Evaluation of Microencapsulated Preparation Formulation of Ethanol Extract of Moringa Leaves (Moringa Oleifera)

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Indonesia has abundant natural resources, one of which is the moringa plant (Moringa oleifera), known as “The Miracle Tree” due to its high nutritional content and pharmacological properties as an antioxidant, antimicrobial, and anti-inflammatory agent. This study aims to formulate ethanol extract of moringa leaves into microencapsulated preparations to improve the stability of active compounds and protect the active ingredients from stomach acid. The extract was obtained through maceration using 96% ethanol solvent with a yield of 24.42%. The characterization of the crude drug met the Indonesian Herbal Pharmacopoeia standards, and phytochemical screening showed the presence of alkaloids, flavonoids, triterpenoids, steroids, and tannins. The microencapsulation formulation was made using a combination of sodium alginate polymer and chitosan coating with CaCl2 hardening agent in four different extract concentrations. The evaluation of the preparations included stability testing (cycling test), moisture content testing, and buoyancy testing (floating). The stability test results showed that the preparation remained organoleptically stable without significant physical changes during 6 cycles. All formulas met the <5% moisture content requirement, with the 0.75% concentration formula (F4) having the lowest moisture content of 0.26%. In the adhesion test using rat gastric mucosa, the preparation showed good mucoadhesive ability, where the interaction of chitosan in an acidic environment prolonged the retention time of the microcapsules in the stomach. This study concluded that microencapsulation of moringa leaf extract has great potential as a stable and effective drug delivery system.

Keywords: Moringa leaves (Moringa oleifera), Microencapsulation, Sodium Alginate, Chitosan, Antioxidants

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1. Introduction

Indonesia boasts abundant biodiversity, a diverse natural resource with high potential for human benefits, such as moringa leaves. Moringa is known as "The Miracle Tree" because it offers benefits as a source of nutrients with medicinal properties due to its abundance compared to other plants (Marhaeni, 2021). Nearly all parts of the moringa plant, from the roots and fruit to the leaves and even the wood, have been used traditionally and as food to treat various ailments. Numerous studies have shown that moringa leaves possess numerous pharmacological activities, significantly influencing the effects of traditional medicine. Various parts of the moringa plant are known to contain secondary metabolites such as alkaloids, tannins, flavonoids, triterpenoids, and others (Riskianto, 2021).

Previous research has shown that moringa leaf extract has a strong antioxidant effect. However, the secondary metabolites contained in the extract are still in dosage forms with weak potency. Therefore, to prevent damage to important compounds in natural ingredients, several modern techniques can be used to protect these compounds, such as microencapsulation (Ahmadian, 2019).

Microencapsulation is a dosage form formed from microcapsules by coating or coating solid particles, droplets, or a mixture of these materials with a thin layer measuring up to thousands of nanometers. Microencapsulation works synergistically to produce capsules measuring microns to millimeters. Various methods are used in the formulation process for microencapsulated preparations, ranging from simple processes such as emulsion to more complex methods such as suspension polycondensation. Other methods include granulation, spray drying, freeze-drying, dispersion, and polymerization, among many others (Pratama, 2021). Microencapsulation aims to increase the phytochemical stability of active ingredients, protect them from gastric acid, and reduce side effects of irritation to the gastric mucosa. Another goal is to ensure the coating material effectively protects the core ingredients. There are three methods available for microencapsulation: chemical, physical, and physicochemical. Therefore, this study aims to formulate a microencapsulated formulation using ethanol extract of Moringa leaves and evaluate the preparation through stability, water content, and buoyancy tests. The results of this study are expected to serve as a basis for further research on Moringa leaves through delivery, keeping pace with technological developments in the world of drug formulation.

2. Method

This research employed an experimental method encompassing the characterization, screening, formulation, and evaluation of a microencapsulated preparation containing moringa leaf extract. The research was conducted at the Integrated Laboratory of the University of North Sumatra and the Pharmacology Laboratory of the University of Prima Indonesia. The study was conducted from June 2025 to October 2025, encompassing all stages from planning to completion. The sample used was moringa leaves, collected using a purposive sampling method. A total of 10 kg of samples were collected from Jalan Mahoni, Gaharu Village, Medan Timur District, Medan City, North Sumatra. Subsequently, the preparation of the simplex, characterization of the simplex powder, phytochemical screening, formulation, and evaluation of the microencapsulated preparation were carried out. Tools and Materials

The tools used in this study included laboratory glassware (Pyrex®), a blender, a densicator, a disintegration tester, filter paper, a magnetic stirrer (Model SS30), an oven (Memmert®), a 10 cc injection syringe, and an analytical balance (Precisa XB 220A®). The materials used in this study included distilled water, CaCl2, 96% ethanol, chitosan, sodium alginate, and 0.1N HCl solution as a medium to simulate rat gastric fluid in the floating test.

