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Chitosan/bentonite nanocomposites: a new approach for the protection of essential oil from *Commiphora leptophloeos*

Nanocompósitos de quitosana/bentonita: uma nova abordagem para a proteção do óleo essencial de *Commiphora leptophloeos*

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The entrapment of essential oils in the lamellae of a polymer/clay nanocomposite allows, in addition to their protection, facilitation of their release. Therefore, tablets consisting of chitosan/bentonite/*Commiphora leptophloeos* essential oil (EO) were produced and their physical and physicochemical properties were studied. The EO, extracted by hydrodistillation, with a density of 1.0466 g mL^{-1}, presented as the main components of Germacrone and Germacrene B, terpenes of great biological importance. The tablets, in turn, obtained by direct compression of biodegradable film fragments of the clay nanocomposite, were characterized by infrared spectroscopy (FTIR), X-ray diffractometry (XRD), hardness, friability, and disintegration. The FTIR spectrum showed interactions between the chitosan matrix, bentonite, and functional groups of the EO, while XRD suggested the formation of an intercalated nanocomposite. The tablets had a mass of 0.397 ± 0.004 g, tolerance to a maximum force of 151.21 ± 4.36 N, and a disintegration time of 11 min ± 27 s. These results indicate the successful production of chitosan/bentonite tablets containing the essential oil from *C. leptophloeos*, opening up space for a range of applications for these tablets containing EO.
Keywords: nanocomposites, chitosan, bentonite.

O aprisionamento de óleos essenciais nas lamelas de um nanocompósito polímero/argila permite, além de sua proteção, a facilitação de sua liberação. Portanto, foram produzidos comprimidos constituídos de quitosana/bentonita/óleo essencial (OE) de *Commiphora leptophloeos* e estudadas suas propriedades físicas e físico-químicas. O OE, extraído por hidrodestilação, com densidade de 1,0466 g mL^{-1}, apresentou como principais componentes a Germacrona e o Germacreno B, terpenos de grande importância biológica. Os comprimidos, por sua vez, obtidos por compressão direta de fragmentos de filme biodegradável do nanocompósito de argila, foram caracterizados por espectroscopia no infravermelho (FTIR), difratometria de raios X (DRX), dureza, friabilidade e desintegração. O espectro de FTIR mostrou interações entre a matriz de quitosana, bentonita e grupos funcionais do OE, enquanto a DRX sugeriu a formação de um nanocompósito intercalado. Os comprimidos tinham massa de 0,397 ± 0,004 g, tolerância a uma força máxima de 151,21 ± 4,36 N e tempo de desintegração de 11 min ± 27 s. Esses resultados indicam a produção bem-sucedida de comprimidos de quitosana/bentonita contendo o óleo essencial de *C. leptophloeos*, abrindo espaço para uma gama de aplicações para esses comprimidos contendo EO.
Palavras-chave: nanocompósitos, quitosana, bentonita.

1. INTRODUCTION

Essential oils are volatile compounds produced by plants, containing 20-60 different components in varying concentrations [1], with two or three of these components present in high concentration (20-70%), which confer their physiological properties [2]. These oils have attracted interest from the scientific community due to their potential biological applications, including antimicrobial, anti-inflammatory, and antioxidant properties [3, 4].

Commiphora leptophloeos (Mart.) J. B. Gillett, a native Brazilian plant, contains an essential oil rich in volatile compounds [5] that demonstrate efficacy against pathogenic microorganisms

[6], making it a promising alternative to conventional antimicrobial agents. Additionally, the oil exhibits notable biological activity against *Aedes aegypti* larvae, with a median lethality rate (LC_{50}) of 99.4 ± 2.7 μg mL^{-1} [5]. However, its volatility, oxidation, and low chemical stability [7] hinder its direct application in biological systems. To protect essential oils, sensitive systems for control and active protection, such as clay nanocomposites, can be designed [8].

