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PHYSICOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF ALGINATE-ENCAPSULATED ESSENTIAL OILS OF *ORIGANUM VULGARE* L. AND *THYMUS VULGARIS* L. AGAINST CANINE AND FELINE UROPATHOGENS

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ABSTRACT

Urinary tract infections are common in small-animal practice, with Escherichia coli and *Klebsiella pneumoniae* as the primary causative agents. Bacterial resistance to synthetic antimicrobials has driven the search for natural alternatives, such as essential oils, whose direct application is limited by their volatility and instability. The objective of this study was to characterize, both physicochemically and morphologically, alginate capsules containing a mixture of essential oils from *Origanum vulgare* L. and *Thymus vulgaris* L., as well as to evaluate, in a complementary manner, their antibacterial activity against uropathogenic bacteria isolated from dogs and cats. The capsules were obtained via ionic gelation and characterized for physical properties, water activity, moisture content, and morphology. Antibacterial activity was evaluated using the agar diffusion method. The encapsulated systems exhibited parameters consistent with good flowability, physicochemical stability, and a water activity below 0.7. Furthermore, the capsules containing the essential oils maintained antibacterial activity, as evidenced by the formation of inhibition zones against the evaluated strains. In conclusion, alginate encapsulation is a promising strategy for protecting and applying essential oils, with potential use in controlling uropathogenic pathogens in veterinary medicine.

***Keywords*:** Alginate capsules, Carvacrol, Thymol, Uropathogenic bacteria, Companion animals.

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1. INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial diseases diagnosed in small animal practice, particularly in dogs and cats, with *Escherichia coli* and *Klebsiella pneumoniae* as the primary causative agents (Sousa et al., 2019; Silva et al., 2025). Treatment of these infections often relies on synthetic antibiotics, which are frequently administered empirically and repeatedly, thereby favoring the selection of multidrug-resistant strains in the veterinary setting (Sellera et al., 2025). This scenario has been widely described in urinary isolates from small animals and is associated with treatment failure, recurrent infections, and increased costs of clinical management (Scarpellini et al., 2025).

In this context, essential oils have attracted growing interest as natural alternatives due to their antibacterial activity and efficacy against diverse bacterial groups (Matte et al., 2023; Brenda et al., 2024; Mustafa et al., 2025; Dao et al., 2026). These plant-derived compounds have the potential to inhibit the growth and reproduction of pathogenic microorganisms. Furthermore, their use does not promote the development of microbial resistance, as they possess a complex structure that targets multiple cellular targets within microorganisms, making them effective in controlling clinically relevant pathogens (Nuță et al., 2021; Cui et al., 2024; Khair et al., 2026).

The essential oils of *Origanum vulgare* and *Thymus vulgaris* have been studied for their activity against bacterial pathogens, including Gram-negative species, which is attributed primarily to phenolic compounds such as thymol and carvacrol (Cui et al., 2024). These constituents act by disrupting the bacterial cell membrane, resulting in the inhibition of microbial growth or death.

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Despite their recognized antibacterial potential, the direct application of essential oils has limitations, including high volatility and instability under environmental conditions, which can compromise their efficacy (Nuță et al., 2021), particularly in complex biological systems such as the urinary tract. Consequently, encapsulation has been employed as a strategy to protect bioactive compounds, increase stability, and promote controlled release, thereby expanding the application possibilities of these systems (Nuță et al., 2021; Štrbac et al., 2021).

The objective of this study was to characterize the physicochemical and morphological properties of the encapsulation systems employed and to evaluate capsules containing a mixture of essential oils from *Origanum vulgare* L. and *Thymus vulgaris* L. for their antibacterial activity against strains isolated from urinary tract infections in dogs and cats.

2. MATERIALS AND METHODS

This study was approved by the Animal Ethics Committee of the Federal University of Campina Grande (CEUA/CSTR/UFCG, protocol number: 022026).

2.1. Location of the Experimental Tests and Test Substances

The experimental tests were conducted at the Microbiology and Biochemistry Laboratories of the Central Laboratories of the Academic Unit of Biological Sciences (UACB) at the Federal University of Campina Grande (UFCG), Patos Campus, Paraiba, Brazil. The essential oils of *Origanum vulgare* L. (oregano) and *Thymus vulgaris* L. (thyme) were purchased from a certified commercial supplier (Quinarí®, Ponta Grossa, Paraná, Brazil). The samples were stored in amber vials, refrigerated (4°C), and protected from light until use.