Characterization of Moringa leaf extract

Determination of Water Content

a. Toluene Saturation
   200 ml of toluene was placed in a round-bottom flask, followed by 2 ml of distilled water. The apparatus was then installed and distillation was carried out for 2 hours. The distillation was stopped and allowed to cool for approximately 30 minutes. The volume of water in the receiving tube was then read to the nearest 0.1 ml.
b. Determining the water content of the medicinal plant
   The toluene-filled flask was filled with 5 g of accurately weighed medicinal plant powder and heated carefully for 15 minutes. After the toluene boiled, the toluene flow rate was adjusted to 2 drops per second until most of the water had distilled off. Then, the flow rate was increased to 4 drops per second. After all the water had distilled off, the inside of the condenser was rinsed with toluene. The distillation was continued for 5 minutes, and the receiving tube was allowed to cool to room temperature. After the water and toluene had completely separated, the water volume was read to the nearest 0.1 ml. The difference between the two water volumes corresponded to the water content

of the material being tested. The water content was calculated as a percentage. (ARISTA WAHYU NINGSIH, 2022) Water content formula:

% Water content of simple drugs = $\frac{\text{(water volume)}}{\text{sample weight}}$ x 100%

Determination of Water-Soluble Extract Content

Five grams of powdered medicinal plants were placed in a stoppered flask and macerated with 100 ml of water-chloroform (2.5 ml of chloroform in 1000 ml of water) for 24 hours, with occasional shaking for the first 6 hours, and then allowed to stand for 18 hours. After filtering, 20 ml of the filtrate was evaporated to dryness in a shallow, flat-bottomed, tared dish. The remainder was heated at 105°C until a constant weight was obtained. The water-soluble extract content was calculated based on the dried material. (ARISTA WAHYU NINGSIH, 2022)

Formula for Water-Soluble Extract Content:

% Water soluble content = $\frac{\text{weight of juice(g)}}{\text{sample weight (g)}} \times \frac{100}{20} \times 100\%$

Determination of Ethanol-Soluble Extract

5 g of powdered medicinal plants were placed in a stoppered flask, macerated with 100 ml of 96% ethanol for 24 hours, with occasional shaking for the first 6 hours, and then allowed to stand for 18 hours. After filtering, 20 ml of the filtrate was evaporated to dryness in a shallow, flat-bottomed, tared dish. The remainder was heated at 105°C until a constant weight was obtained. The ethanol-soluble extract was calculated based on the dried material. (ARISTA WAHYU NINGSIH, 2022)

Ethanol-Soluble Extract Formula:

% Ethanol-Soluble Extract = $\frac{\text{weight of juice (g)}}{\text{sample weight(g)}} \times \frac{100}{20} \times 100\%$

Determination of Total Ash Content

A 2 g of accurately weighed powder was placed in a tared porcelain crucible, then leveled. The crucible was heated until a constant weight was obtained. The ash content was calculated for the air-dried material. (ARISTA WAHYU NINGSIH, 2022)

Ash Content Formula:

Ash Content = $\frac{\text{Ash weight}}{\text{sample weight (g)}} \; x \; 100\%$

Determination of Acid-Insoluble Ash Content

The ash obtained in the total ash content determination was boiled in 25 ml of 2 N hydrochloric acid for 5 minutes. The acid-insoluble portion was collected, filtered through ash-free filter paper, and washed with hot water. The residue and filter paper were heated to constant weight, then cooled and weighed. The acid-insoluble ash content was calculated against the dried material. (ARISTA WAHYU NINGSIH, 2022)

