation rate variation from 0 to 500 s^{-1} (ascending curve) and from 500 to 0 s^{-1} (descending curve), at 1 min and 25 points for each curve. The readings were performed in triplicate and a new sample was used in each measurement.

The Differential Scanning Calorimetry (DSC) curves and Thermogravimetry (TG) were obtained in a differential scanning calorimetric module DSC 910 (TA - Instruments) (heat flow type), coupled to a thermal analyzer TA2000 (TA - Instruments), according to the method proposed by Bazzo and Silva (2005). In the analyses, sample support of aluminum, air and nitrogen atmosphere was used. Temperature calibration was previously performed in the equipment using as standards the metallic fusion points of indium (156.4 °C) and zinc (419.5 °C), with purity of 99.99 %. The energy calibration was based on the fusion enthalpy of metallic indium, ΔHfus = 28.5 Jg^{-1} and the TG curves were obtained, in triplicate, by means of thermobalance, model DTG- 60H coupled to the DTA-TG apparatus, under nitrogen atmosphere with flow of 50 mL.min^{-1}, with the heating rate 10 °C.min^{-1}. The sample masses (about 5 mg), were packed in aluminum crucible. The calibration of the instrument was verified using a calcium oxalate sample.

2.5. Spectroscopy analyses

2.5.1. X-ray diffraction

The X-ray diffraction analysis was performed according to Pumacahua-Ramos et al. (2015). The samples were fixed on a glass support with a thin layer of solvent-free powder material. The diffractometers of the ESDC and ESIN were obtained using Shimadzu® diffractometer, model XRD6000, equipped with copper anode, using 40 kV voltage and 30 mA current. The sample was analysed in the angle range 2θ of 5 − 75° at a scanning speed of 2°/min.

2.5.2. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) analyses were performed according to the methodology proposed by Pumacahua-Ramos et al. (2015). The ESDC and ESIN infrared spectra were obtained from Perkin Elmer® Spectrum 400 with attenuated total reflection device (ATR) (Miracle ATR, Pike Technologies Spectroscopic Creativity) with selenium crystal. The resolution used was 4 cm^{-1} in the 4000–500 cm^{-1}

range, using the KBr tablet technique.

2.5.3. High efficiency liquid chromatography with photodiode array detector coupled to sequential electrospray ionization and ion trap mass spectrometry (HPLC-PDA-ESI-IT-MS^n)

The dry extracts ESDC and ESIN were analysed by high efficiency liquid chromatography with photodiode array detector coupled to sequential *electrospray* ionization and ion trap mass spectrometry (HPLC-PDA-ESI-IT-MS^n), according to Cuyckens and Claeys (2004), using a Luna reverse phase column C18 (2) HST (100 × 3.0 mm, 2.5 μm; Phenomenex, Torrance, CA, USA).

The samples were filtered with 0.22 nylon filter μm (Millipore, Sao Paulo, Brazil) and 20 μL were injected into the HPLC system, Shimadzu (model LC-20ADX Proeminence; Kyoto, Japan) equipped with two high pressure pumps and PAD coupled to the Bruker mass spectrometer (model Amazon Speed; Billerica, MA, USA) with ion trap analyser and *electrospray* ionization (ESI) operated in negative mode under the following condition: capillary voltage, 3.5 kv; dry temperature, 230 °C; desolvation gas flow (N2), 360 L/h; the *m/z* range was 100–1000. The mobile phase consisted of water with 0.1 % formic acid (A) and acetonitrile (B), and was performed at a flow rate of 0.5 mL min^{-1}. A 22-minute gradient program was used in the study, starting with 0.1 % formic acid and acetonitrile (95:5, v/v), followed by acceleration to 8 % acetonitrile in five minutes, increasing to 15 % in eight minutes, then increasing to 20 % acetonitrile in 12 min, then to 35 % in 15 min and finally there was a decrease in the acetonitrile proportion to 5 %. The samples were injected in triplicate. The chemical compounds identification was performed using strategies alongside an analysis of the mass spectra obtained by the HPLC-PDA-ESI-IT-MS^n, comparison to a database of mass spectrum like the Massbank® (https://massbank.eu/MassBank/) and literature data (Cechinel Filho, 2009; Farag et al.; 2015; Ferreres et al., 2012; Silva-López and Santos, 2015).

2.6. In vitro simulation of gastrointestinal digestion

The simulated digestion process of nanocapsulated dry extracts occurred in four stages: oral, gastric, duodenal and simulation of the enzymatic action of colonic microbiota, according to protocols of Fogliano et al. (2011) and Minekus et al. (2014). Simulated Salivary Fluid (FSS), Simulated Gastric Fluid (FGS) and Simulated Intestinal Fluid (FIS) were composed by solutions of electrolytes, enzymes, $CaCl_2$ and water.

In the oral phase, 0.5 g of both the ESDC and ESIN was dissolved in 2.5 mL of MilliQ water (1:4, m/v) and 3.5 mL of salivary physiological solution, 0.5 mL of α-amylase, 25 μL of $CaCl_2$ and 975 mL of water were added and placed in a MilliQ water bath for two minutes at 37 °C, pH 7.0. Later the falcon tube containing the sample was centrifuged at 2177.28 g for 40 min, the material digested with α-amylase was stored for further analysis while the sediment was mixed with 7.5 mL of FGS, 1.6 mL of pepsin solution, 5 μL of 0.3 M $CaCl_2$, 0.2 mL of HCl 6 M to reach pH 3.0 and 695 μL of water (water bath for two hours at 37 °C, pH 3.0).

After digestion, the tube was centrifuged at 2177.28 g for 40 min, the material digested with pepsin was removed for further analysis, while the sediment was dissolved in 11 mL of FIS, 5 mL of pancreatin, 2.5 mL of bile, 40 μL of 0.3 M $CaCl_2$, 0.15 mL of 1 M NaOH until it reached pH 7.0 and 1.31 mL of water (water bath for two hours at 37 °C, pH 7.0). The digested sample was then centrifuged and the supernatant stored. 2 mL of protease was added to the residue from this phase (water bath for one hour at 37 °C, pH 8.0). After centrifugation and separation of the supernatant, the digested sample was finally treated with 100 μL g^{-1} Viscozyme (a multi-enzyme complex containing a wide range of carbohydrases, including arabinase, cellulase, β-glucanase, hemicellulase and xylanase) and placed in the water bath for one hour at 37 °C and pH 4.0. For all digestion steps, a blank preparation was prepared, without addition of samples, to avoid overestimation in the quantification of the analysed compounds (Supplementary material Fig. 1A).

2.7. Total polyphenols (PT) and total flavonoids (FT)

The samples digested at each stage of the *in vitro* digestion process were analysed for total polyphenols and total flavonoids. The total polyphenols were analysed by *Folin Ciocalteu* in alkaline medium, measured at 720 nm with UV–vis spectrophotometer and expressed in mg of gallic acid. g^{-1} of sample, according to Swain and Hills (1959). Total flavonoids were quantified by the aluminum trichloride ($AlCl_3$) method according to Zhishen, Mengcheng and Jianming (1999) and were measured at 510 nm with UV–vis spectrophotometer and expressed in mg of catechin. g^{-1} sample.