A promising approach to achieve the protection of essential oils is the use of clay-polymeric nanocomposites. These materials combine thermal and chemical stability with processability and flexibility [9], making them suitable for the development of drug encapsulation systems. Cationic clays, especially bentonites, exhibit remarkable adsorption and dispersion capacities [10], while chitosan, a polysaccharide derived from chitin found in the exoskeleton of insects and crustaceans [11], possesses properties such as biocompatibility, biodegradability, and non-toxicity [12].

Nanocomposites can be applied in different forms, such as films and tablets. Both films and tablets provide precise dosage due to standardized amounts of the active substance [13], reducing waste. Additionally, tablets are easy to handle, store, and transport, and are resistant to moisture and impacts [14], increasing their stability and prolonging their shelf life. The combination of bentonite and chitosan enables the creation of nanocomposites with suitable characteristics to produce films and tablets containing essential oils.

These systems can have applications in the pharmaceutical, cosmetic, and food industries [15]. In pharmaceuticals, they can be used for controlled release of bioactive compounds, protecting them from degradation. Compared to conventional antimicrobial agents, the encapsulation of essential oils in nanocomposites can help mitigate resistance, a growing problem due to the overuse of antibiotics [16].

In this context, the objective of this study was to develop a tablet that explores the additional advantages offered by chitosan/bentonite nanocomposites in the incorporation of bioactive essential oils, such as that of *C. leptophloeos*. The choice of this oil stems from its significant pharmacological properties, including antimicrobial and larvicidal activities, which are essential for developing effective bioactive materials. Through this approach, we focused on evaluating the physical and physicochemical properties of these tablets, which are relevant to their potential biological applications.

2. MATERIAL AND METHODS

2.1 Material

The following reagents were used in this study: chitosan (Sigma-Aldrich®) with a molecular weight of 60.05 g mol^{-1} and an experimentally obtained degree of deacetylation of 79.37%; acetic acid (Proquimios); and bentonite (Bentec S.A. Argilas).

2.2 Obtaining and characterization of the essential oil from *C. leptophloeos*

2.2.1 Obtaining the essential oil

The leaves of *C. leptophloeos* were collected at the Federal University of Vale do São Francisco, Agricultural Sciences Campus (9° 19' 39.4" S 40° 32' 50.0" W), on May 17, 2021, in the morning. Botanical confirmation was performed by the Vale do São Francisco Herbarium (HVASF), and access to the species was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under the number A0E9FB9. The leaves were cleaned, dried in an air circulation oven (SL 102 - SOLAB) at 40 ± 0.5 °C (SPL 059 - Spolu) for seven days, and ground in an industrial blender.

According to de Carvalho et al. (2023) [17], the mixture, consisting of dried leaves and distilled water, was heated to 100 °C for 2 h in a round-bottom flask attached to a Clevenger apparatus for hydrodistillation. The essential oil was collected, separated from the aqueous phase, and stored in a light-protected, refrigerated glass vial. The yield was calculated by relating the

mass of plant material ($m_{plant\ material}$, g) used to the mass of essential oil (m_{oil}, g) obtained using Equation 1:

$$\text{Equation 1} \qquad yield = m_{oil} / m_{plant\ material} * 100\%$$

2.2.2 Characterization of the essential oil

The density of the essential oil was determined using a picnometer, following the methodology described in the Brazilian Pharmacopoeia (2019) [18]. The empty container and the container filled with the essential oil (V_{oil} = 5 mL) were weighed at 20 °C to calculate the mass of the oil (m_{oil}, g). Then, the container filled with water was weighed at the same temperature to calculate the mass of water (m_{water}, g), and the mass density (d) and relative density (ρ) were calculated using Equations 2 and 3, respectively:

$$\text{Equation 2} \qquad d = m_{oil} / V_{oil}$$

$$\text{Equation 3} \qquad \rho = m_{oil} / m_{water}$$

A gas chromatograph coupled to a Shimadzu® mass spectrometer (Kyoto, Japan), model GC/MS-TQ8040, was used for GC/MS analysis of the essential oil to identify its volatile constituents, including monoterpenes and sesquiterpenes. The analysis was performed with an SH-RTX-5SILMS column (30 m x 0.25 mm ID, 0.25 µm film thickness), ultra-pure helium as the carrier gas (99.999% purity, White Martins S.A) at a flow rate of 1 mL min^{-1}, and an autoinjector (split/splitless). The identification of the essential oil components was performed by comparing the retention indices (RI) with tabulated values and by comparing the compound spectra with references from the Nist 107, 21, and Wiley 8 libraries.