Acid-Insoluble Ash Content Formula

Ash Content = $\frac{\text{Ash weight}}{\text{heavy until (g)}} \; x \; 100\%$

Making Moringa Leaf Ethanol Extract

The 5 kg of collected Moringa leaves underwent a wet sorting process, which involved washing and draining the leaves to reduce the water content. The leaves were then dried in a drying cabinet. A 1.2 kg

sample of the dried and ground Moringa leaves was placed in a glass bottle and 12 liters of 96% ethanol (1:10) were added. The maceration process, also known as re-maceration, was carried out for 3 days, stirring every 6 hours and filtering until a clear filtrate was obtained. The filtrate was then evaporated using a rotary vacuum evaporator at 60°C and heated in a water bath until a thick extract was obtained. (Husni, 2019)

$$\% \text{ rendemen} = \frac{weight\ of\ thick\ extract}{dry\ weight\ of\ simple\ drugs} x\ 100$$

Phytochemical Screening

Several compounds in plants are considered important for health. Phytochemical testing is performed to determine the compounds present in a plant that can be used as medicine.

Flavonoids

The phytochemical test is performed by weighing 0.1 g of the crude drug and dissolving it in 2.5 mL of water. Place it in a water bath. Then, transfer it to a test tube and add 100 mg of magnesium powder, 1 mL of concentrated hydrochloric acid, and 3 mL of amyl alcohol. Shake vigorously and allow to separate. The flavonoid compounds in Moringa leaves are identified by the presence of yellow, red, and orange spots on the amyl alcohol layer. (ARISTA WAHYU NINGSIH, 2022)

Tannin

A phytochemical test to determine the tannin compounds in Moringa leaves is performed by weighing 1 gram of the medicinal herb, adding 5 drops of 10% NaCl, filtering, and adding 1% gelatin and 10% NaCl. The formation of a white precipitate indicates the presence of tannins. (ARISTA WAHYU NINGSIH, 2022)

Steroids & Terpenoids

A phytochemical test to determine the terpenoid compounds in Moringa leaves is performed by placing the sample in a test tube, then adding glacial CH3COOH and concentrated H2SO4. The formation of a red color indicates the presence of terpenoids, while a blue or purple color indicates the presence of steroids, indicated by the appearance of brownish-pink spots using the H2SO4 reagent. (ARISTA WAHYU NINGSIH, 2022)

Alkaloids

A phytochemical test to determine the presence of alkaloids in Moringa leaves was carried out by weighing 0.1 g of the crude drug, adding 5 ml of 10% hydrochloric acid, stirring thoroughly, and then adding 5 ml of 10% ammonia solution. Extract using 10 ml of chloroform. To the evaporation residue, add 1.5 ml of 2% hydrochloric acid, then add 3 drops of Dragendorff's reagent to the second tube until a brick-red precipitate forms, indicating the presence of alkaloids. (ARISTA WAHYU NINGSIH, 2022)

Saponin

A phytochemical test to determine the saponin content in Moringa leaves involves weighing 0.1 g of the crude extract, dissolving it in 2.5 mL of water, placing it in a water bath, transferring it to a test tube, and shaking it vertically for 10 seconds until a stable foam forms. Let it stand for 10 minutes. Add 1 drop of hydrochloric acid. If the foam persists, saponin is present. (ARISTA WAHYU NINGSIH, 2022)

Microencapsulation Preparation

The microencapsulation preparation process begins with preparing the extract. Prepare 2 grams of alginate, 0.75 grams of chitosan, and 100 ml of distilled water. Place some of the distilled water into a beaker on a magnetic stirrer and add the alginate little by little, evenly and until homogeneous. If there are clumps of

foam in the solution, stir thoroughly or spray it with alcohol. After homogenization, 0.75g chitosan was added. Next, the thick ethanol extract of Moringa leaves was taken with four different concentrations (blank, 0.25%, 0.50%, 0.75%) and put into a beaker glass, stirred until homogeneous. Prepared a 50ml CaCl2 solution with 0.15M as much as 4 beakers. Then, drip the polymer into the CaCl2 solution using a 10cc syringe that has been opened to produce wet microcapsule granules. The wet microcapsule granules were soaked in the CaCl2 solution for 10 minutes then filtered using filter paper and dried for 12 hours at 45oC.

