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In vitro gastrointestinal digestion of microencapsulated extracts of *Flourensia cernua, F. microphylla, and F. retinophylla*

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ABSTRACT

Recently, some species of the genus *Flourensia* have been identified by their potential health effects (*e.g.* anti-inflammatory and apoptotic). Encapsulation of plant extracts is a process that can allow an adequate dosage administration, as well as to protect bioactive compounds and improve their controlled release in the gastro-intestinal (GI) system. Therefore, the aims of this work were: to microencapsulate the ethanol extracts *of F. cernua, F. microphylla*, and *F. retinophylla*; and to evaluate the controlled release of the microencapsuled extracts in an *in vitro* GI system. Leaves of *Flourensia* spp. were collected in wild sites of Coahuila State, and the ethanol extracts were obtained by the Soxhlet method. The encapsulation was performed by the gelation technique, using alginate. The microcapsules formed were characterized in terms of total phenol content (Folin-Ciocalteu method), antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiazo-line-6-sulphonic) diammonium acid (ABTS), and the ferric reducing antioxidant power (FRAP) assays, scanning electron microscopy (SEM), and thermal analysis, and *in vitro* GI digestion. The microcapsules were found to have spherical-shape and a micro-scale dimension in the range of 2.1–68.8 μm. Also, the built of microcapsules was confirmed by the appearance of an exothermic peak centered at ˜600 °C in the DSC analysis. *F. microphylla* noted for its strong antioxidant activity, even in its encapsulated form. In the gastric system the extracts of fresh microcapsules were released from 7.7% to 14.5%, while values of 26.5% to 53.3% were observed for those dried. For the intestinal system, the higher release was observed for dried microcapsules (59.9% to 78.4%) than for those fresh (26.3% to 30.2%). Thus, it was demonstrated that the alginate microcapsule protected the extracts until they were delivered to the target site in the GI model, and this effect was better with the dried micro-capsules of *Flourensia* spp. This study would set the guide for the application of *Flourensia* spp. extracts in order to take advantage of their benefits to human health.

1. Introduction

Flourensia spp. are wild plants that occur on a vast area from the south of USA crossing Mexico, Peru, Bolivia up to Argentina (Dillon, 1984). The genus *Flourensia* is formed by about 42 species, which at least nine of them have been found in Mexico (Rios, 2015), and three are considered as endemic of the state of Coahuila: *F. cernua, F. mi-crophylla*, and *F. retinophylla* (Jasso de Rodríguez et al., 2007). *F. cernua*, commonly known as "hojasén" is the most studied species, as it has proven antifungal, antibacterial, and antioxidant properties (Jasso de

Rodríguez et al., 2017b; Mendez et al., 2012; Castillo et al., 2010; Salazar et al., 2008; Molina-Salinas et al., 2006). The presence of ter-penes such as eudesmol, limonene, and borneol has been reported in its chemical composition (Vines, 1960), as well as alkaloid traces (Téllez et al., 2001). Other phytochemicals of diverse nature have been found for ethanol extracts of leaves of *F. cernua*, such as oxygenated sesqui-terpenes, sesquiterpenes, pyrazines, and steroids (Jasso de Rodríguez et al., 2017b). Also, *F. retinophyll*a (locally known as "yerba de mula") has antifungal activity against phytopathogenic fungi; which is char-acterized by the presence of amides, flavonols, and flavanones within

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its structure (Jasso de Rodríguez et al., 2017b; Stuppner and Müller, 1994; Dillon and Marby, 1977). Meanwhile, *F. microphylla* has shown antifungal, and also anti-inflammatory, and apoptotic effects on HT-29 colon cancer cells; in which were identified polyols, fatty acids esters, phenolic amines, phenolic compounds, and flavonoids (Jasso de Rodríguez et al., 2017b,a).