2.3 Preparation of nanocomposite films

The intercalation method was used to produce chitosan/bentonite (NC) and chitosan/bentonite/essential oil (NC-EO) nanocomposite films with a ratio of 1:2, where the reaction time and polymer/clay ratio were previously determined. Chitosan was dispersed in a 0.25 mol L^{-1} acetic acid solution at a concentration of 10 g L^{-1}. The dispersion was stirred (SL 92 - SOLAB) at room temperature for 24 h before adding 20 g L^{-1} of bentonite and 1 g L^{-1} of the essential oil. The concentration of essential oil was selected based on the findings of da Silva et al. (2015) [5] for larvicidal activity against *Ae. Aegypti*. The beaker was covered with plastic film to prevent solvent evaporation and aluminum foil to protect from light exposure and essential oil volatilization. After stirring for 48 h at room temperature, the dispersion was deposited in Petri dishes and left at room temperature for four days to allow the solvent to evaporate.

2.4 Physical-chemical characterization of the nanocomposite films

2.4.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analyses were performed using the Shimadzu® IRTracer-100 spectrometer in the wavenumber range of 4000-600 cm^{-1}, with 45 scans and a resolution of 8 cm^{-1} [19]. For chitosan and bentonite, KBr pellets were prepared using 1 mg of the sample and 100 mg of KBr, while the ATR module was used for the essential oil and the NC and NC-EO films.

2.4.2 X-ray Diffraction (XRD)

XRD analyses were conducted to characterize the crystalline structure of chitosan, bentonite, and NC and NC-EO films, using a Rigaku® MiniFlex X-ray diffractometer equipped with CuKα radiation (λ = 1.54056 Å) and a nickel filter, with a voltage of 40 kV and a current of 15 mA. The

scanning range (2θ) was set from 5 to 35°, with a scanning speed of 10° min^{-1} and an angular step of 0.02° [20].

2.5 Production of the tablets

The dried films were triturated in a knife mill (SL 31 - SOLAB) and sieved using a granulometric analysis sieve with an opening size of 425 μm and a mesh size of 40. To produce the tablets, 0.4 g of the nanocomposite film powder – composed of chitosan, bentonite, and essential oil in a ratio of 1:2:0.1, without additional excipients – was placed in a circular punch set with a diameter of 8 mm. A force of 1.5 t was applied for 30 s using a benchtop press, adapting the methodology from de Souza et al. (2014) [21], so that each tablet theoretically contained approximately 12.9 mg of essential oil. The individual masses of 20 tablets were measured using an analytical balance to verify mass uniformity, and the average mass was calculated by taking the arithmetic mean according to the Brazilian Pharmacopoeia (2019) [18].

2.6 Characterization of the tablets

2.6.1 Hardness test

The hardness test was performed by adapting the standard procedure established by the Brazilian Pharmacopoeia (2019) [18] and ASTM D 695-15 [22]. Ten tablets were tested in the radial orientation using an EMIC DL-10000 universal testing machine with a load cell capacity of 0.5 tf (1 tf = 1000 kgf), with a load application speed of 2 mm min^{-1}, and the values of force at rupture were measured.

