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Research article

Study of the extraction and encapsulation of *Withania frutescens* (L.) mucilage: towards an innovative formulation

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Abstract

Withania frutescens (L.) is a medicinal plant widely used in phytotherapy. The aim of this study is to extract mucilage from this plant and utilize it for nanoparticle encapsulation. The study begins with the determination of mineral composition using ICP-AES, followed by the analysis of amino acids and vitamins using HPLC-MS. Additionally, mucilage extraction was optimized by defining stabilization parameters. This mucilage was then used as a matrix for the encapsulation of nanoparticles, particularly oils and antibiotics. ICP-AES analysis revealed high levels of iron (58.469 mg/kg), calcium (17.767 mg/kg), and magnesium (43.238 mg/kg). The assessment of nutritional value identified 17 amino acids and 11 vitamins, with maximum concentrations of 14.90 mg/g and 23.69 mg/g, respectively. Mucilage extraction achieved an optimal yield of 65.21% at a liquid-solid ratio of 100 g/L and a shear rate of 3000 rpm. Encapsulation demonstrated a mucilage efficiency and stability of up to 86.35%. The evaluation of the antibacterial activity of encapsulated antibiotics revealed more pronounced inhibition zones with purified mucilage and encapsulated antibiotics. The observed diameters were 12.3 mm for mucilage alone (Muc), 13.8 mm for encapsulated ampicillin (Muc-Amp), 15.6 mm for encapsulated streptomycin (Muc-Str), and 14 mm for encapsulated kanamycin (Muc-Kan). The mucilage extracted from *W. frutescens* exhibits significant potential as an encapsulation material for therapeutic applications and the protection of bioactive compounds.

Keywords: Extraction; Formulation; Mucilage; Nanoparticles

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1. Introduction

Natural mucilage, abundant in various nutrients, has garnered significant attention in recent years for its diverse biological properties, including antimicrobial, anti-algal, and soluble fiber benefits, as well as its excellent lubricating capabilities (Nie et al., 2017; Liu et al., 2014; Hefny et al., 2018). Capsules, which are tasteless and odorless delivery systems designed for powdered medicines, have been used since the late 19th century (Zhang et al., 2017; Nagai et al., 2006). These solid preparations involve filling active drugs or supplemental materials into hard or flexible shells, allowing for controlled content release under specific conditions.

The selection of materials and encapsulation techniques depends on the properties of the active ingredient and the intended application (Shen et al., 2018). Many bioactive substances, such as polysaccharides, polyphenols, and aromatic compounds, are frequently incorporated into capsules, driven by advancements in encapsulation technology and increased production capabilities (Porter et al., 2017; Paini et al., 2015; Lei et al., 2018). Natural polysaccharides, in particular, are valued in drug delivery systems for their biocompatibility, improved bioavailability, and better patient acceptability (Sujitha et al., 2012; Zhang et al., 2013).

The aim of this study was to evaluate the potential of *Withania frutescens* mucilage, combined with alginate, as an alternative encapsulation material for oil extracted from the same plant using external ionic gelation. The resulting capsules were characterized for encapsulation efficiency, moisture content, micrometric and thermal properties, morphology, and oxidative stability. Additionally, the study

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compared the stability of antibiotics encapsulated in this mucilage with their respective crude forms, focusing on commonly used antibiotics such as streptomycin, ampicillin, and kanamycin.

2. Materials and methods

2.1. Plant material

W. frutescens was collected in March 2021 in the city of Sefrou, Morocco, during a period when the plant reaches its maximum development and full flowering. The authentication of the plant material was carried out by a botanist (Prof. Bari Amina), and the sample was registered under the number LBES-069/21 before being deposited in the faculty herbarium. A member of the Solanaceae family, *W. frutescens* is a dicotyledonous plant classified in the order Solanales and included in the *Withania* genus. It is also known by various vernacular names, including Bounouar, Tizhart, and Avadaiz, depending on the region where it is found.

2.2. Nutritional value of W. frutescens

Two grams of *W. frutescens* powder were weighed and placed in porcelain capsules, then heated in a muffle furnace at 550 ± 15°C for 5 hours until a gray ash was obtained. To the ash resulting from incineration of one gram of the powder, 1 mL of hydrochloric acid and 10 mL of distilled water were added. The mixture was heated in a boiling water bath until the ash dissolved, and the solution was then diluted to a final volume of 100 mL. From this solution, the following mineral elements were quantified: Ca, K, Mg, Na, P, Mn, Cu, Fe, and Zn. Mineral analysis was performed using inductively coupled plasma atomic emission spectrometry (ICP-AES) (EL Moussaoui et al., 2019).

Amino acids from leaf and root powder were digested with 6 M HCl for 24 hours at 110°C under a nitrogen atmosphere. The amino acids were then separated by reverse-phase HPLC (Agilent1100, Agilent Technologies, USA) on a Zorbax 80A C18 column (4.6 mm × 180 mm, Agilent) at 40°C, with detection at 338 nm (Jarrett et al., 1986).

Vitamins were analyzed by reverse-phase HPLC. A 15 µL sample was injected, with a mobile phase consisting of acetonitrile and water (90:10), at a flow rate of 2.0 mL/min and a column temperature of 40°C. Detection was carried out at 265-280 nm. For sample preparation, the *W. frutescens* plant extract was accurately weighed and transferred to a 100 mL glass vial, dissolved in 10 mL of purified water with stirring for 30 minutes, followed by the addition of 100 mL of methanol using a calibrated pipette. The solution was stirred for 60 minutes, filtered through a 0.22 µm PTFE syringe filter, and then injected into the HPLC system.