The rich composition of these species represents an outstanding source of bioactive agents to be used in the treatment of inflammatory processes affecting humans. However, their application depends on the chemical stability and bioavailability of the phytochemicals (Fang and Bhandari, 2010). Such is the case of polyphenols, which, due to their chemical nature are easily degradable by the action of light, heat, and pH, among other factors (Cilek et al., 2012; Munin and Edwards-Lévy, 2011). Also, flavonoids are highly sensitive to oxidant environments, pH, and temperature (McClements and Xiao, 2017). Additionally, most of the phytochemicals of interest present in some extracts, have a low solubility in water which limits their application (Pinheiro et al., 2017). This is the case of the extracts of the *Flourensia* spp. Microencapsulation of plant extracts is a good alternative to overcome this problem, which gives stability to the phytochemicals present in their composition (Gupta, et al., 2016; Fabra et al., 2016). Microencapsulation is defined as a technology of micro-scale (1.0–5000 μm) packaging of bioactive materials (solid, liquid or gaseous) in a polymeric matrix, being able to release these materials under controlled conditions (Hu et al., 2019; Cerqueira et al., 2014; Fang and Bhandari, 2010). This technology is widely used in food and pharmaceutical industries, and is constantly improving to develop encapsulation materials, as well as release mechanisms of the active ingredients (Cerqueira et al., 2014; Desai and Park, 2005). The study of the release of bioactive compounds from capsules in the gastrointestinal (GI) tract receives special interest, in such a way that it is possible to guarantee their integrity on their path to the release in a specific site and achieve the maximum effects (Jafari and McClements, 2017). In order to predict the release mechanisms of the bioactive compounds from microcapsules are used kinetic models of zero-order (K_o), first-order (K_1), and Higuchi (K_H) (Safdar et al., 2019). These models may optimize the extract release during the passage in the GI tract.

There is a constant interest in the study of the properties of plants of the Mexican semi-desert, and considering the potential of the bioactive compounds present in the species of *Flourensia* mentioned above, the aims of this work were: (1) to microencapsulate the ethanol extracts *of F. cernua*, *F. microphylla*, and *F. retinophylla*; and (2) to evaluate the controlled release of the microencapsuled extracts in an *in vitro* gastrointestinal system.

2. Materials and methods

2.1. Plant collection

Samples of *F. cernua*, *F. microphylla*, and *F. retinophylla* were randomly collected in a semi-arid zone south of the state of Coahuila, Mexico. The samples were placed in plastic bags and taken to the Phytochemistry Laboratory of the Universidad Autónoma Agraria Antonio Narro (UAAAN). Then, the leaves were separated from the branches, and dried at 60 °C for 48 h (Mapsa, Mexico). Subsequently, the leaves were ground in a Thomas Wiley mill (Swedesboro, NJ, USA) using a 2 mm mesh. The ground material was stored in polyethylene bags until further analyses.

2.2. Ethanol extracts of Flourensia spp.

The extracts were obtained by the Soxhlet method as described Jasso de Rodríguez et al. (2017b). Briefly, 14 g of a sample of dried and ground leaves were placed in a Whatman no. 1 filter paper inside a Soxhlet extractor and refluxed with 200 mL of ethanol for 72 h. After the extraction process, the solvent was separated by rotary evaporation

(Büchi Laboratoriums-Technik, Switzerland). Finally, the extract was subjected to drying in an oven at 50 °C for 24 h to eliminate the remaining solvent. The obtained extracts were weighed and stored in amber bottles and kept in desiccator at 25 °C and 0% of relative humidity (RH) until further tests.

2.3. Microencapsulation of Flourensia spp. extracts

The microencapsulation of extracts was carried out as previously reported by Calero et al. (2008) with some modifications. Firstly, 3 g of sodium alginate were dissolved in 100 mL of distilled water and adjusted to 200 mL; then, 500 mg of *Flourensia* spp. extract was added (Solution A). This procedure was carried out for each *Flourensia* extract. The mixture was constantly stirring until complete dissolution. Then, 3 g of calcium chloride were added to 200 mL of distilled water (Solution B). Solution A was placed in a separatory funnel and dropped into the Solution B for the formation of the microcapsules under continuously stirred at 600 rpm. The obtained capsules were separated into two batches, one of which was subjected to drying at room temperature (d = dried microcapsules) and the second was kept in suspension (f = fresh microcapsules) until further analyses.

In order to clarify, the concentration of the extracts used in the encapsulation process was selected according to preliminary tests (data not shown).

2.4. Extracts and microencapsulated extracts characterization

2.4.1. Total Phenolic Content (TPC)
The content of total phenols was determined by the Folin-Ciocalteu (FC) method described by Singleton et al. (1999), adapted to microplate. For all analyzes, 5 μL of sample (*i.e.* extracts or microencapsulated extracts) were placed in a 96-well microplate. Then, 60 μL of the FC reagent, 15 μL of Na_2CO_3 solution (7.5%, w/v), and 200 μL of distilled water were added to each well. The reaction was incubated at 60 °C for 5 min. Immediately, the absorbance was measured in a UV/Vis spectrophotometer (Synergy HT, BioTek Instruments, Inc. USA) at 700 nm. A calibration curve of gallic acid was prepared (0.2, 0.4, 0.6, 0.8, and 1.0 mg L^{-1}, $R^2 = 0.9919$). The TPC was expressed as milligrams equivalents of gallic acid per 100 mg of sample (mg GAE 100 mg^{-1} extract). Experiments were carried out in triplicate.