2.6.2 Friability test

The friability test was conducted using a friabilator (Nova Ética - Mod. 300), following the procedure described in the Brazilian Pharmacopoeia (2019) [18], with 20 samples rotated at 25 rpm for 4 min. The percentage of lost powder was calculated according to Equation 4:

Equation 4 $$\text{Friability} = ((\text{initial mass} - \text{final mass}) / \text{initial mass}) * 100\%$$

2.6.3 Disintegration test

The methodology for the disintegration test was based on the Brazilian Pharmacopoeia (2019) [18] with adaptations. Six tablets were placed in beakers containing 2 L of mineral water (composition: water and 30.0 mg L^{-1} of sodium bicarbonate, pH = 7.2) in a water bath (SL - 150), and the time for complete disintegration at 34 ± 0.5 °C was observed, as this temperature simulates the environmental conditions in which the biolarvicidal tablets would be applied.

3. RESULTS AND DISCUSSION

3.1 Obtaining and characterization of the essential oil from *C. leptophloeos*

3.1.1 Obtaining the essential oil

The yield of the essential oil extraction process is influenced by environmental factors, climate, plant age, collection conditions, and time [23]. Thus, it is common to find divergent data in the literature regarding this parameter due to the cultivation and collection circumstances of the botanical species. The yield for the essential oil from *C. leptophloeos* (EO) was 0.68%, higher than the yield of 0.08% obtained by da Silva et al. (2015) [5] for the hydrodistillation of leaves of the same species collected in March 2012 in the Catimbau National Park, located in the municipalities of Buíque, Ibimirim, and Tupanatinga - PE. However, this yield was lower than

the 2.05% reported by Pinto et al. (2022) [24] for the hydrodistillation of leaves collected in the "Barra da Onça Settlement" in Poço Redondo – SE.

3.1.2 Characterization of the essential oil

Essential oils are composed of terpenes, which have different compositions, reflecting in their physicochemical characteristics, such as density. These differences are observed among species of the same genus or different ones [25]. This justifies the mass density obtained for the EO of 1.0466 g mL^{-1}, higher than the relative density of 0.98 obtained by Mohamed et al. (2014) [26] for the essential oil from *Commiphora myrrha*. Although they belong to the same genus, the composition was determinant for the difference in density.

The EO presents Germacrone (50.41%), Germacrene B (23.94%), and Germacrene D (4.05%) as its main components (Table 1), which are also present in other *Commiphora* species [27]. However, the chemical composition of a volatile oil extracted from the same organ of the same plant species can vary depending on the time of collection, climatic conditions, and soil [28]. Da Silva et al. (2015) [5], for example, identified α-Phellandrene (26.3%), (E)-Caryophyllene (18.0%), and β-Phellandrene (12.9%) as the main constituents of the essential oil from *C. leptophloeos*, diverging from what was found in this study.

Table 1: Chemical composition of the essential oil from C. leptophloeos determined by GC/MS.

Retention Index (RI)	Area (%)	Compound
1588	1.71	β-Elemene
1616	2.45	α-Gurjunene
1628	1.89	β-Caryophyllene
1657	3.15	(−)-Allo-aromadendrene
1678	4.05	Germacrene D
1685	1.58	α-Guaiene
1693	3.13	γ-Elemene
1757	23.94	Germacrene B
1775	3.00	Spatulenol
1797	1.88	β-Elemenone
1802	2.81	Ledol
1894	50.41	Germacrone

3.2 Physical-chemical characterization of the nanocomposite films

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

Figure 1A shows the FTIR spectra for chitosan, bentonite, EO, and the NC and NC-EO films. The FTIR spectrum of chitosan shows absorption bands at 3454 cm^{-1} (O-H stretching), 2922 cm^{-1} (C-H stretching), 1638 cm^{-1} (amine and amide groups), 1429 cm^{-1} (C=O stretching), 1376 cm^{-1} (N-H and C-N bending), 1158 cm^{-1} (asymmetric C-O-C stretching), and 1053 cm^{-1} (C-O skeletal stretching vibrations) [29]. The FTIR spectrum of bentonite exhibits absorption bands at 3795-3190 cm^{-1} (3630 cm^{-1} and 3437 cm^{-1}, related to the stretching of OH in water), 1643 cm^{-1} (bending of OH in water), 1041 cm^{-1} (Si-O-Si stretching) and 794 cm^{-1} (Al-Al-OH deformation) [30].