2.3. Optimizing mucilage extraction

2.3.1. Extraction of W. frutescens mucilage

The leaves of the plant studied were dried for 3 to 5 days in the dark, at a temperature of around 40°C. Once dry, they were ground to a powder using an electric blender. The mucilage from *W. frutescens* was extracted using high-speed shearing with a 0.3 mol/L sodium hydroxide solution, adjusting the liquid-solid ratio, extraction temperature, extraction time, and centrifugation speed as needed. The supernatant was filtered and then centrifuged at 8000 rpm for 30 minutes. The filtrate was concentrated to one-third of its original volume using a rotary evaporator under reduced pressure, after which the mucilage was obtained by drying in an oven at 60°C. The effects of the independent processing parameters—extraction temperature (°C), liquid-solid ratio (g/L), extraction time (min), and centrifugation speed (rpm)—on mucilage extraction yield (the dependent variable) were systematically investigated.

2.3.2 Physico-chemical characterization of mucilage

The physicochemical characteristics of the extracted mucilage were assessed, and the pH of the mucilage was measured in a 1% w/v aqueous solution using a pH meter (Thanatcha et al., 2011).

2.3.3. Swelling index measurement

The swelling characteristics of the mucilage were evaluated in distilled water. The swelling index was defined as the volume of distilled water (in mL) occupied by 1 g of the substance. Specifically, 1 g of mucilage was added to 25 mL of distilled water, and the mixture was stirred for 10 minutes every hour over a period of 10 hours. After stirring, the solution was allowed to stand for 24 hours at room temperature. Following this resting period, the volume occupied by the mucilage was measured. This procedure was repeated three times, and the average value was calculated (Shubham et al., 2010).

2.3.4. Water holding capacity

Water holding capacity was measured using the modified method of Thanatcha et al., (2011). 0.25 g of the sample was added to 25 mL of distilled water, stirred with the magnetic stirrer for 15 min and centrifuged at 10 000 rpm for 30 min. The supernatant obtained was removed and then the wet sample was weighed to determine the water holding capacity according to the following equation:

$$\mathbf{WRC} = \frac{\mathbf{WMW} - \mathbf{DMW}}{\mathbf{WMW}} \quad (\mathbf{1})$$

Where **WRC**: Water retention capacity; **WMW**: wet mucilage weight; **DMW**: dry mucilage weight.

2.3.5. Oil absorption

The mucilage powder was used to evaluate the absorption of the oil extracted by the Clevenger method from the leaves of this plant (EL Moussaoui et al., 2021). A 0.5 g sample was added to 10 mL of refined oil and mixed using a vortex mixer for 1 minute. The mixture was left at room temperature for 30 minutes, after which it was centrifuged at 10.000 rpm for 30 minutes. The tube was then inverted for 1 minute to allow the remaining oil to drain away. The oil absorbed by the supernatant was removed and the weight of absorbed oil was measured to calculate the oil's absorption capacity.

2.3.6. Emulsifying capacity

Powdered mucilage was used to evaluate emulsification capacity. Samples of 1 g were accurately weighed and dissolved in 50 mL of distilled water before being added to

50 mL of almond oil. The emulsion was prepared by homogenizing the mixture for 1 minute and then centrifuging it at 4100 rpm for 5 minutes. Finally, the height of the emulsified layer was measured and compared with the total height to calculate the emulsification capacity (Thanatcha et al., 2011).

2.3.7. Conductivity measurement

Conductivity measurements were performed to determine the class of surfactants (anionic, cationic, or non-ionic). Surfactant solutions of varying concentrations (0.05, 0.3, 0.5, 1, 1.5, and 2 g/L) were prepared, and their conductivities were measured using an EC214 conductivity meter (Shubham et al., 2010).

2.3.8. Accelerated ageing test by centrifugation

The emulsion stability can be checked with the creaming index. It is measured using a centrifuge set to 3000-4000 rpm at different times, whereby the phase separation and sedimentation of the mucus are checked (Thanatcha et al., 2011; Shubham et al., 2010).

2.4. Encapsulation of the essential oils of W. frutescens in mucilage

2.4.1. Properties and performance of W. frutescens mucilage alginate as an encapsulation matrix

2.4.1.1. Preparation for encapsulation of W. frutescens oil in mucilage

The total mass of *W. frutescens* oil, mucilage-alginate, and Tween 80 (emulsifier) was kept constant at 40 g for all design trials, irrespective of the amount of mucilage-alginate used. Dispersions of sodium alginate and mucilage in a 50:50 weight/weight ratio were prepared in distilled water at concentrations of 1%, 2%, and 3%.

The alginate-mucilage dispersion was degassed for 12 hours prior to use. For the emulsions, 10 g of the alginate/mucilage mixture, along with 1 mL of *W. frutescens* oil and Tween 80, were mixed for several minutes. The emulsions were then pumped at a flow rate of 10 mL/min to a distribution disk, where they were deposited into 400 mL of calcium chloride solution (1%, 2%, and 3%).

The disk was equipped with eight polyethylene spiral-threaded conical nozzles (internal diameter 1.4 mm). The calcium chloride solution was stirred at 350-400 rpm using a wedge-shaped magnetic bar measuring 80 mm in length. After the desired curing time (10, 20, or 30 minutes) at room temperature, the wet capsules were washed with demineralized water to remove any excess calcium chloride. The capsules were then stored at 4°C until further use.

