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Formulation and characterization of nanoparticles combination of binahong leaves and bay leaves as a thin oral herbal preparation for diabetes prevention

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ABSTRACT

Diabetes mellitus is a chronic metabolic disease with a steadily increasing prevalence globally. Binahong leaves (Anredera*cordifolia*) and bay leaves (Syzygium*polyanthum*) contains flavonoids, such as kaempferol, which have the potential as antidiabetics, but its use is limited by the low solubility, stability, and bioavailability of the active compounds. This study aims to formulate and characterize a nanoparticle herbal medicine combining the two leaves using PlantCrystal technology, and develop it into an oral thin herbal preparation. The simplicia was processed through sorting, washing, drying, grinding, and sieving, then characterized and screened for phytochemicals. The nanoparticle herbal medicine was evaluated based on particle size, polydispersity index, zeta potential, and particle morphology. The membrane lysis release test showed an increase in the release of active substances compared to the simplicia. Furthermore, the formulation was developed into an oral thin herbal and evaluated for its physical properties, including organoleptic, water content, pH, film thickness, fold resistance, weight uniformity, and disintegration time.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease which is currently a serious challenge to global health and is characterized by hyperglycemia due to impaired insulin secretion or function (ADA, 2024). The prevalence of DM continues to increase with 589 million sufferers aged 20–79 years worldwide (IDF, 2025). In Indonesia, the prevalence increased from 10.9% (RISKESDAS, 2018) to 11.7% (SKI, 2023), and reached 11.3% at the age of 20–79 years (IDF, 2025). One of the early stages that is often overlooked is prediabetes, which is a condition where blood glucose levels are above normal but do not yet meet the criteria for type 2 diabetes mellitus. Prediabetes can be identified by HbA1c levels of 5.7–6.4%, fasting blood glucose of 100–125 mg/dL, or glucose two hours after the OGTT of 140–199 mg/dL (ADA, 2024). Prediabetes is characterized by insulin resistance and pancreatic β-cell dysfunction, which can accelerate the progression to type 2 diabetes if left untreated (Alvarez et al., 2023). Globally, in 2024 there were 487.7 million people with GDPT and 634.8 million with TGT, estimated to increase to >1.4 billion by 2050 (IDF, 2025). In Indonesia, the prevalence of prediabetes reached 13.4% GDPT and 18.6% IGT (SKI, 2023).

The use of medicinal plants as alternative therapies is growing, including the use of binahong leaves (Anredera cordifolia) and bay leaves (Syzygium polyanthum), which contain flavonoids, saponins, and polyphenols. However, the use of traditional medicinal plants is still hampered by the low solubility, stability, and bioavailability of the active compounds (Hu et al., 2025). Nanoparticle technology such as PlantCrystals can increase solubility, stability, and bioavailability by destroying plant cell walls, down to micro and nano sizes(Abraham et al., 2021). In addition, the development of thin herbal oral preparations, namely thin film preparations that dissolve in the oral cavity and allow direct absorption through the oral mucosa, has the potential to increase stability, bioavailability, comfort of use, and mask the bitter taste of herbal ingredients (Ambarwati et al., 2024; Rajagopalan et al., 2024; Sevinç Özakar & Özakar, 2021). Therefore, Research combining PlantCrystal-based nanoparticle technology with oral thin film formulations for herbal ingredients, particularly binahong and bay leaves, is still very limited. Therefore, this study aims to develop and characterize nanoparticles combining these two plants and evaluate their potential in the form of an oral thin herbal preparation for diabetes prevention.

RESEARCH METHOD

Tool

The tools used in this study include a mesh sieve no. 200, mortar and stamper, oven, analytical balance, chamber, glassware commonly used in laboratories, filter paper, dropper pipettes, spatulas, stopwatches, including glassware, magnetic stirrers, sonicator probes, Particle Size Analyzer (PSA) (Malvern instruments Ltd), Zetasizer microcentrifuge (Zetasizer Nano ZS; Malvern), spray bottles, analytical balance, Transmission Electron Microscopy (TEM), Microplate reader, UV-Vis spectro, pH meter and thin film oral mold.

Material

The materials used for this research include binahong leaves (Anredera cordifolia (Ten.) Steenis) and bay leaves (Syzygium folyanthum), Plantacare, Tween 80 and hydroxypropyl methylcellulose (HPMC), gelatin film-forming polymers, sucralose and nipagin.