2.8. Evaluation of antioxidant activity in vitro

The total antioxidant capacity (CAT) of the ESDC and ESIN, after *in vitro* digestion, was estimated by two different tests: ferric reducing/antioxidant power (FRAP) and oxygen radical absorption capacity (ORAC). The evaluation of FRAP was performed according to Arnous et al. (2002), in which the samples reacted for 30 min with iron chloride solution (3 mM) in a 37 °C water bath and were later added to the acidic solution of 2, 4, 6 - tripyridyl −1, 3, 5 - triazine (TPTZ), the results being expressed in TEAC, in μmol of Trolox.g^{-1} sample.

The ORAC assay was performed according to the methods described by Prior et al. (2003) and Dávalos et al. (2004), with modifications. The results were expressed in Trolox equivalents (μmol of Trolox. mg^{-1} sample).

2.9. Assessment of the bioaccessibility index of antioxidant compounds

To evaluate changes in bioactive compounds during the simulated gastrointestinal digestion *in vitro*, the bioaccessibility indices (IB) were calculated according to Equation (4), according to Ortega et al. (2011).

$$IB(\%) = \frac{100xB}{C} \quad (4)$$

Where B is the content of polyphenols or flavonoids or antioxidant activity obtained from the FRAP and ORAC tests, quantified in the supernatant after the digestion process, and C, the amount of these compounds before digestion, expressed in the same units.

2.10. Statistical analysis

The results of bioactive compounds and antioxidant activity were presented as mean ± standard deviation. The data were analysed using the *t*-student test, two-way analysis of variance (ANOVA), followed by Tukey's test using OriginPro software (version 8) and GraphPad Prism (version 6.0), at a 95 % confidence level ($p < 0.05$).

3. Results and discussion

3.1. Physical-chemical analyses

The results of drying performance, moisture, water activity, density, Hausner ratio and Carr index of ESDC and ESIN are shown in Table 1. The encapsulation efficiency, which is evaluated by the extract yield, is one of the most important parameters of this technique. The general average yield of *B. forficata* spray drying extracts was 62.73 ± 3.93 % (ESDC) and 57.30 ± 1.80 % (ESIN), which is considered efficient, according to Bhandari et al. (1997), as it is>50 %, which shows an adequate interaction between the core and the encapsulating agents, reflecting in an effective nanoencapsulation (Parvez et al., 2022).

It is worth noting that the loss of powder during spray drying may be due to the deposit of some particles of dust on the wall of the drying chamber and the filter of the dryer. A similar result was obtained by

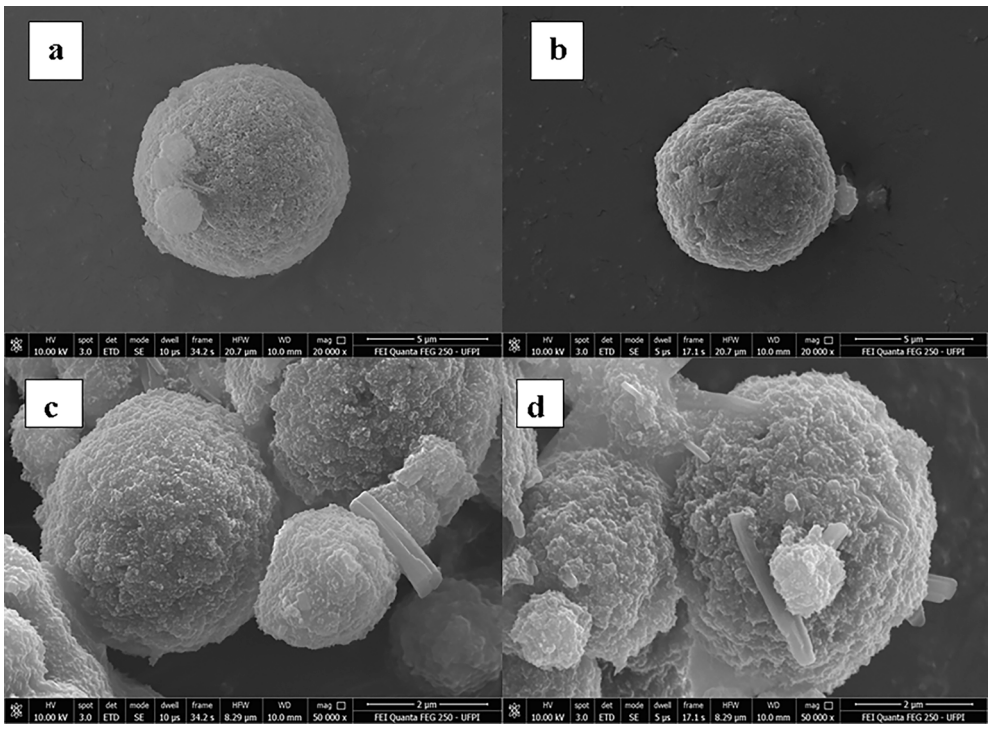

Fig. 1. Photomicrographs of dry extracts ESDC and ESIN. Legend: (a) ESDC: 20000×. (b) ESIN: 20000×. (c) ESDC: 50000×. (d) ESIN: 50000×.

Table 1
Results of drying performance, moisture, water activity, density, Hausner ratio and Carr index of dry decoction (ESDC) and infusion (ESIN) extracts of *Bauhinia forficata* Link leaves.

Parameters	ESDC	ESIN
Yield (%)Humidity	62.73 ± 3.93[a]	57.30 ± 1.80[a]
(%)	5.38 ± 0.08[a]	7.79 ± 0.12[b]
AwBulk density	0.37 ± 0.01[a]	0.49 ± 0.01[b]
(g/cm^3)	0.31 ± 0.05[a]	0.29 ± 0.04[a]
Compacted density (g/cm^3)	0.38 ± 0.01[a]	0.36 ± 0.02[a]
Hausner's ratio	1.25 ± 0.10[a]	1.24 ± 0.17[a]
Carr Index (%)	18.61 ± 11.02[a]	19.00 ± 6.66[a]

Results expressed as means ± standard deviations (n = 3). ESDC: Dry extract of decoction. ESIN: Dry extract of the infusion. Aw: Water activity. Different letters, in the same line, indicate significant statistical difference between extracts. *T*-student test ($p < 0.05$).

Fang and Bhandari (2011), in which the recovery rate of *bayberry* dry powder was 55 ± 3 %. Infusions of *Cinnamomum zeylanicum* and *Quercus resinosa* encapsulated with maltodextrin had a lower yield, 49.60 % and 25 %, according to studies performed by Gallegos-Infante et al. (2013) and Santiago-Adame et al. (2015).