2.4.1.2. Encapsulation efficiency before drying

Unencapsulated oil prior to drying was determined by measuring the weight of free oil remaining on the surface of the gelling solution as well as on the surface of the wet beads. Filter paper was used to absorb surface oil from the wet beads, and these were dried in an oven until a constant weight was reached.

The difference between the initial amount of oil used (W1) and the unencapsulated oil before drying (W2) gives the amount of encapsulated oil (W3):

$$W3=W1-W2 \quad (2)$$

The encapsulation efficiency before drying (EEBD) was expressed as the percentage of encapsulated oil in relation to the initial quantity of oil used:

$$EEBD\ (\%) = W3/W1 * 100 \quad (3)$$

Where EEBD: encapsulation efficiency before drying; W3:

2.4.3. Encapsulation efficiency after drying

The capsules were placed on drying trays and dried in an oven at 60°C until a constant weight was achieved. The amount of unencapsulated oil, which had escaped from the beads during the drying process, was determined by weighing the free oil remaining on the drying tray and on the surface of the dried beads. The difference between the amount of encapsulated oil before drying (W3) and the amount of unencapsulated oil after drying (W4) gives the amount of encapsulated oil after drying (W5):

$$W5=W3-W4 \quad (4)$$

Encapsulation efficiency after drying (EEAD) was expressed as the percentage of encapsulated oil (W5) relative to the amount of encapsulated oil (W3):

$$EEAD\ (\%) = W5/ W3*100 \quad (5)$$

2.5. Encapsulation of W. frutescens mucilage nanoparticles with antibiotics: in vitro stability and functional evaluation

2.5.1. Encapsulation of antibiotics in a mucilage matrix

80 mg mucilage was dissolved in 4 mL distilled water using a magnetic stirrer for complete dissolution for up to 6 hours in a small beaker and separately 20 mg antibiotics (ampicillin, kanamycin and streptomycin) were dissolved in 2 mL distilled water. The antibiotic solution was then added drop by drop using a propette under continuous agitation for 16 hours to completely encapsulate the respective antibiotics in the mucilage. Next, the antibiotic-laden mucilage was freeze-dried to completely remove the water. Experiments were then carried out to confirm antibiotic loading.

2.5.2. Physical characterization

The swelling properties of the mucilage-antibiotic hydrogel were determined in a pH 7.4 buffer solution at 37°C. Dry mucilage (St) was accurately weighed and immersed in the buffer solution. A few hours later, swollen mucilage (Se) was weighed and excess water removed with absorbent cotton. The degree of swelling (%) of the mucilage at time (t) was determined using the following formula:

$$\text{Degree of swelling } (\%) = [(St-Se)/St] \times 100 \quad (6)$$

Where (St) and (Se) are the weights of mucilage at time (t) and in the dry state (t=0), respectively.

2.5.3. Evaluation of McAb's antimicrobial properties

The antibacterial properties of the mucilage-antibiotic complex (McAb) were tested against pathogenic strains known to pose a threat to human health due to their resistance to antibiotics, specifically *Escherichia coli* and *Staphylococcus aureus* (El Atki et al., 2019).

2.6. Statistical analysis

Data were expressed as Mean±SD and subjected to analysis of variance (ANOVA) and Tukey's test ($p < 0.05$). Statistical analyses were performed using GraphPad-prism version 7.0 software.

3. Results and discussion

3.1. Nutritional value of W. frutescens

The results of ICP-AES analyses are presented in Table 1. These results show elevated levels of minerals, in particular Fe, P, Ca and Mg, in the leaf powder (EFWF). However, the root powder was rich in P, followed by Mg, K and Fe. We also observed a difference in mineralogical composition between the two powders. Trace elements or mineralogical elements are essential for life and the human body cannot produce them. And in the human body, they present less than 1 mg/kg body weight. Their absence or insufficient concentration in the human body can cause functional or structural abnormalities, as they play essential roles in catalytic function (enzyme cofactors), vitamin structure, expression of hormonal signals and defence of the organism. The consumption of plant parts (leaves, roots, fruits, etc.) can prevent a deficiency, either to compensate for a deficit or to obtain a therapeutic effect.

Table 1. Mineral composition of the two extracts in mg/kg.

	Ca	P	K	Cu	Mg	Mn	Fe	Na	Zn	Si
EFWF	17767	20489	13473	41.27	43238	652.81	58469	7886	148.41	2458
ERWF	105.21	24792	13894	50.91	14177	456.05	6947	2713	323.73	1584