2.4.2. Antioxidant activity by DPPH assay
The quantification of the capacity of free radical capture of the extracts and microencapsulated extracts, was determined by measuring the degree of discoloration caused by the components of the sample to a methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to Ballesteros et al. (2015) with slight modifications. The reaction was carried out in a 96-well microplate, placing in each well 25 μL of sample and 200 μL of DPPH solution (150 μM, dissolved in methanol to an absorbance value from 0.70 ± 0.01 to 515 nm), and incubating at room temperature in the dark for 1 h. Then, the absorbance was measured in a microplate spectrophotometric reader (Synergy HT, BioTek Instruments, Inc., USA) at 515 nm. Methanol was used as control. The antioxidant activity was expressed as percentage of DPPH-scavenging activity relative to the control using the following equation:

$$\% \, Inhibición = \left(1 - \frac{A_s}{A_0}\right) \times 100$$

(1)

where A_0 and A_s are the absorbance of the control solution and the absorbance of the sample solution, respectively. All experiments were performed in triplicate.

2.4.3. Antioxidant activity by FRAP assay
The antioxidant capacity of the samples by the ferric reducing antioxidant power (FRAP) was carried out according to the method of

Benzie and Strain (1996). Briefly, a FRAP solution of 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl, 20 mM ferric chloride hexahydrate solution, and 300 mM buffer acetate (3.1 g sodium acetate trihydrate and 16 mL glacial acetic acid, pH 3.6) in a ratio 1:1:10, was mixed and incubated 10 min at 37 °C. The FRAP solution was freshly prepared before tests. Subsequently, 200 μL of sample were mixed with 3 mL of the FRAP solution, incubated at 37 °C for 30 min, and the absorbance was measured at 593 nm in spectrophotometer (Varian Cary 50, Palo Alto, CA, USA). The FRAP values were expressed in mM Fe^{2+} L^{-1}, using a calibration curve of iron (Fe^{2+}) sulphate solution. Experiments were conducted in triplicate.

2.4.4. Antioxidant activity by ABTS assay

The cation radical discoloration test (ABTS) of the extracts or microencapsulated extracts, was determined as described by Ballesteros et al. (2015) with some modifications. The ABTS radical cation was prepared by mixing 7 mM of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic) diammonium acid (ABTS) dissolved in distilled water with a 2.45 mM potassium persulfate solution, mixed and left at 4 °C in the dark during 14–16 h, until to achieve a stable oxidative state. After this time, the ABTS radical cation solution was diluted in a solution of 20 mM acetate buffer (pH 4.5) to an absorbance of 0.70 ± 0.01 at 734 nm. The assays were performed by mixing 10 μL of sample with 200 μL of ABTS radical cation solution. The mixture was maintained for 30 min in the dark at room temperature and then the absorbance was measured at 734 nm. Ethanol was used as control. The percentage of inhibition of the ABTS radical cation was calculated using the same equation used for DPPH assay. All experiments were performed in triplicate.

2.5. Characterization of microencapsulated extracts

2.5.1. Encapsulation efficiency
Encapsulation efficiency (%EE) was calculated according to the following equation:

$$EE(\%) = \frac{TPC_{total} - TPC_{free}}{TPC_{total}} \times 100 \tag{2}$$

where TPC_{total} represents the total amount of initial total phenolic compounds present in the sample added during microcapsule production, and TPC_{free} the free total phenolic compounds detected in the microcapsule.

2.5.2. Scanning electron microscopy (SEM) analysis
The structure and size of the microcapsules was determined through scanning electron microscopy (SEM) technique, using a Phillips XL30 ESEM microscope (Eindhoven, Netherlands). In order to record the SEM micrographs, the microcapsules were placed on a stainless-steel holder, following by a drying at room temperature; then they were coated with a fine layer of gold (40–50 nm) and examined using an acceleration voltage of 5 kV.

2.5.3. Thermal analysis
Thermal transition properties of neat alginate and dried microcapsules (20 mg) were analyzed using a Differential Scanning Calorimeter (DSC) with thermogravimetric analysis (TGA) in a simultaneous thermal analysis SDT Q600 analyzer V20.9 (New Castle, DE, USA). The thermograms were obtained at a scanning rate of 10 °C min^{-1} in 30–900 °C temperature range.