2.2. Bacterial Strains and Culture Media

Clinical strains of *Escherichia coli* isolated from dogs (Ecc 49) and cats (Ecg 13) were used, as well as Klebsiella pneumoniae (Kp 04) strains isolated from dogs, obtained from urine samples of animals with a clinical diagnosis of urinary tract infection. The bacterial strains were maintained on Mueller-Hinton agar (MHA) at 4°C until testing. Inocula were prepared from fresh cultures on MHA incubated at 35 ± 2°C for 18–24 h and subsequently diluted in 0.9% sterile saline until a turbidity equivalent to 0.5 on the McFarland scale was obtained, corresponding to approximately 1.5×10^8 colony-forming units per milliliter (CFU/mL) (Bona et al., 2014).

2.3. Analysis by Gas Chromatography Coupled with Mass Spectrometry (GC-MS)

The chemical composition of the essential oil was analyzed using a gas chromatograph coupled with a quadrupole mass spectrometer (GC-MS); Shimadzu, model GC-QP2010 (Shimadzu, Kyoto, Japan). A DB-5 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm) was employed.

The chromatographic conditions employed were as follows: injector temperature of 220°C, split ratio of 1:10 (3.0 minutes), helium (He) carrier gas at a flow rate of 0.6 mL/minute, interface temperature of 250°C, and an electron impact ionization source operating in the 35–350 m/z range. The oven temperature ramp was programmed to start at 40°C (held for 2 minutes), then increase at 3°C/min to 240°C, and hold at that temperature for 5 minutes. The injection volume was 1 µL of a 1% solution in dichloromethane.

Compound identification was performed by comparing fragmentation patterns with mass spectra from the NIST 14 database (Mass Spectral Library, 2014) available on the CG-EM system. Additionally, the compounds were identified by comparison with literature data (Adams, 2007) and by linear retention indices (LRI), calculated from the retention times of a homologous series of hydrocarbons (C8 to C26) injected under the same chromatographic conditions as the sample. The linear retention index was calculated using the methods described by Van den Dool and Kratz (1963) and Adams (2007). For identification by comparison with database spectra, only matches exceeding 90% were considered significant.

2.4. Encapsulation of oregano and thyme essential oils in alginate

2.4.1. Formulations and concentrations: The encapsulation of the essential oils of *Origanum vulgare* L. (oregano) and *Thymus vulgaris* L. (thyme) was performed according to the methodology described by Pagani et al. (2014), with modifications. Initially, 2% (w/v) sodium alginate and 2% (w/v) calcium chloride solutions were prepared using distilled water that had been previously heat-treated to remove potential contaminants.

Sodium alginate-based capsules were prepared by individually incorporating essential oils into the polymer solution at a concentration of 20% (v/v). After complete homogenization, the mixture was dispensed into the calcium chloride solution using an automatic pipette, resulting in the formation of capsules through ionic gelation.

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2.4.2. Gelation and Drying Process: The capsules were immersed in the calcium chloride solution for 60 s, after which they were collected and washed with distilled water to remove excess calcium ions. The capsules were then dried in a forced-air oven at 50 ± 2°C for 32 h. After drying, the capsules were packaged in sterile containers and stored under appropriate conditions until use (Ferreira et al., 2021).

2.5. Physicochemical and Morphological Characterization of Capsules Containing Oregano and Thyme Essential Oils

2.5.1. Measurement of Capsule Diameter and Sphericity Factor (SF): The diameter of the capsules was determined by measuring a sample of 30 capsules from each formulation using a digital caliper with an accuracy of 0.01 mm (Mitutoyo®). For each capsule, the major diameter (dM) and minor diameter (dm) were measured, as described by Asha et al. (2011).

The sphericity factor of the capsules was calculated from the values of the major and minor diameters using the following equation:

$$SF = (dM - dm) / (dM + dm)$$

2.5.2. Density, Compressibility Index, and Hausner Factor: The compressibility index (CI) was determined from the values of bulk density (BD) and compacted density (CD), according to the equation proposed by Carr (1965) and adopted by official compendia such as the United States Pharmacopeia (USP): $CI = (CD - BD) / CD \times 100$.

The Hausner factor (HF) was determined as the ratio of compacted density (CD) to bulk density (BD), as described by Hausner (1967) and in accordance with the United States Pharmacopeia (USP, 2022) recommendations. HF was used as an indicator of the flow and compressibility properties of the materials.