EFWF: leaf powder of *W. frutescens;* **ERWF:** the root powder *W. frutescens*

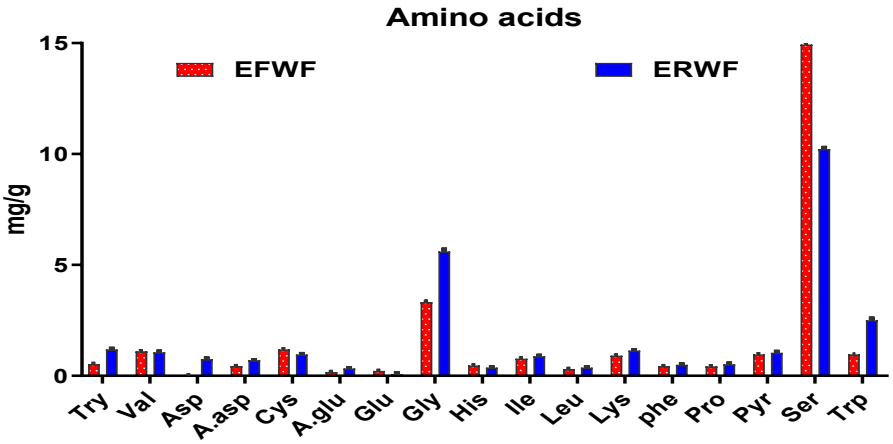

Figure 1. Concentration of amino acids in root and leaf powder

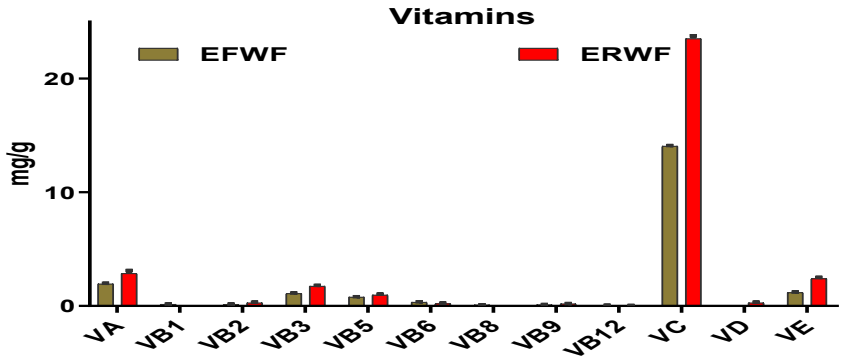

Figure 2. Concentration of vitamins in root and leaf powder

Amino acids were identified by liquid chromatography (HPLC), and the results show that leaf and root powder are rich in amino acids. 17 amino acids were identified in roots and leaves, with Ser and Gly dominating in both parts at concentrations of 10.28 mg/g and 14.90 mg/g, and 5.69 mg/g and 3.35 mg/g respectively (Figure 1).

The amino acid Serine is involved in the biosynthesis of several biomolecules required for cell proliferation, including amino acids, nitrogenous bases, phospholipids and sphingolipids. Serine also plays an essential role in several cellular processes, such as the metabolism of monocarbon (C1) units (Kalhan et al., 2012), or in signalling mechanisms, where it is one of the three amino acids phosphorylated by

kinases. This explains the dominance of this amino acid in the leaves and roots of *W. frutescens*.

In human health, serine is essential for normal embryonic development, in particular for brain morphogenesis (Tabatabaie et al., 2010; Yoshida et al., 2004). This amino acid also has a central role in the control of cell proliferation, and has been implicated in cancer progression (Locasale et al., 2011; Pollari et al., 2011; Bachelor et al., 2011). Serine is an allosteric activator of enzymes such as pyruvate kinase M2, which is overexpressed in cancer cells (Chaneton et al., 2013). It also induces metabolic remodelling in cancer cells in relation to the tumour suppressor protein p53, leading researchers to suggest a potential role for Ser depletion in the treatment of p53-deficient tumours (Maddocks et al., 2013).

Since amino acids are nutritionally important, vitamins also play a role in plant growth and development (Figure 2). Phytochemical analysis revealed that the leaves and roots of *W. frutescens* are rich in vitamins. 11 vitamins were found in the leaves and 10 vitamins in the roots, with vitamin C (VC) dominating at concentrations of 14.09 mg/g and 23.69 mg/g, respectively. On the other hand, the concentration of vitamins A and E was found to be 1.90 mg/g and 1.20 mg/g (leaves), and 3.05 mg/g and 2.48 mg/g (roots), respectively. Numerous studies have revealed that vitamins play a role in the synthesis and use of hormones (Blatt et al., 2001; Mustacich et al., 2007; Manor and Morlet, 2007).

Vitamin A is a biological antioxidant that acts as a detoxifier, immunopotentiator and immune activator (Ames et al., 1993). Similarly, vitamin E (tocopherol) contributes to the body's defense system against lipoprotein oxidation and may help improve sperm motility. Healthy fertile men with normal sperm parameters contain adequate amounts of vitamins A and E (Aitken et al., 1987). Impaired antioxidant activity in men with sperm dysfunction may reflect low seminal levels of vitamins A and E (Rajasekharan et al., 1995).

Ascorbic acid (vitamin C) is a water-soluble vitamin with potent reactive oxygen species (ROS) scavenging activity. Seminal plasma is very rich in ascorbic acid, with a concentration reported to be ten times higher than in serum (Dawson et al., 1987), underlining its importance for fertility. Previous studies have also reported reduced levels of vitamin C in infertile men (Lewis et al., 1997). Treatment with *W. somnifera* and *W. coagulans* improved levels of vitamins A, C and E, indicating the strength of these medicinal plants in protecting against infertility due to vitamin loss (Ahmed et al., 2010).

3.2. Extraction, purification and physico-chemical characterization of mucilage

Mucilage yield increased as the liquid-solid ratio increased in the range of 60 to 100 g/L and the extraction temperature increased from 80 to 100 °C. Mucilage yield increased with increasing extraction time and liquid-solid ratio, but then decreased as these two variables increased further. Evaluation of the effect of shear rate and liquid-solid ratio on mucilage yield. Maximum yield (around 65.21 %) of mucilage was obtained when the liquid-solid ratio was set at around 100 g/L and the shear rate at around 3000 rpm (Table 2 and Figure 3).