Ingredients	Formula				Uses
	F1	F2	F3	F4	
Moringa leaf extract	0 gr	0,25 gr	0,50 gr	0,75 gr	Active ingredient
CaCl2	4,16 gr	4,16 gr	4,16 gr	4,16 gr	Binding/hardening agent
Chitosan	0,75 gr	0,75 gr	0,75 gr	0,75 gr	Coating material
Sodium alginate	2 gr	2 gr	2 gr	2 gr	Polymer

Evaluation of Microencapsulated Preparations

a. Stability Test (Cycling Test)
The stability test relates to the durability of the preparation during storage. The test is conducted by storing the preparation at 4°C and 40°C for 24 hours. The process is repeated six times, and the changes observed are observed. (Anggun, 2020)

b. Moisture Content Test
The test is conducted by placing a Petri dish in an oven at 110°C for 1 hour, then cooling it in a densicator for 30 minutes, and then weighing the initial weight of the Petri dish (Wo). Next, 2 grams of microcapsules are weighed and placed into the dish (Ws), which is then heated in the oven at 110°C for 2 hours. After the heating process, the Petri dish containing the granules is removed from the oven, cooled again in the densicator for 30 minutes, and reweighed to obtain its weight (Wi). The heating and weighing process is repeated until a stable weight is achieved. (Mulun, 2025)

$$\text{Water content } (\%) = \frac{(\text{Wo} + \text{Ws}) - \text{Wi}}{Ws} x\ 100$$

c. Wash-off test
The test was conducted by first preparing gastric fluid. The fluid was prepared by mixing 2 grams of NaCl with 250 ml of distilled water and transferring it to an Erlenmeyer flask. Then, 7 ml of concentrated HCl was added and stirred until the solution was homogeneous. The solution was then diluted with distilled water to a volume of 1 liter. Once complete, the pH of the solution was measured, with a range of 1.2 ± 0.1. Healthy mice with an average weight of 250 grams were prepared, which had been fasted for 24 hours. The mice were anesthetized with ether and surgically removed to remove the stomach, which was then cleaned with physiological NaCl solution. After the stomach was cleaned, 50 microencapsulated granules were inserted into the mouse's gastric mucosa. The stomach was then placed in a disintegration tester filled with loaded gastric fluid. The instrument was operated at a frequency of 30 cycles per minute at a temperature of 37 ± 0.5°C. Observations were made every 30 minutes for 2 hours. (Wardatun, 2020)

3. Results and Disccusion

Characterization of Medicinal Plants

Analysis of the solubility of Moringa leaf powder in specific solvents was performed. Determination of the solubility in water and ethanol solvents aims to estimate the quantity of active compounds based on their polarity, namely polar compounds that are soluble in water and nonpolar (or less polar) compounds that are soluble in ethanol. This test provides an initial overview of the chemical profile of the natural extract.

Furthermore, determining the percentage of solubility is useful for determining extraction effectiveness, thus facilitating the optimal formulation of drug preparations according to desired specifications. Specifically, the ethanol-soluble solubility is used to assess the solubility of the material in organic solvents, while the water-soluble solubility indicates the solubility of the active components in aqueous media. High water content can facilitate microbial growth in the medicinal plant. Ash content determination is performed to obtain an overview of the internal and external mineral content from the initial process to extract formation. At this stage, the extract is heated until the organic compounds and their derivatives are destroyed and evaporated, leaving only the mineral and inorganic elements. This test resulted in the percentage characterization of Moringa leaf medicinal plants that all meet the requirements according to the Indonesian Herbal Pharmacopoeia standards.

Parameters	Result	Terms/conditions	Information
Water Content	6,66%	Not more than 10.0%	Qualify
Water-Soluble Essence Content	32,57%	Not less than 4.9%	Qualify
Ethanol-Soluble Essence Content	19,35 %	Not less than 5.0%	Qualify
Total Ash Content	8,22%	Not more than 11.4%	Qualify
Acid-Insoluble Ash Content	0,80%	Not more than 0.9%	Qualify

Phytochemical Screening

Phytochemical screening is conducted to provide an overview of specific secondary metabolite compounds present in a medicinal herb. Phytochemical screening tests are conducted using a chemical reagent with a specific solvent or reagent. Based on the results of the phytochemical screening test on Moringa leaf medicinal herb, it was found to contain alkaloids, flavonoids, triterpenoids, steroids, and tannins. The saponin test did not produce the required results, which is foaming after shaking. This indicates that the Moringa leaf extract does not contain saponins.