2.5.3. Water activity (Aw) and moisture content of the capsules: The water activity (Aw) of the capsules was determined using an AquaLab® (Dew Point Water Activity Meter 4TEV) instrument, in accordance with the official methodology (NOM, 2008). Aw represents the ratio of the vapor pressure of the water present in the sample to that of pure water at the same temperature (Badui, 2006). Moisture content was determined according to the AOAC (1997) method 925.01 by drying the samples in an oven at 105°C until a constant weight was reached. The moisture percentage was calculated using the equation:

$$\%U = (m_{(a,i)} - m_{(a,f)}) / m_{(a,i)} \times 100$$

where: %U = moisture percentage; $m_{(a,i)}$ = initial mass of the sample; $m_{(a,f)}$ = final mass of the sample.

2.5.4. Scanning electron microscopy (SEM): Morphological analysis of the samples was performed using scanning electron microscopy (SEM) according to Rosenberg & Young (1993). Samples were dehydrated in 70, 90, and 100% ethanol at 1 mL per sample for 10 minutes, then dried in a critical point dryer (Quorum/K 850) using anhydrous CO_2 (1100 psi/31°C). The samples were mounted on aluminum stubs with colloidal silver and coated with a gold–palladium alloy using a metallizer (Quorum/Q 150R ES). After metallization, samples were examined using a scanning electron microscope (Philips XL30 ESEM) operating at 10 kV.

2.5.5. Infrared Spectroscopy Analysis (FTIR/IR): The spectra of the samples were analyzed using a mid-infrared FTIR-ATR spectrometer (Cary 630 FTIR, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an attenuated total reflectance (ATR) accessory, according to the manufacturer's specifications (Agilent Technologies Inc., 2015). Analyses were performed in the spectral range of 4000–600 cm^{-1}, and spectra were obtained in absorption mode, a method widely used for material characterization by infrared spectroscopy (Smith, 2018).

During analysis, the ambient temperature was maintained at 25°C, and a background spectrum was recorded prior to each measurement to correct instrumental interferences. Subsequently, 0.5 g of the sample was placed onto the diamond crystal surface for attenuated total reflectance spectral measurement (Smith, 2018). Spectral data were acquired using Microlab PC software provided by the manufacturer (Agilent Technologies Inc., 2015).

2.6. Antibacterial Activity of Oregano and Thyme Essential Oil Capsules

The assay was performed according to Bona et al. (2014), with modifications. The bacterial strains were inoculated onto Petri dishes containing Mueller–Hinton agar, and 6-mm-diameter wells were made in the center of the agar using a sterile template. Capsules containing the essential oils of *Origanum vulgare* L. and *Thymus vulgaris* L. were aseptically placed into the wells, and the plates were incubated at 35 ± 2°C for 24 h. After incubation, the plates were examined for uniform bacterial growth, and inhibition zones were measured using a millimeter ruler. All tests were performed in duplicate, and results are expressed as mean values.

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3. RESULTS

The analysis of the total ion chromatograms obtained by GC-MS, as shown in Table 1, allowed the identification of the main chemical constituents of the evaluated essential oils. A predominance of phenolic monoterpenes was observed, notably thymol in thyme essential oil (67.5%) and carvacrol in oregano essential oil (83.8%).

Table 1: Chemical constituents of oregano and thyme essential oils obtained by GC-MS

Essential oil	Compound	TR (min)	Area (%)
Oregano	p-cymene	12.2	7.1
Oregano	γ-terpinene	13.8	3.0
Oregano	Carvacrol	25.8	83.8
Oregano	Caryophyllene	30.5	6.0
Thyme	p-Cymene	12.4	24.0
Thyme	Linalool	16.0	3.9
Thyme	Thymol	25.4	67.5
Thyme	2-Ethyl-4,5-dimethylphenol	25.6	4.5

The physicochemical characterization of the capsules containing Origanum vulgare and Thymus vulgaris essential oils, as presented in Table 2, revealed parameters consistent with stable encapsulated systems suitable for biological applications.

Scanning electron microscopy analysis shown in Fig. 1 revealed that the alginate spheres containing oregano and thyme essential oils exhibited a rough, irregular, and heterogeneous surface.

The FTIR-ATR spectra of pure sodium alginate and alginate capsules containing oregano and thyme essential oils, prepared from the stock solution, showed similar profiles, with no significant changes in the characteristic bands of the polymeric matrix, including those attributed to the $–COO^-$, –C–O–C, and –OH groups. Regarding antibacterial activity, the combination of Origanum vulgare and Thymus vulgaris essential oils at 20% inhibited the growth of the evaluated bacterial strains, as evidenced by inhibition zones ranging from 12 to 16 mm, as shown in Table 3.