Table 2. Results of the optimization of mucilage extraction from *W. frutescens*

	Step 1			Step 2			Step 3	
	Aqueous extraction			Ethanol extraction			Purification	
Exp	T (°C)	Ratio (g/L)	Extraction (min)	Ratio in % (V:V)	C (tr/min)	R (%)	Mucilage (10%) and TCA: 5% (V:V)	Rf (%)
1	70	40	15	60	2000	32.127	100	84.251
2	80	30	20	70	3000	25.14	100	83.441
3	80	50	20	50	2000	16.458	100	80.220
4	90	40	20	60	4000	42.814	100	84.109
5	100	40	30	60	8000	43.62	100	87.942
6	100	100	30	80	3000	65.219	100	92.147
7	80	40	20	60	8000	27.141	100	88.527
8	60	30	10	70	6000	22.014	100	89.528
9	60	40	15	60	8000	15.811	100	90.140
10	90	50	15	50	6000	37.846	100	90.201
11	100	60	20	80	4000	62.358	100	91.204
12	70	30	30	70	2000	36.518	100	90.428
13	100	40	60	90	3000	61.024	100	89.473
14	100	50	30	80	2000	64.047	100	91.386
15	70	50	30	50	6000	19.804	100	90.375

Exp: experiments; **T**: temperature (°C); **R**: yield (%); **C**: centrifugation (rpm)

Among the variables, liquid-solid ratio was the most important factor affecting mucilage extraction yield, the next most important factor was shear rate, followed by extraction time, and finally extraction temperature. The optimum extraction conditions to obtain the maximum mucilage extraction yield were as follows: An extraction temperature of 92 °C, a liquid-to-solid ratio of 71 g/L, an extraction time of 34 min and a shear rate of 2500 rpm. Further experiments were carried out under these conditions, with slight modifications: an extraction temperature of 90°C, a liquid/solid ratio of 70 g/L, an extraction time of 30 minutes and a shear rate of 2500 rpm. Under these conditions, the actual mucilage yield obtained was 71.48%, close to the theoretical yield estimated at 73.44%. The purified mucilage was yellow in color, had an irregular rough texture, and was odorless. Its pH was approximately 6.81 ± 0.06, and it showed a swelling index of 72.24 ± 2.14%, which is considered favorable for this mucilage as it exceeds 60% (Deve et al., 2014). The water retention capacity was around 9.64 ± 1.02 g/g, while the oil retention capacity was 1.26 ± 0.51 g/g. The emulsification capacity was 81.41 ± 5.73%, indicating a strong ability to emulsify. This stability was confirmed through centrifugation tests, where the emulsion was subjected to speeds of 3000 rpm and 4000 rpm for 10, 15, and 30 minutes. No sedimentation or creaming was observed after these tests. To assess the stability of the mucilage powder, exposure tests at high temperature, high humidity, and intense light were conducted, providing baseline data for capsule packaging and storage using mucilage (Figure 4). The results revealed visible changes in mucilage appearance under high temperature (60°C), high humidity (95% ± 5%), and intense light exposure, while its weight and content remained unchanged. For instance, the mucilage melted and coagulated at high temperatures and gradually darkened with high humidity and light exposure. These results suggest that mucilage should be stored at low temperatures, in low humidity, and protected from light.

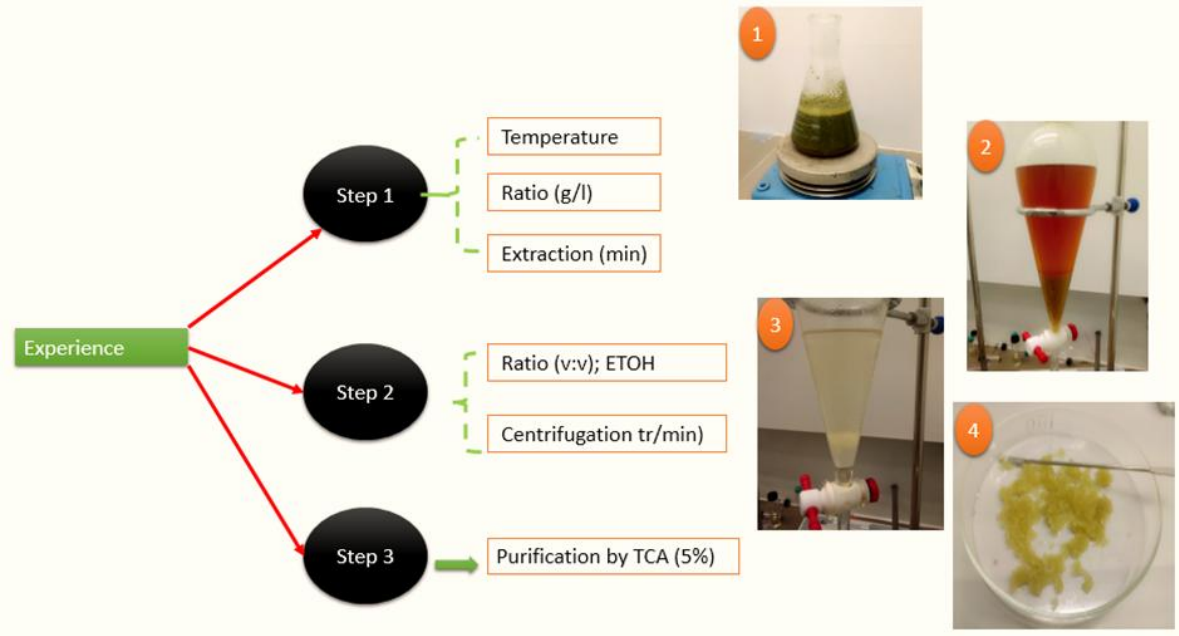

Figure 3. Steps followed for the extraction and purification of *W. frutescens* mucilage