The moisture content of the powders obtained was 5.38 ± 0.08 g. 100 g^{-1} and 7.79 ± 0.12 g. 100 g^{-1} for ESDC and ESIN, respectively, with statistically significant difference between both, corroborates with studies conducted by Araújo et al. (2010), Marreto et al. (2006) and Souza et al. (2007) that also showed moisture values within the range of 3 to 8 % for dry extracts. Regarding water activity, the values were lower than 0.5, which is considered low (≤0.75) according to the Brazilian Pharmacopoeia (Brasil, 2010), showing chemical and microbiological stability. When evaluating the moisture and water activity of both extracts, it can be affirmed that the ESDC presented a higher yield, as the decoction extracts more compounds than the infusion, forming more hydrogen bonds, consequently, it has less hydroxyl groups available. to adsorb moisture. Therefore, ESIN presented higher humidity than ESDC and, consequently, higher water activity, which contributed to a greater adhesion of the extract in the spray dryer, during the encapsulation process, which influenced its yield (Table 1).

The density values were in the range of 0.31 to 0.38 g/cm^3. Similar results were obtained by Medina-Torres et al. (2016) with spray-dried *Litsea glaucescens* infusions, with density values in the range of 0.30 to 0.33 g/cm^3 and by Couto et al. (2011) in *Eugenia dysenterica* dry extracts (0.18 to 0.38 g/cm^3). This physical parameter confirms that ESDC is denser than ESIN, due to the higher concentration of compounds, after extraction, with less air space between particles, decreasing susceptibility to oxidation, being more stable during storage. Referring to the compacted density, this was higher than the gross density, in both extracts, as the voids between the larger particles were occupied by the smaller particles (Parvez et al., 2022).

Hausner's ratio and Carl's index, which are indirect measures of powder density, were also evaluated. Hausner's ratios (HR) were similar among the extracts, having a regular flow (Hr ≤ 1.25). The Carr index (%IC) ranged from 18.0 to 20.0 %, with satisfactory compression (18–21 %). These values of Hr and %IC characterize the powder obtained in this study as non-cohesive, with regular flow and good compression, according to Borini, Andrade and Freitas (2009). These characteristics are suitable for the use of these nanocapsulated extracts in nutraceutical formulas. Islam et al. (2017), in a study of orange juice powders, obtained % IC values of 22.50–23.59 % and HR in the range of 1.23–1.30, which indicates a non-cohesive and free flowing powder.

According to the photomicrographs of the dry extracts of ESDC and ESIN, the nanocapsules showed a spherical shape with a tendency to agglomerate, which may be related to the Van der Walls forces of the particles interacting with the water molecules, typical of spray dried particles (Medina-Torres et al., 2016) (Fig. 1a and b).

Morphological irregularities on the surface of the nanocapsules can be attributed to rapid evaporation of liquid droplets, which causes the particles to shrink with the release of small amounts of air that were trapped inside the nanocapsules during the drying process (Medina-Torres et al., 2016) (Fig. 1c and d). Da Cunha et al., (2010) also characterized by SEM a spray drying extract of *B. forficata* Link and observed agglomerated, spherical particles with smooth surfaces, irregular shape and surface roughness.

The granulometric distribution study was performed by the sieving

technique and the results showed that ESDC and ESIN extracts had a mean particle size of 0.179 and 0.202 μm, respectively, representing 44.8 and 42.2 % of the retained mass, without statistical difference between them ($p > 0.05$) (Fig. 2).

According to Arpagaus et al. (2017) capsules smaller than 1 μm in diameter can be classified as nanocapsules, so both extracts were suitable for incorporation into capsules and tablets, which were classified as fine powder by the Brazilian Pharmacopoeia (Brasil, 2010). However, it is worth highlighting the tendency of these materials to form clusters, as observed in morphological SEM analyses, which may justify the greater resistance of the particles in passing through the sieve with smaller mesh opening.

The rheogram with the flow curves in single shear, shows, for all samples, a pseudoplastic behavior ($n < 1$) (Fig. 3). ESIN showed higher viscosity, in relation to ESDC, under the same drying conditions, which may be associated with the fact that it presented more moisture, with a greater tendency to form agglomerates. This absorptive property of a solid can interfere, both in the yield and in the quality of the extract, being an important parameter to be evaluated for this type of product (Baratto, 2021; Parvez et al. 2022). This higher viscosity of ESIN may also be due to the presence of larger modal particles, promoting more resistance to flow, as well as the influence of the molecular weights of the bioactive compounds present in this extract, according to Hill and Carrington (2006). At high shear rates ($\gamma > 400\ s^{-1}$)' both samples overlapped, and it was observed that their apparent viscosities decreased, making them more fluid, characteristic behavior of pseudoplastic fluids. Studies on the spray drying of *Litsea glaucescens* and *Cinnamomum zeylanicum* infusions reported similar results (Medina-Torres et al., 2016; Santiago-Adame et al., 2015).

In the TG, derivative TG (DTG) and DSC curves, it was observed that, at temperatures up to 400 °C, the degradation range of phenolic compounds, the ESDC showed six processes mass loss and the ESIN displayed four, before 100° C, which corresponds to the adsorbed water. Being the same plant, but submitted to different aqueous extraction processes (decoction and infusion), it can be affirmed that extraction by decoction resulted in a greater variety of organic compounds (Silva et al., 2019). The percentage of mass of both samples, at temperatures of 100 and 400 °C, was practically the same, and the ESDC sample exhibited only 0.12 % more mass (1.2 mg/g), in relation to ESIN (Fig. 4).

Mass loss in the temperature range 100 to 400 °C occurs due to the volatilization of organic compounds extracted from *B. forficata* Link. In the temperature range from 100 to 200 °C, two mass loss processes are observed in the ESDC, with maximum losses at 150 and 175 °C, while in the ESIN, the mass loss in this range occurs in a single process, with loss maximum at 160 °C. The volatilization of kaempferol, in ESDC, occurs in the thermal degradation process observed between 200 and 293 °C, with maximum loss at 262 °C, while in ESIN, the same process occurs between 200 and 270 °C, with maximum loss at 245° C. The volatilization

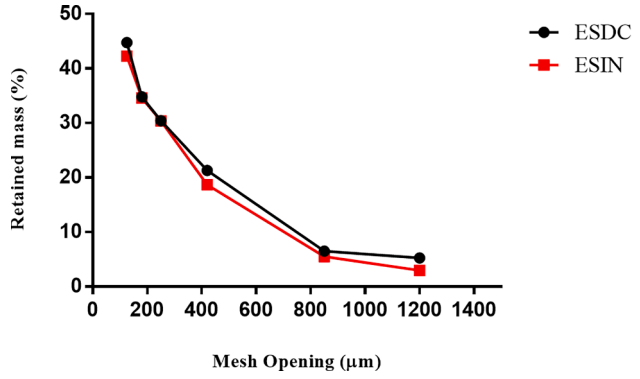

Fig. 2. Granulometry of dry extracts ESDC and ESIN. Legend: *T*-student test ($p < 0.05$), there was no statistically significant difference between extracts.