2.5.4. Extracts release assays
In order to simulate the passage and the delivery of the microencapsulated extracts through the GI tract the following assays were performed: for gastric conditions, 20 g of each sample were placed in a solution of 0.5% NaCl in flasks, and the pH was adjusted to 1–3 using 0.1 M HCl. The mixture was incubated at 37 °C, under shaking at 110 rpm for 2 h (Gbassi and Vandamme, 2012). Then, the microencapsulated extracts were taken from the gastric juice model and filtered under vacuum through a membrane filter (0.45 μm). Subsequently, they were placed in the intestinal juice model, a solution that consisted of NaCl (6.5 g L^{-1}), KCl (0.8 g L^{-1}), $CaCl_2$ (0.2 g L^{-1}), and $NaHCO_3$ (1.4 g L^{-1}) with a pH of 6.5-7.5. The mixture was incubated under shaking at 110 rpm at 37 °C for 20 h. The release of the extracts was measured by spectrophotometry at 2, 4, 6, 8, 10, and 22 h at the wavelength of maximum absorption observed for these extracts previously determined (194 nm), using a Spectrophotometer (DR5000-03, Hach, USA) (Calero et al., 2008).

2.6. Statistical analysis

Statistical analysis was performed through analysis of variance (ANOVA) ($p < 0.05$), and the mean comparison test was conducted using the Tukey test ($p = 0.05$) with the software "R" V 3.2.0. Models were fitted to *in vitro* release data by non-linear regression, using the software STATISTICA™ v7.0 (Statsoft Inc., USA).

3. Results and discussion

3.1. TPC and antioxidant activity of extracts and microencapsulated extracts of Flourensia spp.

The results showed that the concentration of polyphenols and the antioxidant activity of the microencapsulated extracts follow the same trend as the crude extracts (Tables 1 and 2). For TPC of the ethanol extracts of the species of *Flourensia* under study, the ANOVA showed statistical differences ($p < 0.05$), being higher for *F. retinophylla*, followed by *F. microphylla*, and *F. cernua*, with values of 63.5 ± 2.0, 46.9 ± 1.7, and 30.1 ± 0.2 mg GAE 100 mg^{-1} extract, respectively (Table 1). Similarly, the highest content of phenolic compounds was observed for the microcapsules of *F. retinophylla* extract (Table 2). However, the stronger ($p < 0.05$) antioxidant activity was detected for both, the extract and microcapsules of *F. microphylla*, being this behavior presented with all the different methods used for antioxidant determination (DPPH, ABTS, and FRAP). It is noteworthy that this activity remained similar for the crude extract and in its encapsulated form; which may be associated to the presence of certain compounds (*e.g.* flavonols and caffeic acids) identified in the extract with elevated stability and potential antioxidant (Jasso de Rodríguez et al., 2017a).

The microencapsulated extracts presented a phenolic content nearly two orders lower than the crude extracts. This may be due to the presence of an interaction between the polymeric structure of alginate and the chemical compounds present in the extracts, which generates a protection of microcapsule to the extract (Li et al., 2007a,b).

3.2. Encapsulation efficiency

The encapsulation efficiency (%EE) of microencapsulated extracts

Table 1
Total phenolic content (TPC) and antioxidant activities of ethanol extracts of *Flourensia* spp. leaves.

Ethanol extract	TPC (mg GAE 100 mg^{-1})	Antioxidant activity		
		DPPH (%)	ABTS (%)	FRAP (mM Fe^{2+} L^{-1})
F. cernua	30.1 ± 0.2[c]	43.3 ± 1.6[b]	32.5 ± 1.3[b]	1.2 ± 0.2[b]
F. microphylla	46.9 ± 1.7[b]	85.2 ± 0.1[a]	92.5 ± 0.3[a]	4.0 ± 0.2[a]
F. retinophylla	63.5 ± 2.0[a]	44.1 ± 1.0[b]	39.0 ± 8.3[b]	1.3 ± 0.0[b]

Values in the same column followed by different letters are statistically different ($p < 0.05$).
Values reported are the mean ± standard deviation.

Table 2
Total phenolic content (TPC) and antioxidant activities of microencapsulated ethanol extracts of *Flourensia* spp.