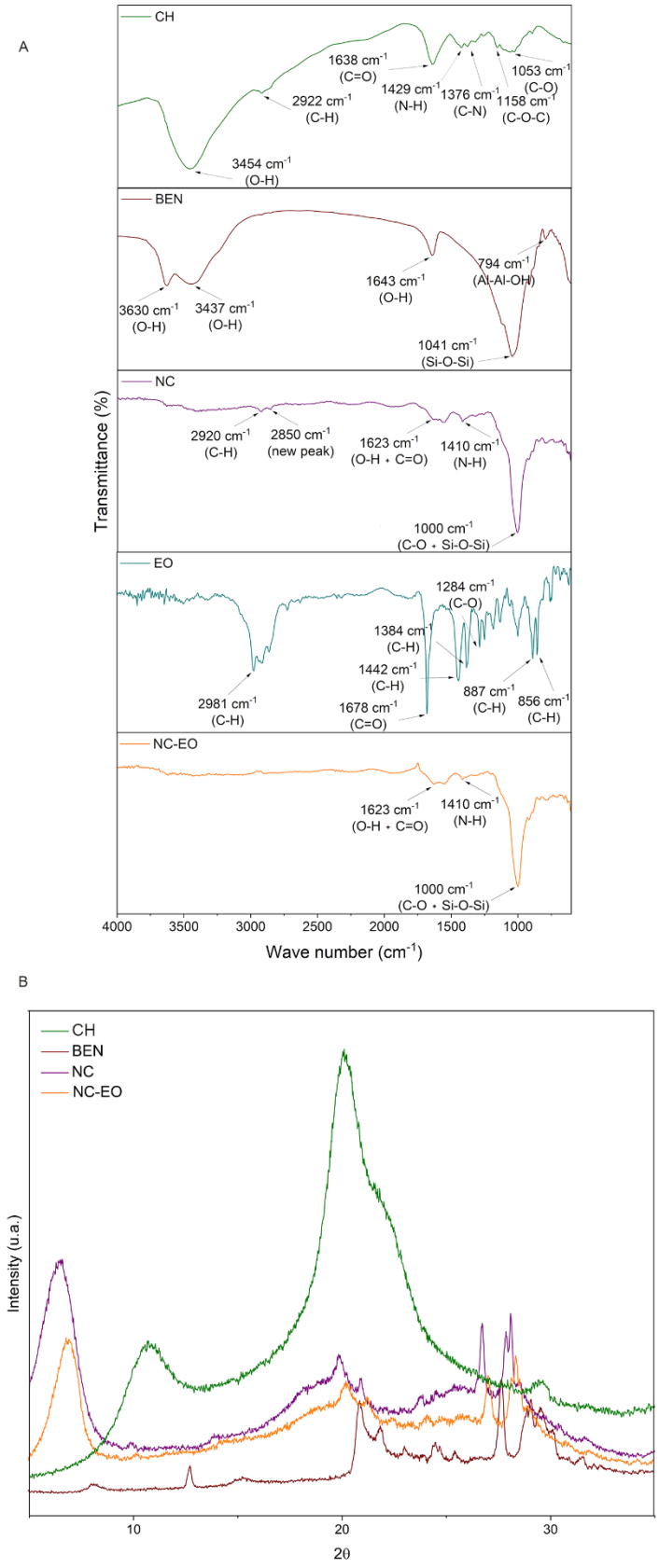

Figure 1: (A) FTIR spectra for chitosan (CH), bentonite (BEN), essential oil from C. leptophloeos (EO), chitosan/bentonite (NC) and chitosan/bentonite/essential oil (NC-EO) films. (B) diffractograms for CH, BEN, NC and NC-EO films.

The FTIR spectrum of the NC film shows bands at 2920 cm^{-1}, 2850 cm^{-1} (new band, attributed to C-H stretching), 1680-1500 cm^{-1}, 1410 cm^{-1}, and 1000 cm^{-1}, indicating interactions between the functional groups of chitosan and bentonite matrix [31].