Phytochemical test	Reagents	Observations	Result
Flavonoids	2mg Mg powder, 1ml concentrated HCl	The results show a yellowish-orange color in the amyl alcohol layer, indicating a positive flavonoid content.	+
Alkaloids	Dragendroff	The results show a brick-red precipitate, indicating a positive alkaloid content.	+
Triterpenoids	2ml 98% chloroform, 5 drops 98% anhydrous acetic acid, 3 drops 98% H2SO4	The results show a color change to orange, indicating a positive triterpenoid content.	+
Steroids	2ml 98% chloroform, 5 drops 98% anhydrous acetic acid, 3 drops 98% H2SO4	The results show a color change to greenish-blue, indicating a positive steroid content.	+
Saponins	1ml 2N HCl	No foam was found when shaken, indicating a negative saponin content in the sample.	-
Tannin	5% FeCl3	The results show a color change to blackish-blue, indicating a positive tannin content in the sample.	+

Extraction

The extraction process in this study used a maceration method using 96% polar ethanol solvent for 24 hours.

Table 1. Results of the Moringa leaf extract yield calculation.

Moringa Oleifera Leaf Sample	Simple weight	Extract weight	% yield
Moringa Oleifera Leaf Sample	1200 grams	293 grams	24.42%

The yield calculation of the extract percentage was performed to compare the amount of extract obtained to the initial weight of the medicinal plant and to determine how many compounds were absorbed into the thick extract of the medicinal plant.

Microencapsulation Formulation

From the resulting thick extract, a microencapsulation formulation process was carried out with four different concentrations, resulting in the weights shown in Table 2.

Table 2. Results of Microencapsulated Granule Weight

Formula (%)	Dosage Weight
F1	5 gr
F2	5.83 gr
F3	6.18 gr
F4	6.42 gr

Stability Test

Stability testing of the preparation was conducted to observe the physical changes in the microencapsulated preparation organoleptically under storage conditions at two different temperatures and for six cycles. The results of the physical stability test are shown in Table 3.

Table 3. Results of the stability test (Cycling Test) during 6 cycles

Time	Parameters	F1	F2	F3	F4
Cycle 1	Color	White	Light greenish brown	Dark greenish brown	Blackish brown
	Texture	Hard	Hard	Hard	Hard
Cycle 2	Color	White	Light greenish brown	Dark greenish brown	Blackish brown
	Texture	Hard	Hard	Hard	Hard
Cycle 3	Color	White	Light greenish brown	Dark greenish brown	Blackish brown
	Texture	Hard	Hard	Hard	Hard
Cycle 4	Color	White	Light greenish brown	Dark greenish brown	Blackish brown
	Texture	Hard	Hard	Hard	Hard
Cycle 5	Color	White	Light greenish brown	Dark greenish brown	Blackish brown
	Texture	Hard	Hard	Hard	Hard
Cycle 6	Color	White	Light greenish brown	Dark greenish brown	Blackish brown
Time	Texture	Hard	Hard	Hard	Hard

Stability testing using the cycling method simulates extreme temperature changes in a preparation, such as freezing at low temperatures and melting at high temperatures. Stability testing is conducted to assess the product's durability during storage (Anggun, 2020). The results of the stability test in this study showed no significant changes, indicating that the organoleptic condition of the preparation remained stable and remained the same from cycle 1 to cycle 6.

Moisture Content Test

Moisture content testing was conducted on each microencapsulation formula using the thermogravimetric method to support the stability of the preparation. The results of the moisture content test on the microencapsulated preparations are shown in Table 4.

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Table 4. Results of the Water Content Test for Microencapsulated Preparations

Concentration	Initial weight (gr)	Final weight (gr)	Water content (%)
Blank	94,96 gr	94,65 gr	0,32 %
0.25%	97,63 gr	97,33 gr	0,30 %
0.50%	98 gr	97,75 gr	0,25 %
0.75%	95 gr	94,75 gr	0,26%

To achieve the desired water content, a drying process or step is necessary for a preparation (Santoso, 2020). Water content can be defined as the amount of water contained in a material or preparation, which can then affect its quality and characteristics in terms of texture and appearance. The higher the water content in a material, the lower its shelf life because it can act as a good growth medium for microbes (Dewi, 2023). In this study, the water content of each formula met the requirements of <5%, as research by (Sadiah, 2022). The low water content results were influenced by the large amount of chitosan added to the microcapsule formula and its interaction with the alginate polymer. Chitosan can form a denser and tighter microcapsule wall structure, thus better resisting water evaporation. In contrast to alginate, which is hydrophilic, alginate can increase water content due to its better water absorption. The interaction between the two forms a polyelectrolyte gel structure that strengthens the microcapsule walls and reduces permeability to water vapor (Ezra Agitian, 2025). From the calculation of the percentage water content, Table 4 shows that the 0.75% formula has the lowest water content compared to the other three concentrations. This means that the 0.75% formula has better shelf life due to its lower water content. This contrasts with the blank formula, which has the highest water content, which tends to absorb more water and has a lower shelf life than the other three formulas.