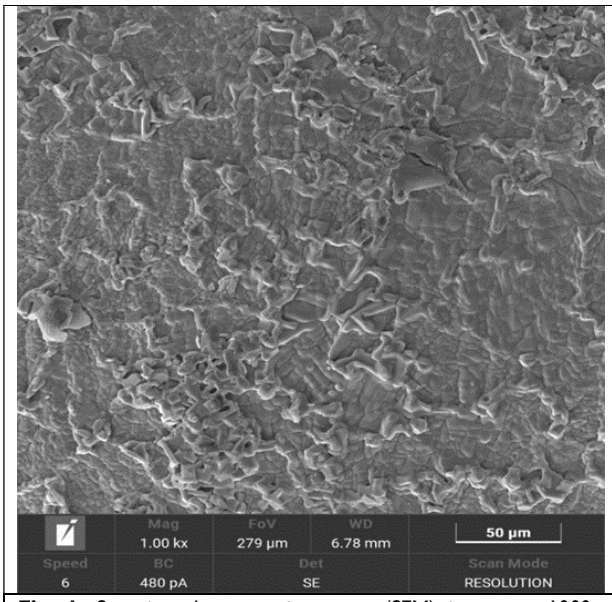

Fig. 1: Scanning electron microscope (SEM) images at 1000× magnification of the alginate sphere containing oregano and thyme.

Table 2: Physicochemical properties of capsules containing a mixture of essential oils

Parameter	Measured Value
Bulk density (BD)	0.80 g/mL
Tapped density (TD)	0.83 g/mL
Average diameter	1.27 mm
Sphericity factor (SF)	0.24
Compressibility index (Carr)	3.61 %
Hausner factor	1.04
Moisture content	17.58 %
Water activity (aw)	0.66

Table 3: Inhibition zones (mm) obtained by the combination of oregano and thyme essential oils encapsulated at a concentration of 20% against the tested bacterial strains

Bacterial strain	Animal species	Identification	Inhibition zone (mm)
Escherichia coli	Cat	ECG 13	14
Escherichia coli	Dog	ECC 49	16
Klebsiella pneumoniae	Dog	KP 04	12

4. DISCUSSION

Compounds such as thymol and carvacrol are widely recognized for their antimicrobial properties, reinforcing the biological potential of the essential oils studied (Cui et al., 2024). Furthermore, identifying the major constituents is essential for establishing reference parameters and evaluating the efficiency of the encapsulation process in subsequent stages.

Regarding the physicochemical characterization of the capsules, the bulk and tapped densities showed little variation, directly reflecting a low compressibility index and a Hausner factor close to unity. These results indicate good flowability and low cohesiveness of the material, which are fundamental characteristics for the processing, storage, and standardization of encapsulated systems (Carr, 1965; Hausner, 1967; Brubaker & Moghtadernejad, 2024).

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The average capsule diameter, combined with the sphericity factor, suggests the formation of relatively homogeneous particles, although they are not perfectly spherical. This morphology is observed in encapsulated systems obtained via droplet formation and ionic gelation, such as alginate capsules, and is related to the characteristics of the particle-formation process (Martins et al., 2017). Uniform dimensions and a shape close to a sphere favor a more controlled release of bioactive compounds and contribute to the physical stability of the encapsulated system (Karim et al., 2022).

The observed moisture and water activity values indicate conditions favorable for the physicochemical and microbiological stability of the capsules. A water activity below 0.7 indicates low availability of free water in the system, a parameter directly related to reduced microbial growth risk and slowed chemical degradation reactions (Labuza, 1980; Guadarrama et al., 2014). This aspect is particularly relevant in encapsulated systems containing essential oils, as low water activity helps protect volatile and phenolic compounds, reducing oxidation and volatilization losses during storage (Guadarrama et al., 2014). Furthermore, recent studies have shown that alginate-based polymer systems exhibit greater structural stability and better preservation of the biological activity of the encapsulated compounds when maintained within narrow ranges of water activity, reinforcing the validity of the values obtained in this study (Dodero et al., 2021).

The rough, irregular, and heterogeneous surface of the analyzed capsule indicates that this morphology is primarily associated with the differential contraction of the polymer matrix during the gelation and drying processes, which promotes intense water removal and consequent structural shrinkage, resulting in the formation of surface roughness and microcracks (Vargas et al., 2018).