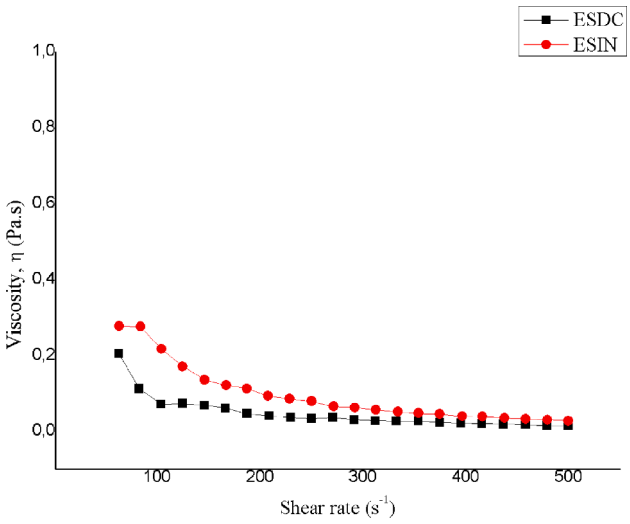

Fig. 3. Rheology of dry extracts ESDC and ESIN.

process of quercetin and its derivative isorhamnetin probably occurs in the thermal degradation processes starting at 295 °C for ESDC and 270 °C for ESIN and both go up to 365 °C with maximum losses at 317 and 300 °C, respectively, based on DTG curves. Thus, it is inferred that ESDC showed greater thermal stability of the markers used than ESIN.

The analysis of the amount of mass loss in each process was performed based on the TG curve. It was observed that, in the temperature range 100 to 200 °C, there was a mass loss of 15.49 % in ESDC and 12.78 % in ESIN. At the stage of probable volatilization of kaempferol, ESDC lost 22.28 % mass and ESIN 15.07 %, while at the stage of possible volatilization of quercetin and its derivative isorhamnetin, ESDC lost 11.96 % mass and ESIN lost 19.76 %. According to Chen et al. (2010), these losses occurred through exothermic processes as can be observed in the comparison of the TG curve with the DSC curve of each sample. The TG curve falls coincides with elevations, even if mild in the DSC curves. Such thermal events occur through the volatilization of the compounds present in the sample, and the thermal behavior of the ESIN sample is indicative of a more crystalline sample. In addition, from 400 °C onwards, there was the decomposition of the material in ESDC and ESIN through the degradation of phenolic compounds associated with depolymerization and branching of carbohydrates, as evidenced by Ballesteros et al. (2017), who thermally analysed samples of coffee beans dried by spray drying.

3.2. Spectroscopy analyses

3.2.1. X-ray diffraction

Diffractograms of ESDC and ESIN extracts present characteristics of amorphous substances, common in spray drying plant extracts. There is also a greater inclination in the initial angles of the diffractograms, forming an "amorphous aureole", according to Newman and Byrn (2003), a fact that can be explained by the presence of carbohydrates, which were unable to return to their crystalline form and were organized in an amorphous form during the drying process (Fig. 5). Diffractograms similar to the present study were observed in dry extracts of *Schinopsis brasiliensis* and *Hamamelis virginiana*, with and without drying aids, whose conformation was amorphous and formed by an intense noise, which suggests that the presence of the adjuvant did not affect the amorphous aspect of *spray drying* extracts (Fernandes et al., 2013; Gallo et al., 2011).

3.2.2. Ftir

The ESDC and ESIN samples were analysed by FTIR (Fig. 6), in which the spectra of both extracts showed similar characteristics, with more

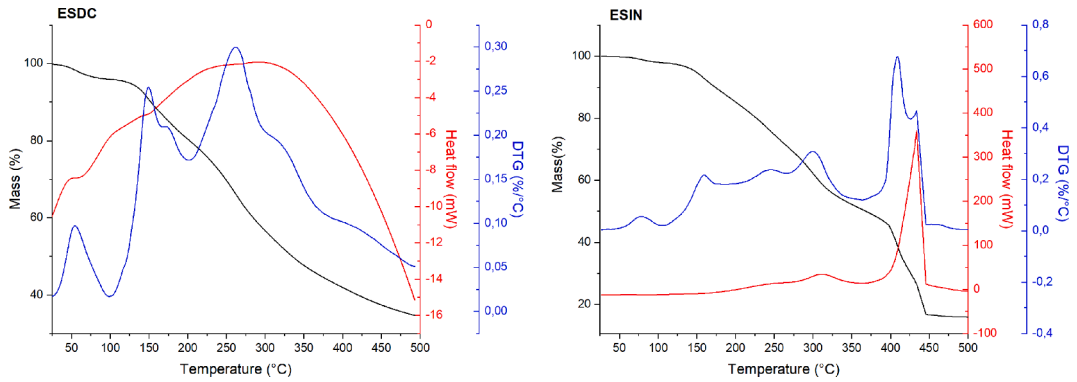

Fig. 4. Differential calorimetry and thermogravimetry curves of dry extracts ESDC and ESIN.

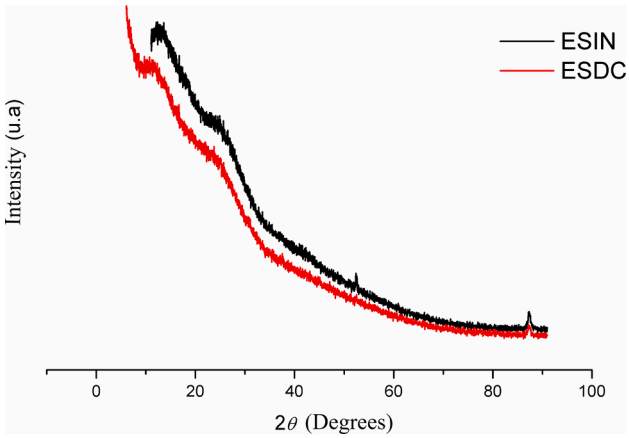

Fig. 5. Diffractograms of dry extracts ESDC and infusion ESIN.