Preparation and Making of Simple Drugs

Binahong leaves (Anredera cordifolia) and bay leaves (Syzygium polyanthum) were first identified to ensure the correctness of the plant species used. Next, the fresh leaves were wet sorted to remove dirt and foreign materials, then washed, dried, and dry sorted. The dried simplicia was ground using a Dry Food Grinder FGD-Z300 to form a powder, then sieved using a Sieve Shaker with a mesh of 200 to obtain a simplicia powder with a fine and uniform particle size.

Characterization of Binahong Leaf and Bay Leaf Simplicia

Characterization of binahong leaf and bay leaf simplicia includes determination of drying loss, total ash content, acid-insoluble ash content, ethanol-soluble extract content, and water-soluble extract content. Drying loss was determined by drying 2 g of simplicia powder in an oven at 105 °C until a constant weight was obtained. Determination of total ash content was carried out by heating 2 g of simplicia powder in a silica crucible until all the charcoal burned and reached a constant weight. The ash obtained was then reacted with 10% HCl, filtered, and the residue was re-heated at 600 °C until a constant weight was reached to determine the acid-insoluble ash content. Determination of ethanol-soluble extract content was carried out by extracting 5 g of simplicia powder using 95% ethanol, while the water-soluble extract content was determined by extraction using chloroform water P. The filtrate was evaporated and the residue was heated at 105 °C until a constant weight was reached, then calculated as a percentage of the initial weight of the simplicia powder (% w/w) (Ministry of Health of the Republic of Indonesia, 2017).

Phytochemical Screening of Simplisia Powder

Phytochemical screening was conducted to identify the alkaloid, tannin, flavonoid, quinone, and steroid/triterpenoid content in the powdered simplicia. The alkaloid test was conducted by basification using 10% NH_3 , chloroform extraction, the addition of 2N HCl, and Dragendorff's reagent, which was indicated by a yellow-orange precipitate. The tannin test was conducted by boiling in hot distilled water and the addition of 1% gelatin, which resulted in a white precipitate. The flavonoid test was conducted by adding ethanol: HCl (1:1), Mg powder, and amyl alcohol, which was indicated by a red, orange, or yellow color. The quinone test used the addition of KOH, which resulted in a red-yellow color. Meanwhile, the steroid/triterpenoid test was conducted by ether extraction and the addition of Liebermann-Burchard's reagent, with purple indicating triterpenoids and green-blue indicating steroids (Safutri et al., 2022; Shitta et al., 2024).

Making PlantCrystal-Based Nanoparticle Herbal Medicine Using a Combination of Binahong and Bay Leaves

Fine powder of binahong leaf and bay leaf simplicia is weighed at 0.5% (w/w) each, then mixed into distilled water containing Plantacare or Tween-80 surfactants at various concentrations, so that the particles do not easily clump and obtain initial stability.(Abraham et al., 2021)The mixture was then stirred with a magnetic stirrer until homogeneous and lump-free. Afterward, it was sonicated using a probe sonicator at the specified time and intensity until the particle size was smooth and homogeneous, resulting in PlantCrystals.

Characterization of Nanoparticle Herbal Medicine

The herbal nanoparticles were characterized to determine their physicochemical properties. Particle size and polydispersity index (PDI) were measured using a Malvern ZSP Zetasizer Particle Size Analyzer (PSA) after the samples were diluted with distilled water. Zeta potential was analyzed using the same instrument to evaluate system stability. Particle morphology was observed using Transmission Electron Microscopy (TEM) after the samples were dropped onto a grid and dried. These parameters were used to assess the size, distribution uniformity, stability, and shape of the herbal nanoparticles (Jafar et al., 2021a: Jafar et al., 2021b).

Active Substance Release Test using Dialysis Membrane Method

In vitro release studies were conducted using the dialysis membrane method. A total of 1 mL of the PlantCrystal-based nanoparticle herbal preparation, a combination of binahong leaves and bay leaves, was placed into a dialysis membrane bag (MWCO 12,000–14,000 Da) and placed in a phosphate buffer medium pH 6.8 at 37 ± 0.5°C with constant stirring. Samples were taken at certain time intervals and replaced with fresh media to maintain a constant volume. The concentration of released kaempferol was analyzed using a UV-Vis spectrophotometer based on a calibration curve, then the cumulative release percentage was calculated to evaluate the release profile of the preparation (Suhandi et al., 2023).