The results, showing no significant changes in the characteristic bands of the polymer matrix, indicate that the incorporation of essential oils did not promote structural modifications detectable by this technique, suggesting that the interaction between the bioactive compounds and sodium alginate occurred predominantly in a physical manner without the formation of new chemical bonds.

The absence of bands corresponding to the major constituents of essential oils, such as carvacrol and thymol, may be attributed to their incorporation into the sodium alginate polymer matrix and to their low concentration in the system. Owing to their hydrophobic nature, essential oil compounds tend to disperse as droplets within the matrix, potentially interacting with the polymer and becoming difficult to identify by FTIR. Similar results have been reported in the literature for active polymeric films and materials containing extracts or essential oils, in which the incorporation of bioactive compounds did not significantly alter the FTIR spectra, especially at low concentrations (Atarés & Chiralt, 2016; Johnson et al., 2023).

Encapsulated essential oils may retain their ability to inhibit microbial growth, as evidenced by inhibition halos, compared with free-form oils (Bona et al., 2014; Mostaghimi et al., 2023). Furthermore, the reduction in alginate and $CaCl_2$ concentrations led to greater essential oil release due to the formation of a less compact polymeric network with a lower degree of ionic crosslinking. This more porous structure facilitates diffusion of encapsulated compounds, thereby promoting faster, more intense release of essential oils into the culture medium (Mostaghimi et al., 2023; Yammine et al., 2023).

The variation in halo diameters observed among strains may be related to intrinsic differences in bacterial susceptibility, as well as to the composition and concentration of phenolic compounds in the essential oils, particularly thymol and carvacrol (Cui et al., 2024). These compounds are described in the literature as capable of acting in a complementary manner, promoting cell membrane disruption, increasing permeability, and causing leakage of intracellular constituents, which may result in an additive antimicrobial effect when used in combination (Hamza et al., 2023; Mráz et al., 2025).

The correlation between the physicochemical parameters of the capsules and the observed antibacterial activity against uropathogenic strains indicates that the encapsulation process primarily protects essential oils and modulates their release, rather than directly enhancing antibacterial potency. Encapsulation in an alginate matrix may help preserve bioactive compounds, thereby reducing losses due to volatilization, oxidation, and degradation during storage and application (Bakry et al., 2016; Dodero et al., 2021).

In addition, encapsulated systems have the potential to promote the gradual release of active ingredients, helping to maintain effective concentrations over time and increasing the viability of these compounds as natural alternatives for controlling uropathogenic pathogens isolated from dogs and cats, especially in light of the growing problem of antibiotic resistance (Štrbac et al., 2021).

5. CONCLUSION

The physicochemical characterization of the capsules revealed properties consistent with stable encapsulated systems, including good flowability, homogeneous morphology, and low water activity values, indicating

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conditions favorable to physicochemical and microbiological stability and reinforcing the role of encapsulation in protecting and maintaining the biological activity of essential oils. The combination of essential oils from *Origanum vulgare* L. and *Thymus vulgaris* L., encapsulated in an alginate matrix, exhibited antibacterial activity against uropathogenic strains of *Escherichia coli* and *Klebsiella pneumoniae* isolated from dogs and cats, as evidenced by in vitro inhibition zones. Thus, the encapsulated systems show potential as a natural alternative for controlling uropathogenic pathogens of veterinary importance, given the growing scenario of antimicrobial resistance in small animals, highlighting the need for future studies to optimize formulations and evaluate them in more complex biological models.

Declarations

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Data Availability: All data are available in the article.

Ethics Statement: This research was reviewed and approved by the Animal Ethics Committee of the Federal University of Campina Grande (CEUA/CSTR/UFCG), under protocol number 022026.

Author's Contributions: CTP conducted the essential oil encapsulation experiments, performed the physicochemical and morphological characterization of the capsules, conducted the antibacterial activity assays, analyzed and interpreted the data, and drafted the manuscript. BS and MMS assisted in sample preparation, capsule processing, and laboratory analyses. JHAF contributed to the microbiological procedures and the collection of experimental data. MSA and FMCM contributed to the interpretation of the microbiological results and reviewed the methodology applied. WFBS and AAT performed sample preparation, image acquisition, and morphological analysis by scanning electron microscopy (SEM). MAAM participated in the discussion of the data and the critical review of the manuscript. AAOF supervised the study, contributed to the experimental design, and critically reviewed the final version of the manuscript. All authors read and approved the final version of the manuscript.

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