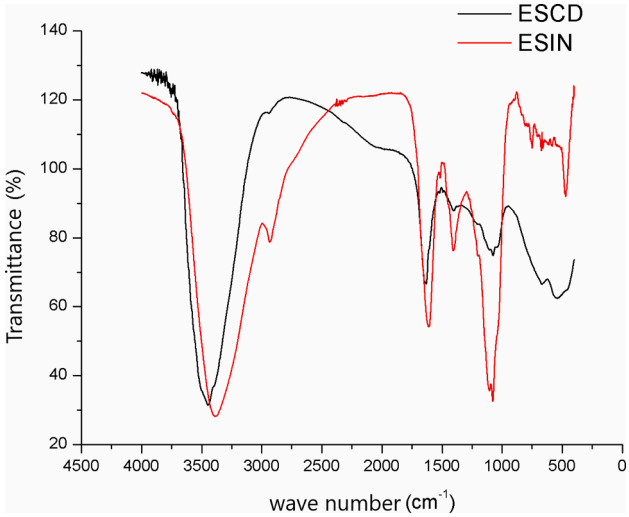

Fig. 6. Infrared absorption spectral of ESDC and ESIN.

differentiated spectral absorption only around the wave number of 1000 cm-1 in ESIN. In the spectra of these extracts, the presence of characteristic bands of maltodextrin is also observed, appearing in the region of 3400, 1620 and 1100 cm^{-1}, as already identified in the literature by Sritham and Gunasekaran (2017) and Moradi et al. (2022), who identified the characteristic bands in the region of 3400, 1600 and 1000 cm^{-1}, which correspond to elongation of the O—H bond, bending of the O—H bond or elongation of the C═C bond and elongation of the C—O bond of the alkoxy group. Therefore, with this observation, it is shown that the structure of maltodextrin did not change, inferring that, in the encapsulation process, there was no reaction between maltodextrin and the compounds of the extracts. In the ESCD and ESIN spectra, the band in the region of 1400 cm^{-1} corresponds to an asymmetric deformation of CH_3, according to Agarwal, Tandon and Gupta (2006), as in the structure of maltodextrin there is no CH_3 group, this band indicates the presence of other organic compounds from extracts of *B. forficata*. Lu et al. (2011), Medina-Torres et al. (2016) and Ragupati Raja Kannan, Arumugam and Anantharaman (2011), in their studies on phenolic content in *Allium cepa*, *Allium oschaninii*, *Litsea glaucescns* and seaweed, obtained spectra with similar absorption bands.

3.2.3. HPLC-PDA-ESI-IT-MS^n

The ESDC and ESIN chromatograms are shown in Fig. 7. Using High-efficiency liquid chromatography analyses with photodiode array detector coupled with electrospray ionization sequential mass spectrometry and ion trap analyser (HPLC-PDA-ESI-IT-MS^n) detected 25 peaks, which 22 were identified, in the two extracts, some with the same mass to load ratio (*m/z*). Among the identified substances, eighteen are distributed in both extracts, while three are present in ESDC and four are to ESIN.

Simple and chain carbohydrates were identified mainly in the initial region of the chromatogram (time less than eight minutes), phenolic acids and their glycosylated derivatives (between 8 and 10 min) *O*-glycosylated flavonoids, between 10 and 20 min, of the chromatogram, with the exception of peaks 8 and 11 (4.4 and 5.7 min, respectively), which are also flavonoid, this outcome is in line with the results of DSC, TG, XRD and FTIR.

In the study of sequential fragmentations obtained by mass spectrometry, one simple carboxylic acid and its five saccharides were identified. Among the saccharides, five chromatographic peaks with precursor ion $[M - H]^-$ *m/z* of 355 (peaks 5, 7, 9, 10 and 11) were observed. In all these peaks, the first fragmentation referred to a loss of $[M - 146 - H]^-$, giving rise to the ion product *m/z* 209, showing that they are isomers of the glucaric acid (since the other product ions formed are identical to those of peak 4) *O*-glycosylated to a rhamnose, the glucaric acid *O*-rhamnoside. This saccharide can form several constitutional isomers, since the rhamnose part of the molecule can bind to all the hydroxyls available in saccharic acid.

The chromatographic peaks numbers 12, 13, 14, 15 and 18 displayed as a deprotonated molecule $[M - H]^-$ a *m/z* 369 and its second order fragmentation gave rise to a product ion *m/z* 223 (MS^2 [369]) by the loss of $[M - 146 - H]^-$, referring to the fragmentation of the *O*-rhamnose bond. MS^3 of *m/z* 369 (MS^3 [369 → 223]) presented as ions products *m/z* 205 (100), 111 and 85 all belonging to sinapic acid, demonstrating that the molecules deprotonated at peaks 12, 13, 14, 15 and 18 refer to the isomers of sinapic acid-*O*-rhamnoside.

In the phenolic acid category peak 16 showed the *m/z* 399 ion as a

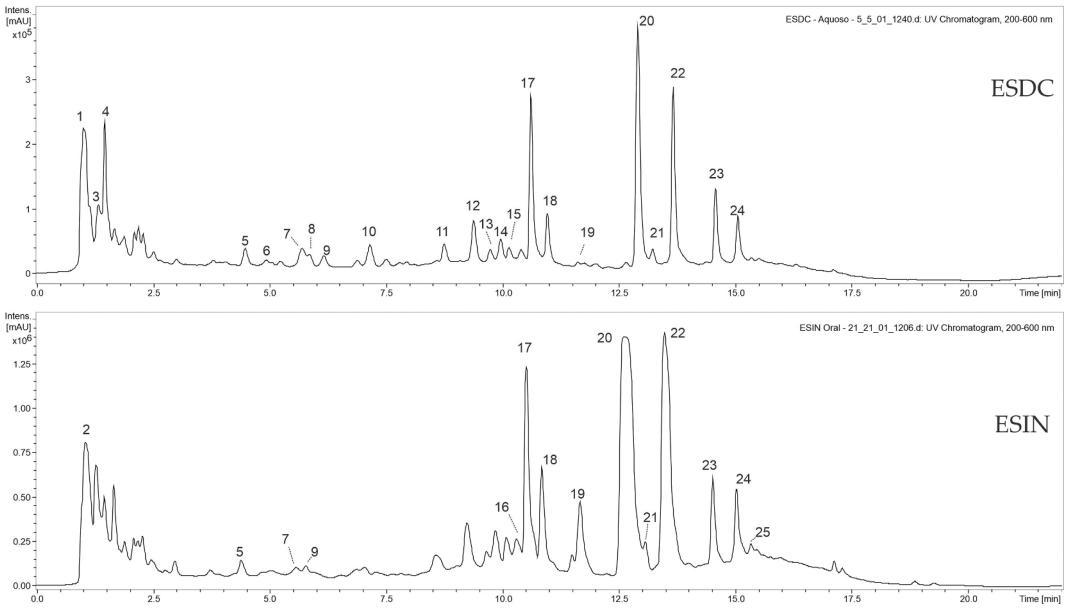

Fig. 7. HPLC-PDA-ESI-IT-MS^n analysis of dry extracts ESDC and ESIN.

deprotonated ion and precursor of the second order product *m/z* 223, formed by the loss of [M − 176 - H]$^-$ concerning the fragmentation of the ferrulate ion (ferulic acid anion), indicating the hydroxyl binding of the hydroxyl acid in the phenolic hydroxyl groups of sinapic acid, since *m/z* 223 and the third order fragment (MS^3 [399 → 223]), *m/z* 205 belong to that acid. Therefore, it possible to proposed the identification of feruloyl-sinapic acid for peak 16 of the ESIN chromatogram.