Mucilage before drying Mucilage after drying Exposure test

Figure 4. Exposure test for *W. frutescens* mucilage at high temperature

3.3. Preparation of capsules by mucilage

The effects of alginate and mucilage concentrations, calcium chloride concentration, and cross-linking time on oil encapsulation efficiency are presented in Table 3. Encapsulation efficiency was assessed immediately after encapsulation (EEBD) and following the drying process (EEAD). In this study, the oil loading represented 34.72% of the total mucilage weight. Encapsulation efficiency, expressed as the percentage of encapsulated oil relative to the total oil used, ranged from 69% to 86% (Table 3 and Figure 5). The results showed that encapsulation efficiency was influenced by the presence of calcium ions in the cross-linking solution, indicating that gel film formation occurs from the outer layer of the capsule core. This behavior can be explained by gel formation controlled by the diffusion of the components involved. Given that the metal cation is

smaller than the polymer molecules, it is primarily the cation that diffuses between the polymer chains, binding to unoccupied sites within the polymers. Cross-linking with calcium resulted in a robust gel network with a mechanical profile indicative of a stable, permanent gel structure.

In this study, a higher concentration of calcium chloride reduced the initial encapsulation efficiency (EEBD). According to Kamaruddin et al., (2014), during capsule formation at elevated $CaCl_2$ concentrations, a rigid gel layer forms quickly, which can facilitate the release of encapsulated material from the core. Conversely, at lower $CaCl_2$ concentrations, a more elastic gel forms, which better retains the encapsulated material within the core.

After drying for 12 hours at 60 °C, the encapsulation efficiency (EEAD) was recalculated to assess the influence of water content on the capsule surface and within the encapsulation matrix. Drying leads to the shrinkage of the hydrogel matrix, causing some surface oil to be expelled from the beads. The EEAD values ranged from 51% to 79% (Table 3), with a decrease observed in certain treatments. This reduction in efficiency may be due to the loss of surface water and oil during drying, resulting in a lower weight and encapsulation efficiency, particularly in capsules with higher water content and surface oil.

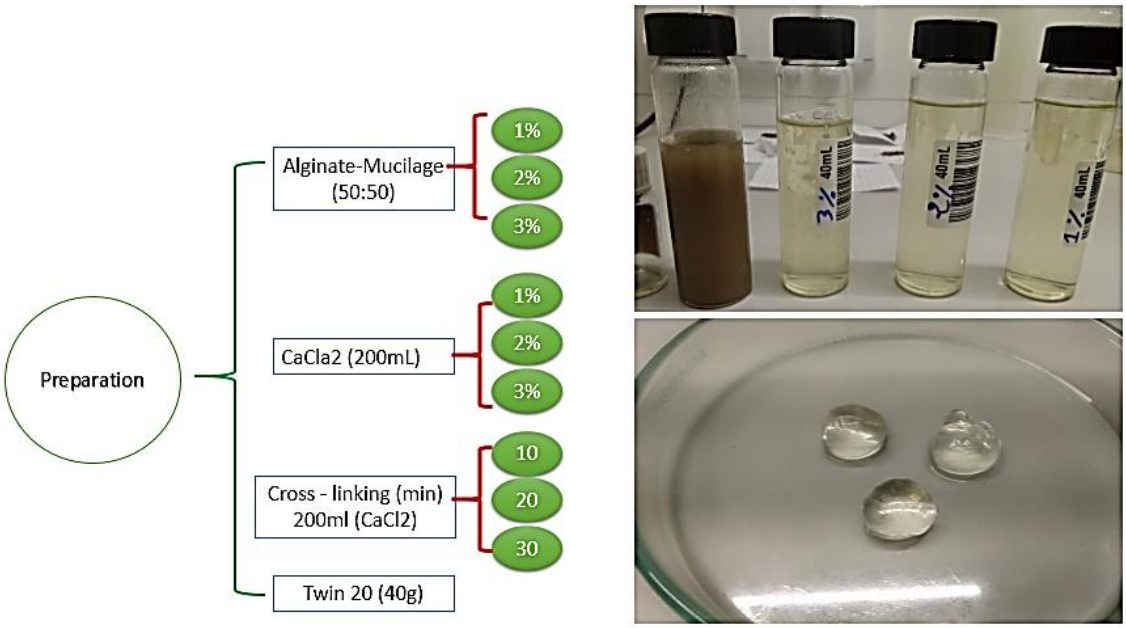

Figure 5. Preparation of encapsulation in an alginate-mucilage matrix

Table 3. Encapsulation efficiency results for *W. frutescens* essential oils in the alginate-mucilage matrix

EXP	Alginate-mucilage (%)	$CaCl_2$ (%)	Reticulation (min)	Eau (%)	EEBD (%)	EEAD (%)
1	1	1	10	59.22	85.34	61.33
2	3	1	10	63.04	86.32	68.24
3	1	3	10	61.43	76.83	51.36
4	3	3	10	63.81	76.41	51.42
5	1	1	30	60.24	84.47	58.46
6	3	1	30	61.91	86.35	58.91
7	1	3	30	64.26	69.86	60.48
8	3	3	30	63.52	69.81	68.68
9	2	2	20	62.58	78.49	79.83

This behavior may be explained by the dependency of EEAD on the degree of cross-linking at the surface of the extruded emulsion droplet. At higher concentrations of alginate-mucilage and $CaCl_2$, when the emulsion droplet enters the $CaCl_2$ bath, there is likely enhanced cross-linking between alginate-mucilage and calcium ions on the droplet's surface. This results in the formation of tight Ca-alginate hydrogel walls with lower internal water content. According to Chan (2011), encapsulation efficiencies above 90% in Ca-alginate beads were achievable with alginate concentrations over 15 g/L and oil loadings up to 40% by volume. However, lowering the alginate concentration below 15 g/L or increasing the oil loading above 40% by volume reduced encapsulation efficiency.