Adhesion Test

The adhesion test (wash-off) in this study used rat stomachs to evaluate the durability of the microcapsule preparation in the gastrointestinal tract with artificial gastric fluid adjusted to gastric pH (pH 1.5–2.5). The results are shown in Figure 1.

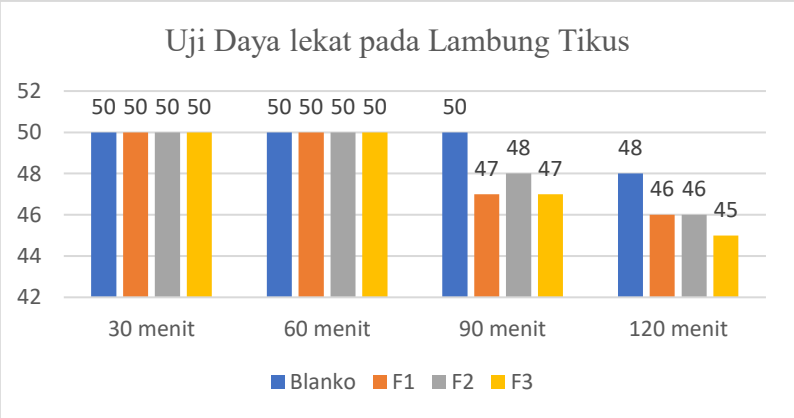

Figure 1. Observation results of the microencapsulation adhesion test.

Microencapsulation testing in the stomach can only be carried out for two hours, as if it were carried out for more than two hours, the integrity of the gastric mucosal tissue would be unsuitable for testing. Figure 1 shows that the buoyancy test results for the blank microcapsule preparation showed a stable adherence to the gastric mucosa for 90 minutes, with a gradual decrease at 120 minutes. F1 and F2 showed a decrease in the number of stably attached microcapsules until the last minute. Meanwhile, F3, with a concentration of 0.75%, showed the highest frequency of decrease, at 45 at 120 minutes. Chitosan's solubility in an acidic environment allows it to interact with the negatively charged gastric mucosa, thereby increasing the retention time of the microcapsules in the stomach. Furthermore, the combination of sodium alginate and

CaCl2 produces granules with mucoadhesive properties that facilitate periodic and measurable release of the active ingredient.

4. Conclusion

This study concluded that the Moringa leaf simplicia used met the quality standards based on the Indonesian Herbal Pharmacopoeia. This was proven through characterization results showing a water content of 6.66%, a water-soluble extract content of 32.57%, an ethanol-soluble extract content of 19.35%, and a total ash content of 8.22%. In addition, phytochemical screening results confirmed the presence of active compounds in the form of alkaloids, flavonoids, triterpenoids, steroids, and tannins, although the preparation did not contain saponins. The extraction process using the maceration method using 96% ethanol succeeded in obtaining a yield of thick extract of 24.42%. The resulting microencapsulated preparation formulation showed organoleptically stable physical quality during storage. Based on the results of the stability test using the cycling test method for six cycles, no significant changes were found in the color or hard texture of all the formulas tested. The success of this formulation is supported by the interaction between the sodium alginate polymer and chitosan which is able to form a dense and cohesive microcapsule wall structure to protect the active ingredients. The final evaluation showed that all microencapsulation formulas met the ideal water content requirement, which is below 5%. The formula with a 0.75% extract concentration had the lowest water content at 0.26%, indicating optimal shelf life due to a lower risk of microbial growth. In the floating test, the preparation was shown to have good mucoadhesive ability on the gastric mucosa of mice for a two-hour interval. The solubility of chitosan in an acidic environment was shown to increase the retention duration of the microcapsules, thus potentially becoming an effective drug delivery system for the periodic release of active substances.

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