Peaks 8, 20 and 23 to 28 showed UV spectra with absorption bands between 254 and 365 nm characteristic of flavonols. Peak number 8 showed a non-protonated ion at *m/z* 447 and its second order fragmentation (MS^2[447]), gave rise to the product ion *m/z* 315 through the loss of an *O*-glycosylated pentose ([M − 132 - H]$^-$), where *m/z* 315 refers to isorhamnetin, a flavonoid aglycone by breaking the Y_0 bond, according to Cuyckens and Claeys (2004). Ferreres et al. (2012), in a study with some plants of the genus *Bauhinia*, found mainly flavan-3-ol type molecules or better known as flavonols.

Through its third order fragmentation (MS^3 [447 → 315]), the most stable ion formed was *m/z* 152 by the fragmentation of the bonds at position $^{1,3}A_0$, showing that the pentose is bonded at position three of the flavonoid, according to Cuyckens and Claeys (2004). Therefore, it can be proposed that peak 8 is isoramnetin-3-*O*-pentose. It was evident that three other peaks (22, 24 and 25) in the chromatograms had the ion *m/z* 315, referring to isorhamnetin, either in the second order fragmentation of its unprotonated molecule (24) or in the third order (peak 22 and 25). The differences between these peaks are found in the bonds and number of saccharides bonded to flavonoid aglycone. Peak 22 has as fragment MS^2, the product ion *m/z* 623, referring to the loss of an *O*-glycosylated rhamnose ([M − 146H]$^-$), while MS^3 forms the ions *m/z* 315, 300, where the first refers to the loss of a rutinosil or a disaccharide, rhamnosyl-hexoside and the second ion refers to the loss of a methyl, present in isorhamnetin.

Therefore, the deprotonated molecule of peak 22, which presented as a precursor ion *m/z* 769, is isoramnetin-3-*O*-ramsosil-rutinoside. This bioactive ingredient was also identified by Farag et al. (2015), in this same plant species. The chromatographic peak in 15 min (peak 24) has as unprotoned molecule the *m/z* 623 ion and because it exhibits the same sequential fragmentations of the previously proposed molecule, it should be isoramnetin-3-*O*-robinobioside. The signal in 15.3 min had [M - H]$^-$ ion at *m/z* 477 and with fragments MS^2 and MS^3 to be *m/z* 357 and 315 respectively, that is, peak 25 showed a $^{0,2}X$ break of *O*-hexose ([M - H − 120]$^-$) in the formation of its product ion MS^2 while its MS^3 ion was formed by the loss Y_0 ([M − 120 -H$^-$]) to demonstrate the unprotoned aglycone of isorhamnetin. Therefore, the deprotonated molecule of peak 25 refers to isoramnetin-3-*O*-hexose.

Another flavonol identified in the nanocapsulated extracts ESDC and ESIN, by this technique was kaempferol. This flavonol displayed as a deprotonated ion at *m/z* 285 and was present in the ions of peaks 17, 20, 21 and 23 with retention times of 10.6, 12.8, 13.1 and 14.5 min respectively. The flavonoid identified at peak 20 had *m/z* 885 as its deprotonated ion and *m/z* 739 (MS^2) and *m/z* 575 and 285 (MS^3) as its derived ions.

When forming the second order ion, the precursor ion *m/z* 885, showed a loss of one *O*-glycosylated rhamnose ([M − 146 - H]$^-$), while the *m/z* 575 ion originated from the fragmentation of an *O*-glycosylated hexose ([M − 162 - H]$^-$) and the *m/z* 285 showed kaempferol's aglycone through successive losses of two other units of rhamnoses, demonstrating that it was kaempferol-3-*O*-rhamnosyl-rutinoside.

Peaks 20 and 21, identified in both extracts, had a deprotonated ion at *m/z* 739 and due to sequential fragmentations similar to peak 20 (MS^2 [739]: *m/z* 593 and (MS^3 [739 → 593]): *m/z* 285), which were found to be isomeric molecules of each other, with kaempferol as aglycone and sequential losses of one rhamnose and one rutinose, therefore, peaks 20 and 21 can be named as two isomers of kaempferol-*O*-rhamnosyl-3-*O*-rutinoside, this is in line with the studies performed by Pizzolatti et al. (2003), Salatino et al. (1999), Ferreres et al. (2012), Farag et al. (2015), Santos, Fortunato and Spotorno (2018).

Peak 23 showed [M - H]$^-$ *m/z* 593, previously described as the product ion of ion 739, thus the difference between the two molecules was found in the assertion that [M - H]$^-$ *m/z* 593 showed one rhamnose less than the *m/z* 739 ion, so it is kaempferol 3-*O*-rutinoside, which is present both in the ESDC and in the ESIN.

Table 2 shows the identifications of the molecules of the ESDC and ESIN nanocapsulated extracts, based on retention time, UV spectrum and fragmentation by sequential mass spectrometry. Some different compounds were observed in both extracts, and it can be inferred that the extraction technique influences the compounds that will be extracted. This can be explained in the case of decoction and infusion, whose contact time and temperature variables are different. As the samples, in the decoction there is greater contact with the solvent, during heating, until boiling, there is a greater and better extraction of hydrophilic compounds, chemically modifying some constituents as well (Silva et al., 2019).

Table 2
Identification of molecules present in dry extracts ESDC and ESIN by HPLC-PDA-ESI-IT-MS^n.

Peak No	Retention time (minutes)			UV (nm)	[M-H]$^-$	Fragments of [M-H]		Identification proposal
		ESDC	ESIN			MS^2	MS^3	
1	1.1	+	-	225/258	377.2	161	143, 101	Unidentified
2	1.1	-	+	208/271	549.1	341	177; 161	Unidentified
3	1.2	+	-	200/270	223.0	205	111; 83	Sinapic acid
4	1.5	+	-	200/257	209	191	85	Glucaric acid
5	4.4	+	+	219/281	355.2	209	191	Glucaric acid-*O*-rhamnoside
6	4.9	+	+	200/270/435	471.2	398	-	Unidentified
7	5.6	+	+	204/227/31	355.1	209	191; 147; 85	Glucaric acid-*O*-rhamnoside isomer
8	5.7	-	+	202/223/313	447.2	315	152; 123	Isoramnetin-3-*O*-pentose
9	6.1	+	+	312/221	355.1	209	191; 147; 85	Glucaric acid *O*-rhamnoside isomer
10	7.1	+	+	226/313	355.3	209	191; 147; 85	Glucaric acid *O*-rhamnoside isomer
11	8.7	+	+	226/314	355.3	209	191; 147; 85	Glucaric acid *O*-rhamnoside isomer
12	9.4	+	+	227/313	369.1	223	205; 111; 85	Sinapic acid *O*-rhamnoside
Peak No.	Retention time (minutes)			UV (nm)	[M-H]$^-$	Fragments of [M-H]$^-$		Identification proposal
		ESDC	ESIN			MS^2	MS^3	
13	9.7	+	+	224/313	369.2	223	205; 111; 85	Sinapic acid *O-rhamnoside*
14	10.0	+	+	221/311	369.3	223	205; 111; 85	Sinapic acid *O-rhamnoside*
15	10.1	+	+	225/269/316	369.3	223	205; 111; 85	Sinapic acid *O-rhamnoside*
16	10.4	-	+	225/271/326	399.3	223	205	Feruloyl sinapic acid
17	10.6	+	+	229/266/347	885.7	739	575; 285	Kaempferol-3-*O*-rhamnosyl-rutinoside
18	11.0	+	+	204/255/353	369.1	223	205	Sinapic acid *O*-rhamnoside
19	11.8	+	+	228/266/342	441.5	395	249	Unidentified
20	12.8	+	+	203/266/346	739.6	593	285	Kaempferol *O*-rhamnosyl-*3-O-rutinoside*
21	13.1	+	+	203/266/346	739.7	593	285	Kaempferol *O*-rhamnosyl-*3-O-rutinoside*
22	13.6	+	+	203/255/353	769.6	623	315;300;255	Isoramnetine 3-O-rhamnosyl-rutinoside
23	14.5	+	+	226/266/343	593.2	285	255	Kaempferol 3-*O*-rutinoside
24	15.0	+	+	225/254/350	623.2	315	300; 271; 255	Isoramnetine 3-*O*-robinobioside
25	15.3	-	+	232/268/334/366	477.4	357	315; 300	Isoramnetine 3-*O*-hexosideo