The high yield of this encapsulation process is advantageous for preserving expensive oils and stabilizing volatile or easily oxidized compounds, as it is conducted at room temperature without exposure to air. Additionally, the drying process can be adjusted to prevent product loss or degradation. This technique offers the flexibility to produce

highly concentrated capsules for controlled oil release, with mucilage acting as a stabilizer for oil-water emulsions. Munoz et al. (2012) reported that mucilage demonstrated an emulsifying activity index of 41.40 ± 0.10 g/L and stabilized emulsions effectively at 78.42 ± 1.96% with 40% oil. Given the extreme sensitivity of oils to oxidation, which can lead to undesirable flavors and quality loss, the emulsion-stabilizing capacity of *W. frutescens* mucilage is valuable. This stabilizing effect may be attributed to mucilage's ability to adsorb onto solid or liquid interfaces, maintaining oil-in-water emulsions without any chemical or enzymatic modification.

3.4. In vitro stability and functional evaluation of encapsulation of W. frutescens mucilage nanoparticles with antibiotics

The antibacterial activity of purified mucilage, crude antibiotics, and mucilage-encapsulated antibiotics on solid media is illustrated in Figure 6. The findings demonstrate that bacterial growth was inhibited across all treatments, with the smallest inhibition zones recorded for crude antibiotics: 10 mm for ampicillin (Amp), 11.5 mm for streptomycin (Str), and 10.6 mm for kanamycin (Kan). In contrast, larger inhibition zones were observed for purified mucilages and mucilage-encapsulated antibiotics, with 12.3 mm for mucilage alone (Muc), 13.8 mm for mucilage-encapsulated ampicillin (Muc-Amp), 15.6 mm for mucilage-encapsulated streptomycin (Muc-Str), and 14 mm for mucilage-encapsulated kanamycin (Muc-Kan).

The minimum inhibitory concentration (MIC) of each antibiotic was also evaluated in its free and encapsulated forms against *E. coli* and *S. aureus*. Results showed that the MIC of encapsulated antibiotics was consistently lower than that of their free counterparts. For example, the MIC of encapsulated ampicillin was 34 µg/mL compared to 56 µg/mL for free ampicillin against *E. coli*, while for streptomycin, it was 17 µg/mL (encapsulated) versus 24 µg/mL (free), and for kanamycin, it was 16 µg/mL (encapsulated) versus 39 µg/mL (free) (Figure 7).

In the case of *S. aureus*, the MIC for encapsulated ampicillin was 37 µg/mL, compared to 46 µg/mL for the free form, and for kanamycin, the values were 15 µg/mL (encapsulated) and 29 µg/mL (free). These results indicate that mucilage-encapsulated antibiotics exhibit enhanced antibacterial efficacy, achieving bacterial inhibition at lower concentrations than their free antibiotic forms. This suggests that using mucilage nanoparticles for antibiotic delivery could enable lower antibiotic dosages, potentially reducing the toxicity associated with the widespread use of antibiotics in modern healthcare.

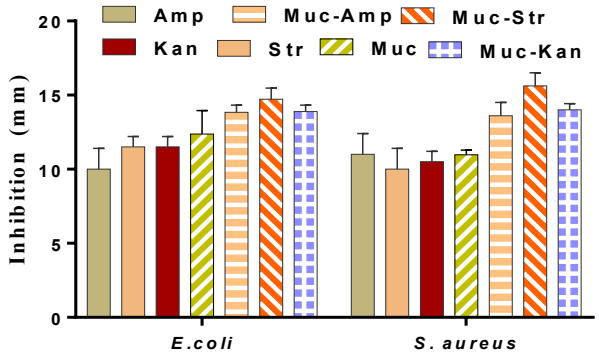

Figure 6. Antibacterial potency of *W. frutescens* mucilage using the disk method on solid medium. Mu: mucilage; Kan: kanamycin; Str: streptomycin; Amp: ampicilin.