For the EO, bands were found in the FTIR spectrum at 2981 cm^{-1} (C-H stretching), 1678 cm^{-1} (C=O stretching of the ketone group in Germacrone), 1442 cm^{-1} and 1384 cm^{-1} (C-H bending), 1284 cm^{-1} (C-O stretching), 887 cm^{-1} and 856 cm^{-1} (out-of-plane C-H bending) [32], confirming its chemical composition as hydrocarbons of the terpene class, consistent with the results presented by GC/MS. The FTIR spectrum of the NC-EO film is like that of the NC film with the disappearance of bands at 2920 cm^{-1} and 2850 cm^{-1}, suggesting the formation of bonds between phenolic compounds from the EO and chitosan [33].

3.2.2 X-ray Diffraction (XRD)

Figure 1B shows the diffractograms for chitosan, bentonite, and the NC and NC-EO films. The diffractogram of chitosan exhibits characteristic peaks at 10.7° (d = 8.26 Å) and 20.1° (d = 4.41 Å), indicating a semicrystalline structure [34], attributed to the presence of O-glycosidic bonds in the carbohydrates [35]. The diffractogram of bentonite displays reflection peaks characteristic of montmorillonite at 2θ = 8.12° (d = 10.88 Å) and 12.78° (d = 6.92 Å) [30].

For the NC film, the disappearance of the peak at 10.7º and an increase in the intensity of the peak at 8.12°, which is shifted to 2θ = 6.41° (d = 13.78 Å), indicates the formation of an intercalated nanocomposite [33]. The NC-EO film showed a similar profile to the NC film, with a reduction in the intensity of the peaks, indicating a decrease in crystallinity due to oil dispersion [30]. The films with EO exhibited higher angles and reduced interplanar distances, as indicated in Table 2. In both films, the characteristic reflection peak of bentonite appeared at lower 2θ values, suggesting some degree of intercalation and exfoliation [34].

Table 2: Main peaks of the diffractograms for the chitosan/bentonite (NC) and chitosan/bentonite/essential oil (NC-EO) films.

NC film		**NC-EO film**	
2θ (°)	**d (Å)**	**2θ (°)**	**d (Å)**
6.41	13.78	6.90	12.80
19.88	4.46	20.23	4.39
26.75	3.33	27.09	3.29
28.08	3.18	28.37	3.14

3.3 Production of the tablets

Seventy tablets were produced from the NC-EO films, which had a dark color and smooth surface, without visible cracks or wear. The tablets had an average mass of 0.397 ± 0.004 g, a diameter of 8.21 ± 0.01 mm, and a thickness of 4.03 ± 0.09 mm. The variation limit for the mass of the tablets was less than ± 10.0%, with the highest variation value being 7.23%, which is within the limit established in the Brazilian Pharmacopoeia (2019) [18]. The tablets were darker than the NC-EO films, which may be attributed to chemical oxidation reactions caused by the increased surface area during the milling process.

3.4 Characterization of the tablets

3.4.1 Hardness test

The NC-EO tablets exhibited a maximum force tolerance of 151.21 ± 4.36 N and a deformation of 0.20 ± 0.08 mm during the hardness test (Table 3). The Brazilian Pharmacopoeia (2019) [18] does not specify a minimum acceptable hardness value for tablets. The hardness of the tablets was comparable to those formulated by Soares et al. (2003) [36], which contained a high concentration of dry extract from *Maytenus ilicifolia* in a ratio of 3.5:1 with microcrystalline cellulose, along with colloidal silicon dioxide (2.2%) and magnesium stearate (1.0%). These tablets were

produced with a force of 10 kN (≈ 1 t) at a speed of 25 rpm, which had a hardness of 148.2-159.4 N.

Table 3: Results of hardness, friability and disintegration tests.