3.3. *In vitro* bioaccessibility of polyphenols and flavonoids

In this study, the bioaccessibility of polyphenols and flavonoids was performed through simulated oral, gastric, intestinal and colonic digestion of the nanocapsulated extracts of the aqueous fraction of *B. forficata* Link leaves, ESIN and ESDC. The total polyphenol and flavonoid contents of ESIN and ESDC, as well as their bioaccessibility indices are presented in Table 3.

The total content of ESIN polyphenols had a statistically significant difference between the oral (17.75 mg EAG. g^{-1}) and gastric (14.80 mg EAG. g^{-1}) phases, but when they reached the duodenum, they became similar (15.79 mg EAG. g^{-1}), decreasing in the colonic phase (0.60 mg EAG. g^{-1}). When evaluating the bioaccessibility index, considering that most of the absorption of these compounds occurs in the duodenum, it was observed that 52.68 % of the polyphenols were bioaccessible in relation to their content before digestion. Pellegrini et al. (2018) and Tagliazucchi et al (2010) obtained similar results, in which they found that the oral phase negatively affects the recovery of polyphenols, being released from the matrix after gastric digestion, due to acid hydrolysis and pepsin action and, in the small intestine, it may have increased, since there is a change in pH, which becomes alkaline, which favors the formation of new metabolites.

Considering ESDC, the content of polyphenols showed a statistically significant increase in oral (7.83 mg EAG. g^{-1}), gastric (13.79 EAG. g^{-1}) and duodenal (16.99 EAG. g^{-1}) phases, with similar levels to ESIN, except in the colonic phase (0.88 EAG. g^{-1}). This can be explained by the structural transformation of polyphenols into new compounds, with different bioavailability or as a result of the release of these phenolics from macromolecules present in the plant matrix (Celep et al., 2018). It is noteworthy that polyphenols that are not absorbed in the duodenum, reach the colon, where the colonic microbiota hydrolyze the glycosides into aglycones and degrade them into simple phenolic acids, whose activity is of great biological importance, according to D'archivio et al. (2010).

Regarding flavonoids, ESIN showed decreased concentrations at each stage of the digestive process (7.19 mg EAG. g^{-1}, 6.28 mg EAG. g^{-1},

Table 3
Spectrophotometric determination of total polyphenols and flavonoids during the simulation of *in vitro* digestion of dry extracts of *Bauhinia forficata* Link and bioaccessibility index.

Polyphenols (mg EAG. g^{-1})			Flavonoids (mg EC. g^{-1})	
	ESIN	ESDC	ESIN	ESDC
ND	29.98 ± 0.08^{A}	21.49 ± 0.14^{A}	9.78 ± 0.28^{A}	3.06 ± 0.03^{bA}
Stages of gastrointestinal digestion				
Oral	17.75 ± 1.77^{aB}	7.83 ± 0.31^{bB}	7.19 ± 0.09^{aB}	3.56 ± 0.38^{bAD}
Gastric	14.80 ± 2.31^{aC}	13.79 ± 0.26^{aC}	6.28 ± 0.74^{aC}	1.13 ± 0.09^{bC}
Duodenal	15.79 ± 1.21^{aBC}	16.99 ± 0.20^{aD}	1.29 ± 0.25^{aD}	4.26 ± 0.32^{bD}
Colonic	0.60 ± 0.07^{aD}	0.88 ± 0.04^{aE}	NI	0.13 ± 0.01^{E}
Bioaccessibility index (%)				
Oral	59.21 ± 6.05^{A}	36.42 ± 1.28^{bA}	73.57 ± 3.12^{A}	116.09 ± 11.86^{bA}
Gastric	49.38 ± 7.79^{A}	64.17 ± 1.0^{bB}	64.08 ± 5.91^{A}	36.61 ± 3.08bB
Duodenal	52.68 ± 4.15^{A}	79.06 ± 1.08^{bC}	13.24 ± 2.88^{aC}	139.03 ± 8.86^{bC}
Colonic	2.00 ± 0.23^{aB}	4.08 ± 0.23^{aD}	NI	4.24 ± 0.33^{D}

Means ± standard deviations (n = 3). EAG: Gallic acid equivalent. CE: Catechine equivalence. ND: Not digested. NI: Not identified. ANOVA two-way, Tukey post-test ($p < 0.05$). Different lower case letters on the same line show statistically significant differences. Different capital letters in the same column represent statistically significant differences. The bioaccessibility index was calculated based on the content of polyphenols and flavonoids before digestion.

1.29 mg EAG. g^{-1}). However, an interesting fact is that 64.08 % of the flavonoids present in these nanocapsules, extracted with infusion, were resistant to acid pH and gastric pepsin, that is, more than half of the flavonoids are bioaccessible, which may be due to nanoencapsulation, such as aglycones, which are easily absorbed in the stomach and small intestine (De Morais Sousa et al., 2021). Referring to digestion in the duodenum, it was observed that 86.76 % of the flavonoids, in alkaline medium, were modified by oxidation or polymerization reactions.

Flavonoids in ESDC nanocapsules decreased from the oral phase (3.56 EAG.g^{-1}) to the gastric phase (1.13 EAG.g^{-1}), increasing after duodenal digestion (4.26 EAG.g^{-1}). These increased flavonoid contents (139.21 %), as observed for this same extract, in relation to polyphenols, can be explained as a result of the action of pancreatin, bile and protease, which extracted more flavonoids from intestinal fluids, when compared to aqueous extraction performed before digestion (De Morais Sousa et al., 2021).