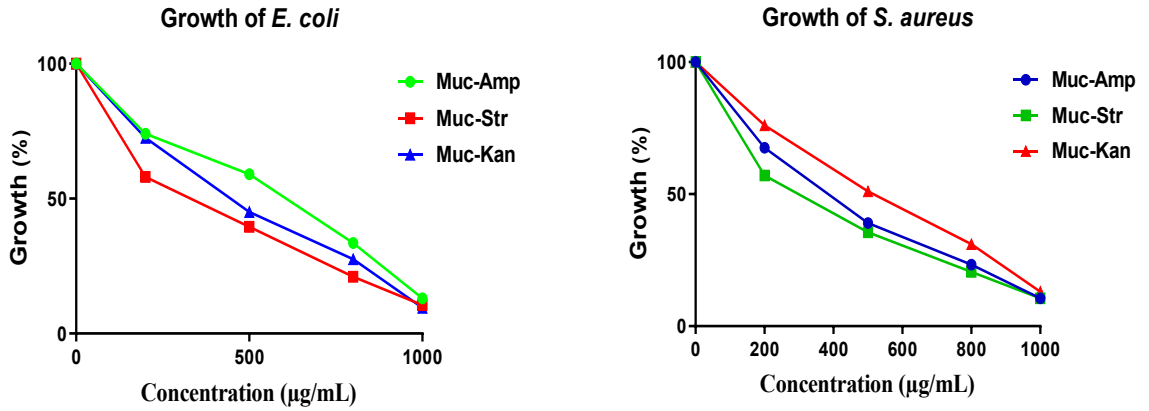

Figure 7. Bacterial growth as a function of nanoparticle concentration

We then examined the stability of mucilage-encapsulated antibiotics compared with free antibiotics. Antibiotics and antibiotic-encapsulated mucilage nanoparticles were incubated at 25-28 °C for different numbers of days before testing. It was found that mucilage nanoparticles encapsulated in antibiotics were able to retain their activity over a long storage period, compared with free antibiotics (Figure 8). In addition, the thermal stress of the two forms of the three antibiotics used for antibacterial activity was measured using an agar diffusion test. All the encapsulated forms showed greater stability at elevated temperatures. In the case of *E. coli,* the activity of the free form of streptomycin decreased significantly at 90 °C, and *S. aureus* also showed a similar trend. In general, the encapsulated antibiotic showed the greatest activity and stability at elevated temperatures.

In all three cases, the free antibiotics were less active than the antibiotics encapsulated in the mucilage. The experiments allow us to conclude that okra mucilage is stable even after long periods of storage and exposure to high

temperatures. Antibiotic-encapsulated *W. frutescens* mucilage nanoparticles could be used to increase the functional stability of commonly used antibiotics.

Nanoparticles encapsulated in ampicillin were used to treat salmonella infected mice and it was observed that the amount of drug used was divided by forty compared to free ampicillin (Nagpal et al., 2017). Several reports indicate that abundant use of antibiotics causes toxic effects (Geller et al., 1986). Ampicillin, kanamycin and streptomycin are widely used in various pathogenic conditions. The rampant use of these antibiotics increases toxicity levels in patients and also leads to the synthesis of multidrug resistance in various pathogenic strains (Li et al., 2014). In addition, these antibiotics are highly susceptible to degradation at elevated temperatures, ultraviolet light and prolonged storage (Bhattacharya et al., 2012).

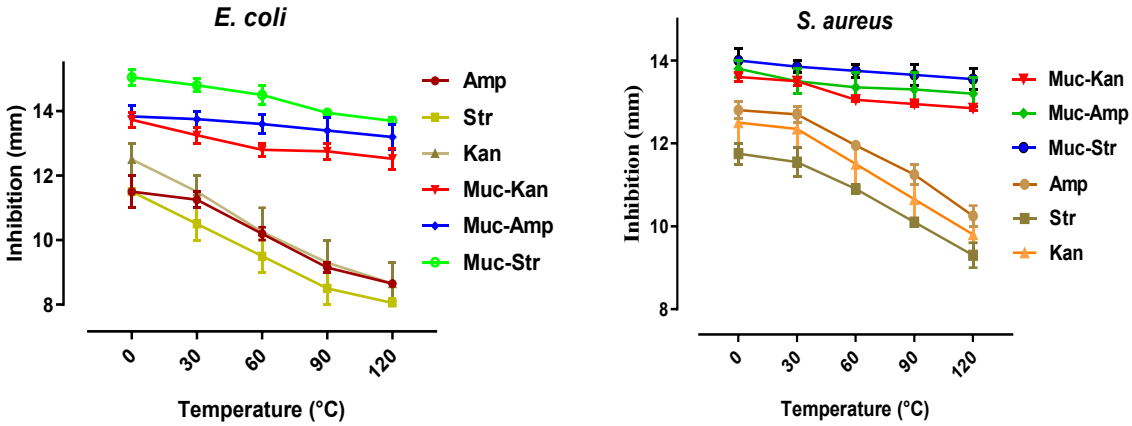

Figure 8. Effect of temperature on nanoparticles and inhibition of bacterial growth.

The main objective is to evaluate the bactericidal efficacy and functional integrity of common antibiotics encapsulated in mucilage on pathogenic bacterial strains. These antibiotics are used worldwide in various pathogenic conditions. But the predominant use of antibiotics causes systemic toxicity and also induces resistance in various pathogenic strains, which is already wreaking havoc in the modern world. Moreover, these antibiotics are susceptible to functional degradation when exposed to unfavorable conditions. Encapsulating these antibiotics in a nanomaterial may be a solution to this serious problem. The conjugation of antibiotics in nanoparticles involves the use of different functionalizing agents that could have undesirable effects on human health. We have designed a simple system that avoids the use of such agents. The resulting conjugate has higher antibacterial activity, suggesting that a small amount of antibiotics is used, reducing toxicity arising from abundant antibiotic use (Bhattacharya et al., 2012; Nagpal et al., 2017; Geller et al., 1986).

4. Conclusion

This work enabled us to optimize *W. frutescens* mucilage extraction conditions by determining key parameters such as high-speed centrifugation, extraction temperature, liquid-solid ratio, and extraction time while maximizing mucilage yield. The encapsulation of active ingredients with alginate demonstrated effective thermal protection, preserving the stability of encapsulated drugs even under high-temperature processing conditions. The results obtained provide a valuable foundation for the development and integration of *W. frutescens* mucilage into medical devices, with potential for large-scale application. This study highlights the *in vitro* performance of mucilage-alginate capsules, emphasizing the need to complement these findings with *in vivo* trials and pilot-scale experiments using suitable food models. This hybrid biomaterial exhibits promising characteristics not only as a carrier for bioactive compounds but also as an innovative element that can be combined with other hydrocolloids or polymers. Its potential thus opens up exciting prospects for applications in the pharmaceutical and food packaging industries.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data availability statement

Data will be available upon request from the corresponding author.

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