Microencapsulated extracts	TPC (mg GAE $100\,mg^{-1}$)	Antioxidant activity		
		DPPH (%)	ABTS (%)	FRAP (mM $Fe^{2+}\,L^{-1}$)
F. cernua	0.5 ± 0.0^c	3.9 ± 0.6^c	12.2 ± 1.3^b	0.6 ± 0.0^b
F. microphylla	0.7 ± 0.0^b	84.4 ± 0.2^a	50.2 ± 1.2^a	1.8 ± 0.1^a
F. retinophylla	0.8 ± 0.0^a	7.6 ± 0.5^b	12.3 ± 1.1^b	0.5 ± 0.0^b

Values in the same column followed by different letters are statistically different ($p < 0.05$).
Values reported are the mean $\pm$ standard deviation.

was in general elevated for all the species, with values of 99.3 ± 0.0, 99.7 ± 0.0, and $99.7 \pm 0.0\%$ for *F. cernua*, *F. microphylla*, and *F. retinophylla*, respectively. da Rosa et al. (2014) suggested the dependency of the %EE with the coating material used for the encapsulation, as they observed different %EE values (about 46.5–75.5%) when encapsulated phenolic compounds from blueberry in diverse matrixes (β-cyclodextrin, chitosan, xanthan, and hydrogel). Also, the composition and type of core material influences the encapsulation efficiency, as it has been reported that molecules with elevated hydrophobicity and smaller size present higher affinity for the coating material (Fang and Bhandari, 2010). Hu et al. (2019) detected %EE in the range of 14.4 to 27.9% depending on the citrus used as source of flavonoids, and associated this effect with the solubility of flavonoids in the encapsulating matrix (whey protein concentrate). Therefore, the high values obtained for the %EE proven the capacity of alginate to load the compounds present in the extracts of *Flourensia* species under study, as well as the interaction between the components; which could be performed through hydrogen bonding (Sanna et al., 2015).

3.3. Microscopy observations of microencapsulated extracts

The morphology and the size were determined by SEM, which showed capsules mostly spherical-shape (Fig. 1). The size distribution showed dimensions ranging from 2.1 to 3.2 μm for capsules of *F. cernua* and *F. retinophylla*, respectively; whereas the capsules of *F. microphylla* presented a greater variability in shape and size, with dimensions between 4.5 to 68.8 μm. In general, the values obtained allowed to classify the capsules formed as microcapsules, as they are in the range for this category (1.0–5000 μm) (Hu et al., 2019). The larger size of *F. microphylla* capsules may be related to having maintained a large part of their antioxidant activity (Table 2), probably due to a greater interaction between the bioactive compounds of the extract and the polymeric structure of the alginate. The results are comparable with those reported by Likitdecharoj and Ratanavaraporn (2018) for green tea encapsulated in gelatin, which had diameters between 66 and 73 μm; and Sanna et al. (2015) for resveratrol microencapsulated in a polymeric matrix of chitosan poly(D,L-lactic-co-glycolic acid) with values from 11 to 20 μm.

3.4. Thermal analysis

The presence of the extracts into alginate microcapsules is demonstrated by the thermal analysis of the samples. Fig. 2 shows the thermogravimetric and DSC analysis of the alginate microcapsules with and without *Flourensia* extracts. The DSC scans of neat alginate capsules (Fig. 2a) showed the characteristics peaks previously reported: one endothermic peak at 70 °C corresponding to the hydration water; two exothermic peaks at 180 °C and 280 °C, and another endothermic signal at 200 °C (Deng et al., 2019; Anbinder et al., 2011). Also, it is observed three exothermic peaks at 280 °C, 425 °C, and 600 °C in the thermogram of alginate capsules. As Anbinder et al. (2011) explained, the shift of the
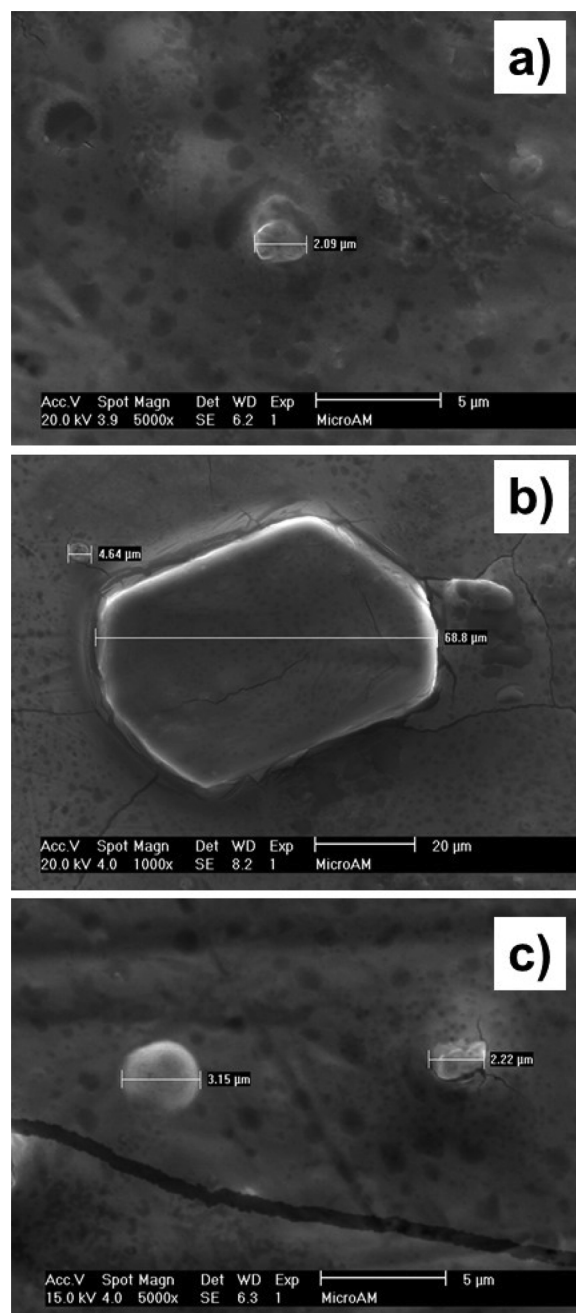