The bioaccessibility of these bioactive compounds, evaluated in these extracts, of the present study, can be released from the food matrix, still in the oral phase, by mastication, even before the action of salivary amylase. The release of these bioactives, during all phases of digestion, varies for each food matrix, as it depends on their physicochemical properties such as molecular size, configuration, lipophilicity, solubility and pKa, so there are divergences in studies regarding the content of these substances. compounds after *in vitro* digestion. For these compounds to be absorbed in the small intestine, their glycosylated hydroxyl groups need to be biotransformed into aglycones, as already observed by Hollman et al. (1999), Kumar and Pandey (2013), Pandey and Rizvi (2009).

3.4. Antioxidant capacity of polyphenols during in vitro digestion simulation

To investigate the influence of the *in vitro* gastrointestinal digestion of ESIN and ESDC from *B. forficata* leaves, on the antioxidant capacity, two assays with different mechanisms of action were used: the methods of reduction of ferric ion (Fe^{3+}) in ferrous (Fe^{2+}) by FRAP assay and oxygen radical absorbing capacity (ORAC) (Table 4).

After digestion, a significant reduction in ESIN's antioxidant capacity was observed in the FRAP assay from the oral phase (13.45 μmol Trolox. g^{-1}) to duodenal (6.06 μmol Trolox.g^{-1}), as well as in ORAC, from 1902.22 μmol Trolox. mg^{-1} to 366.44 μmol Trolox. mg^{-1}, from oral to duodenal. In contrast, in ESDC, iron reduction activity (FRAP) increased from the oral phase (2.94 μmol Trolox.g^{-1}) to gastric (11.27 μmol Trolox.g^{-1}), but decreased in the duodenal phase (7.80 μmol Trolox. g^{-1}). The oxygen radical uptake capacity (ORAC) also decreases from the oral phase (793.47 μmol Trolox. mg^{-1}) to duodenal (88.06 μmol Trolox. mg^{-1}). In both extracts, the colonic phase showed no antioxidant activity in the methods studied, with the exception of ORAC in ESDC (108.11 μmol Trolox. mg^{-1}). This lack of antioxidant activity in the colonic phase is correlated with the reduced concentration of the bioactive compounds analysed in these extracts, either because they were not cleaved from their insoluble bonds during the fermentation process by the microbiota or because the metabolites formed have a low degree of hydroxylation, decreasing the activity capacity.

Similar results were verified by Buniowska et al. (2017), whose total antioxidant activity of a mixture of exotic fruit juice sweetened with *Stevia rebaudiana* decreased in ORAC tests after gastric digestion. Celep et al. (2018) also noticed a reduction in the total antioxidant capacity of *L. stoechas* ssp. *stoechas* leaf extract after *in vitro digestion*. In contrast, Pellegrini et al. (2017), in the FRAP, reported an antioxidant activity, after the oral phase, greater than that of undigested quinoa seeds.

The bioactive ingredient present in the duodenal phase of ESIN showed an increased activity (25.00 %), in the ORAC method, in relation to the ESDC (7.99 %), this is a relevant fact because this method verifies the sequestrating capacity of the hydrophilic and lipophilic fractions proportional to an antioxidant inhibiting the formation of a peroxyl radical, induced by AAPH (2,2′-azobis(2′-amidinopropane) dihydrochloride) at 37 °C, similar to human body temperature, whose peroxyl radical reacts with fluorescein forming a nonfluorescent product, according to Dávalos et al. (2004) and Prior et al. (2003).

Table 4
Antioxidant capacity of the FRAP and ORAC assays of dry extracts ESDC e ESIN, before and after the digestion and bioaccessibility index of the antioxidant action.

FRAP (μmol Trolox. g^{-1})			ORAC (μmol Trolox. mg^{-1})	
	ESIN	ESDC	ESIN	ESDC
ND	118.86 ± 0.82^{A}	57.68 ± 0.93^{bA}	1465.63 ± 1.97^{A}	1102.03 ± 0.92^{bA}
Stages of gastrointestinal digestion				
Oral	13.45 ± 0.11^{aB}	2.94 ± 0.17^{bB}	1902.22 ± 0.83^{aB}	793.47 ± 2.16^{bB}
Gastric	0.37 ± 0.21^{aC}	11.27 ± 0.31^{bC}	770.57 ± 2.74^{aC}	777.50 ± 1.69^{bC}
Duodenal	6.06 ± 0.61^{aD}	7.80 ± 0.85^{bD}	366.44 ± 0.49^{aD}	88.06 ± 2.65^{bD}
Colonic	NA	NA	NA	108.11 ± 1.05^{E}
Bioaccessibility index of antioxidant action (%)				
Oral	11.31 ± 0.02^{A}	5.09 ± 0.23^{bA}	129.83 ± 0.07^{A}	72.00 ± 0.14^{bA}
Gastric	0.32 ± 0.17^{aB}	19.55 ± 0.88^{bB}	52.57 ± 0.11^{aB}	70.55 ± 0.09^{bB}
Duodenal	5.09 ± 0.50^{aC}	13.53 ± 1.45^{bC}	25.00 ± 0.0^{aC}	7.99 ± 0.23^{bC}
Colonic	NA	NA	NA	9.81 ± 0.08^{D}

Means ± standard deviations (n = 3). ND: Not digested. NA: No activity. FRAP: ferric reducing activity power. ORAC: μmol trolox equivalent/mg. ANOVA two way followed by Tukey post-test ($p < 0.05$). Different letters in the same column show significant differences. Different symbols in the same row represent statistically significant differences. The bioaccessibility index was calculated based on the antioxidant activity of the extracts before digestion.

When comparing the antioxidant activity of both analysed methods, it appears that the bioactives present in ESIN and ESDC can act both as chelators of ferric ions (Fe^{3+}), with a reduction in this activity being observed, after digestion, and as scavengers. Of peroxyl radicals (ROO•), generated in ORAC, which is the main mechanism of action of these compounds, in this study, whose antioxidants released from ESIN nanocapsules were more bioaccessible to promote the removal or inactivation of ROO• formed during initiation or propagation of the reaction, through the donation of hydrogen atoms to these molecules, forming inactive species, interrupting the chain reaction, inhibiting oxidative stress (Cianciosi et al., 2022; Sasikala et al., 2018; Stagos, 2019; Stagos, 2019).

4. Conclusions

The nanocapsulated extracts of *Bauhinia forficata* Link leaves showed satisfactory yields, inferring an efficient encapsulation, with low values of humidity and stable water activity, as shown by the thermal analysis. The parameters Hr, %CI, granulometry, SEM and viscosity showed that these extracts are suitable for incorporation into tablets, capsules, sachets. X-ray diffraction revealed that these extracts had amorphous characteristics and FTIR showed that there was no reaction between the materials in the encapsulation process. Phenolic compounds, mainly flavonols and their O-glycosylated derivatives, were identified in the extracts, in addition to carbohydrates, probably maltodextrin, used as an encapsulating agent. The bioaccessible polyphenols and flavonoids of each extract showed antioxidant activity after the oral, gastric and

duodenal phases of *in vitro* digestion. Therefore, these nanocapsulated extracts are promising for application in new nutraceutical formulations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2022.100144.

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