Test	Variable	Mean ± Standard deviation
Hardness test	Maximum Force (N)	151.21 ± 4.36
	Strain at Maximum Force (mm)	0.20 ± 0.08
Friability	Initial mass (g)	0.3721 ± 0.0039
	Final mass (g)	0.3703 ± 0.0040
	Mass loss (g)	0.0018
	Mass loss (%)	0.49
Disintegration	Time (minutes ± seconds)	11 ± 27

The NC-EO tablets exhibited satisfactory resistance and compression time, which depends on the compression force, time, and composition.

3.4.2 Friability test

The NC-EO tablets exhibited a friability of 0.49% (Table 3), which is below the acceptable limit of 1.5% set by the Brazilian Pharmacopoeia (2019) [18]. Their friability was higher than that of the previously mentioned *M. ilicifolia* dry extract tablets [36], which showed a friability of 0.26-0.36%.

Friability is influenced by the compression force [37], as well as by the speed and, consequently, the compression time, as demonstrated by Spaniol et al. (2009) [38]. In their study, tablets composed of 92% of granules with a high load of *Phyllanthus niruri* L. spray-dried extract, 7.92% excipient granules (62.9% microcrystalline cellulose and 37.1% sodium starch glycolate), and 0.08% magnesium stearate were produced on a rotary tablet press at speeds of 15, 22.5, and 30 rpm, exhibiting friability of 0.87%, 0.57%, and 0.09%, respectively.

Therefore, the friability of the NC-EO tablets indicated that the compression force and time used were appropriate.

3.4.3 Disintegration test

The disintegration time of a tablet can be influenced by various factors. Among them, the compression time and force, the presence of additives in the formulation, and the test medium used are noteworthy [13]. The compression process and applied force can affect the density and porosity of the tablet, either delaying or accelerating its disintegration [39]. Additionally, additives added to the formulation can enhance the physical properties of the tablet but may also impact its disintegration by modifying particle cohesion, solubility of active substances, and matrix porosity [40]. Finally, it is important to consider the test medium as disintegration may vary depending on the liquid used for the test [13].

The NC-EO tablets disintegrate in 11 min ± 27 s (Table 3), which is like tablets composed of 31.92% granulated *Calendula officinalis* L. hydroethanolic extract, 33.35% D-lactose monohydrate, 21.80% cellulose, 9.06% Duryea starch, and 3.87% magnesium stearate. These tablets, when produced with a force of 20 kN (≈ 2 t) for 4 min, disintegrated in 13 min using distilled water at 37 ± 1 °C [41]. However, they took less time than that of the *M. ilicifolia* dry extract tablets (25.80-29.32 min) [36] using distilled water at 37 °C. The absence of additives in the tablets leads to rapid disintegration, which is proportional to the force and duration of compression. The obtained disintegration time is suitable for the desired immediate action of the active compound.

4. CONCLUSION

Given the physical instability characteristics of essential oils, the development of structures for their incorporation proves to be an innovative alternative to enhance their applicability. In this regard, tablets were obtained from chitosan/bentonite films containing essential oil from *Commiphora leptophloeos* (EO). The intercalation of EO in clay composite devices, an essential step for composite formation, is possible and was confirmed through the results of FTIR and XRD for the films. From these films, NC-EO tablets were fabricated and exhibited suitable characteristics, including mass, hardness, and satisfactory disintegration, in accordance with current Brazilian legislation.

The development of structures for the incorporation and stabilization of essential oils offers new possibilities for their application. This study highlights the originality in obtaining polymer/clay tablets containing essential oil, as no previous studies on the subject were found. The NC-EO tablets were developed to enhance the stability of essential oils, with potential applications in pharmaceutical and biocidal fields. Due to their composition and properties, these tablets could be particularly useful in antimicrobial formulations and targeted drug delivery systems, and biolarvicidal applications. This work fills a gap in the existing literature and provides a foundation for future research. Further studies can explore new applications and optimize these encapsulation systems, expanding the use of essential oils and promoting the development of innovative solutions for healthcare and pest control.

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