Fig. 1. SEM images of microcapsules of *Flourensia* spp. extracts: a) *F. cernua*, b) *F. microphylla*, and c) *F. retinophylla*.

endothermic peak at 180 °C–190 °C observed in microcapsules with extract, would be evidence of the presence of extract into the encapsulates, guaranteed the interaction between the polyphenols of the extract and the polymeric structure of alginate. On the other hand, the microencapsulated extracts exhibited a considerable increase in the intensity of the exothermic peak centered at 600 °C, which was more marked for microcapsules of *F. microphylla* (Fig. 2a). This may corroborate the presence of specific compounds of the *Flourensia* extracts within the capsules, which vary according the source of extract. The protection of polymeric capsule for each extract and encapsulation were also confirmed, as microcapsules of *Flourensia* extracts presented lower mass loss when compared with that of neat alginate capsules (Fig. 2b) (da Rosa et al., 2014). This effect was more evident for the microcapsules of *F. microphylla* (Fig. 2b), agrees with the stronger intensity at ~550 °C observed in the DSC extracts (Fig. 2a). This corresponds with
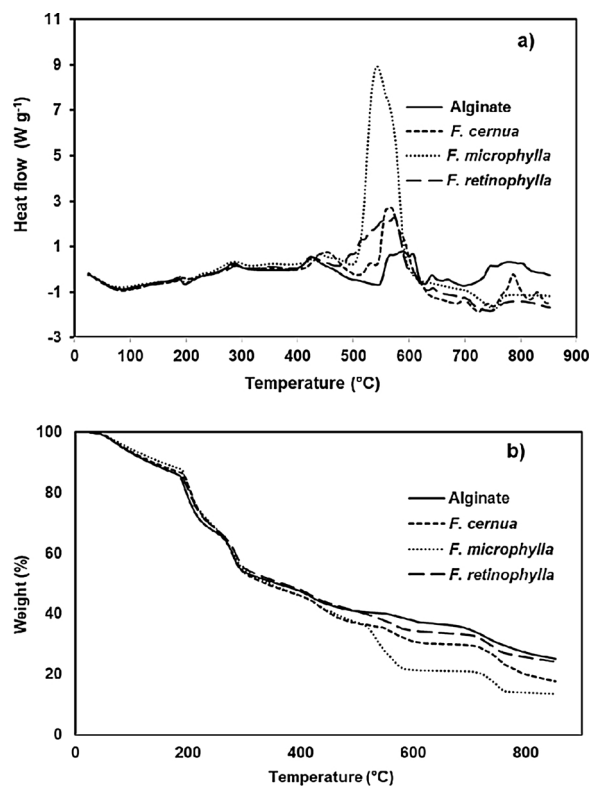
Fig. 2. a) Differential thermal analysis, and b) Thermogravimetric analysis of microcapsules of neat alginate, and extracts of *F. cernua*, *F. microphylla*, and *F. retinophylla*.

the higher antioxidant activity determined for the microcapsules of *F. microphylla* (Table 2), as well as with their larger size observed (Fig. 1b).

3.5. Microencapsulated extract release in simulated gastrointestinal fluids

The extract released (%) from the microcapsules in gastric fluids is presented in Fig. 3. During the test (2 h, pH 1.2), it was observed an extract release from the dry microcapsules of 26.5% to 53.3%, while for fresh microcapsules it was in the range of 7.7% to 14.5%. In the first instance, these data are not encouraging as it is desirable that the release in the stomach of an active ingredient is less than 10%. This is because for a drug to be effective, it must be absorbed in the intestinal
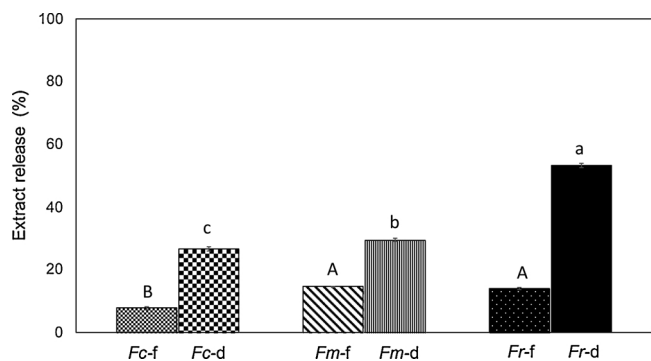
treatment, so the ideal is that the release is as much as possible in the intestine and not in the stomach. However, there are reports of release of natural extracts from other matrices that are even superior to those obtained in this work. Gorbunova et al. (2018) reported a high release (between 29% and 36%) of betacyanins, betaxanthins, and phenolic compounds from encapsulated extracts of alginate in gastric medium (pH 1.2). Likewise, Moreno et al. (2018) also reported a release higher than 20% of grape encapsulated by spray-dry. In this sense, it is interesting to carry out further studies in which some type of coating of the encapsulates is used to allow the release at the desired site (Colilla et al., 2010). Anbinder et al. (2011) demonstrated that the chitosan coating of alginate capsules prevents the release of polyphenols compounds from yerba mate extracts in gastric fluids, promoting the release in intestinal fluids. Also, it seems to be that the microcapsules of alginate enhance the solubility of the extracts of *Flourensia* species in aqueous solutions, and when dispersed it has a greater contact area with the fluid, increasing the percentage of release of the extract in the dry capsules (Zheng et al., 2011).

Regarding the release of *Flourensia* extracts from fresh microcapsules in intestinal fluid (pH 7), the values reached up to 30% at 22 h; whereas for dried microcapsules the release was significantly greater: 58%, 64%, and 80% for *F. cernua*, *F. microphylla*, and *F. retinophylla*, respectively (Fig. 4). In addition, it can be remarked that the release is constant throughout the time interval studied, which may be related to a prolonged and gradual release effect. It has been reported that for some materials the release of anti-inflammatory drugs, such as zoleodrante, is considered slow, as after 4 d a release of only 45% is achieved (Vallet-Regí et al., 2004).

It can be observed that the dried microcapsules of *F. retinophylla* presented the best behavior in the release of the extract (Fig. 4). However, it is necessary to consider that the release in gastric fluids was elevated (up 53%); thus, only about 25% is released in intestinal fluids. While the dried microcapsules of *F. microphylla* released up to 38% of the extract in intestinal fluid. In this sense, it is clear that the encapsulation protects the extract allowing its controlled release in the intestinal fluids.

On the other hand, to determine the reaction order of the release kinetics, the calculations of the release rate constants of the encapsulated extracts were performed (Table 3). This approach is important as it could predict the release behavior of an extract or active compound, and it helps to develop strategies to improve the release (Safdar et al., 2019). Except for *F. microphylla* fresh microcapsules (*Fm*-f), all the encapsulated extracts were fitted with zero-order model (K_o),

Fig. 3. Extract release in gastric fluids (pH 1.2) from fresh (-f) and dried (-d) microcapsules of *F. cernua* (*Fc*), *F. microphylla* (*Fm*), and *F. retinophylla* (*Fr*). Values are the mean and the error bars show the standard deviation.
Uppercase letters are used to compare means of fresh microcapsules; lowercase letters are used to compare means of dried microcapsules.
Means with the same uppercase and lowercase letter did not differ by Tukey's test ($p < 0.05$)
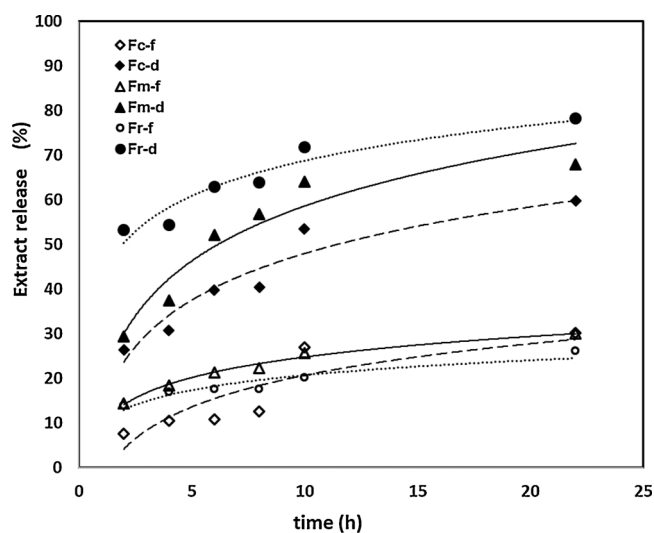

Fig. 4. Extract release in intestinal fluids (pH 7) from fresh (-f) and dried (-d) microcapsules of *F. cernua* (*Fc*), *F. microphylla* (*Fm*), and *F. retinophylla* (*Fr*).

Table 3
Kinetic parameters of extract release of *Flourensia* spp.

Sample	Zero order		First order		Higuchi	
	K_o (h^{-1})	r^2	K_1 (h^{-1})	r^2	K_H (h$^{1/2}$)	r^2
Fc-f	1.13	0.94	0.06	0.91	7.04	0.94
Fm-f	0.70	0.75	0.03	0.66	4.61	0.98
Fr-f	0.57	0.95	0.03	0.89	3.63	0.96
Fc-d	1.58	0.92	0.04	0.84	10.27	0.98
Fm-d	1.68	0.97	0.03	0.94	11.50	0.86
Fr-d	1.23	0.99	0.02	0.98	7.96	0.97

Fc: F. cernua; *Fm: F. microphylla*; *Fr: F. retinophylla*; *-f: fresh microcapsules*; *-d: dried microcapsules.*

as a correlation adjustment greater than 0.9 was obtained. Otherwise, for the model of first-order (K_1) were found correlations less than 0.9. This means that the release rate of the *Flourensia* spp. extracts encapsulated in alginate is constant and independent of the concentration (Safdar et al., 2019). The results obtained agree with those reported by Li et al. (2007a,b), who found that the release of garlic extracts, microencapsulated in ethyl cellulose/cellulose acetate phthalate, follows a zero-order kinetics between 60 and 120 min. Another kinetic model reported to understand the active compounds release was proposed by Higuchi (1963), which describes an empirical process to drug release, and it is based-on Fick Law. Higuchi model (K_H) considers that the release of a drug depends on time, thus, it is possible that an active principle is released following a zero-order model at the beginning of the ingestion and later change to a different behavior (Safdar et al., 2019). Then, from Table 3 it can be seen that the microcapsules of the extracts of *F. cernua* and *F. retinophylla* (fresh and dried) followed a zero-order model, and that a Higuchi model is also adjusted. The behavior of *F. microphylla* is different, as the release of the extract from fresh microcapsules is better fitted to the Higuchi model, while the release from the dry capsules follows a zero-order model. This may be associated to the larger particle size observed in the microcapsules (Fig. 1b), and also to the stronger antioxidant activity of this extract (Table 2).

The results obtained are promising, and suggest the effective protection that the microcapsule of alginate provides to the extracts of *Flourensia* species here studied.

4. Conclusions

Microcapsules of three species of *Flourensia* spp. were obtained by gelation with alginate, using a simple and reproducible methodology. The formation of capsules was demonstrated by the presence of an exothermic peak about 600 °C; while, their microscale size was confirmed by SEM, being in the range of 2.1–68.8 μm. The size dimension varied according to the source of extract. Most of the microcapsules presented a spherical shape, except for those of *F. microphylla*; however, they showed the most outstanding behavior, showing a strong antioxidant activity, even in its encapsulated form. In addition, the microcapsules of *F. mycrophilla* displayed the best release, as about 35% of extract can be profited, following a Higuchi model.

In general, it was proved the protection effect that the microcapsule provides to the extracts, obtaining better results of release in the intestinal fluids with the dry microcapsules than for the fresh microcapsules. Thus, this is a good vehicle of protection and delivery of functional compounds present in the *Flourensia* spp. This is the first scientific report of microencapsulation of *Flourensia* species, and their controlled release under *in vitro* GI digestion.

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