Thin film formulation

The formulation of thin herbal oral formulations based on nanoparticle herbal medicine was carried out using the solvent casting method. Gelatin was dissolved as a film former until homogeneous, then sucralose as a sweetener and nipagin as a preservative were added, and mixed until uniform. Next, the nanoparticle herbal medicine was carefully dispersed into the polymer solution to ensure even distribution within the film matrix (Ambarwati et al., 2024). The mixture is then left to stand for 10 minutes to remove air bubbles, poured into a flat mold, and dried at room temperature for 2 x 24 hours with the mold covered with plastic wrap (Gustana et al., 2025).

Characterization of Thin Film Preparations

Characterization of thin film preparations included organoleptic testing, water content, pH, film thickness, fold resistance, weight uniformity, and disintegration time. Organoleptic testing was carried out by observing the aroma, taste, shape, and color of the film. Water content was measured using a moisture balance at 105 °C for 5 minutes. pH measurements were carried out by dissolving the film in 10 mL of distilled water and measured using a pH meter with a good pH range of 5.5–7.9. Film thickness was measured using a caliper or digital micrometer at five measurement points. Fold resistance was determined by repeated folding, where the film was declared flexible if the fold value was >200. Weight uniformity was determined by weighing six sheets of film from each formula and calculating the average, while the disintegration time was observed visually in 10 mL of phosphate buffer pH 6.8 at 37 °C until the film disintegrated (Ambarwati et al., 2024; Gustana et al., 2025; S et al., 2023).

RESULTS AND DISCUSSIONS

Results of Examination of Simplex Characteristics

Table 1. Characterization results of binahong leaf and bay leaf simples

Type of Determination	Results	
	Binahong Leaves	Bay leaf
Drying Loss	0.706%	5.32%
Total Ash Content	13.82%	1.5%
Acid Insoluble Ash Content	0.675%	1%
Water Soluble Essence Content	15.78%	16%
Ethanol Soluble Essence Level	16.97%	22.33%

Based on the results of the examination of the characteristics of the simplicia, binahong leaves and bay leaves showed drying loss values, total ash content, acid-insoluble ash content, water-soluble essence content, and ethanol-soluble essence content that were still within the limits of the simplicia quality requirements stated in the Indonesian Herbal Pharmacopoeia (Ministry of Health of the Republic of Indonesia, 2017).

Phytochemical Screening Results of Binahong Leaf and Bay Leaf Simplicia

Table 2. Results of phytochemical screening of binahong leaf and bay leaf simplicia

Compound Groups	Results	
	Binahong Leaves	Bay leaf
Alkaloid	(+)	(-)
Tannin	(+)	(+)
Flavonoid	(+)	(+)
Quinone	(+)	(+)
Steroid-Triterpenoid	(+)	(+)

The results of phytochemical screening showed that binahong leaf simplicia contained alkaloids, tannins, flavonoids, quinones, and steroids/triterpenoids, while bay leaf simplicia contained tannins, flavonoids, quinones, and steroids/triterpenoids, but did not show any alkaloids.

The presence of alkaloids in binahong leaves is indicated by the formation of a brick red precipitate after the addition of Dragendorff's reagent (Sudira et al., 2024), and this compound is known to have antibacterial activity (Masniawati et al., 2021). In both simplicia, tannin is indicated by the formation of a white precipitate after the addition of 1% gelatin (Iriany et al., 2021), and is known to have potential as an antioxidant (Safitri et al., 2023). Flavonoids are identified by the appearance of a yellow color in the amyl alcohol layer.(Maulidya et al., 2023), with activity as an antioxidant, antibacterial, anti-inflammatory, and anticancer (Surya Ardiansyah, 2025). Quinones are characterized by a yellow color change after the addition of KOH (Bonor et al., 2024), and is reported to have antibacterial and antifungal activity (Junior et al., 2022). Meanwhile, steroids/triterpenoids are indicated by the formation of a red-purple or green-blue color (Masniawati et al., 2021), and has the potential to be anti-inflammatory and anticancer (Gustana et al., 2025). Overall, these results indicate that binahong and bay leaf simplicia contain secondary metabolites that have the potential to support their pharmacological activity.

Table 3. Formula and characterization results of nanoparticle herbal medicine made from binahong and bay leaves

Code	Formulation						Particle size (nm)	PdI	Zeta Potential (mV)
	SDB (mg)	SDS (mg)	PLA (%)	TWE 80 (%)	HPMC (%)	Aquadest (add)			
PCX F1	11.4	11.4	-	-	-	100 mL	186.10 ± 25.17	0.39 ± 0.06	-28.09 ± 3.01
PCX F2	11.4	11.4	1	-	-	100 mL	456.45 ± 25.24	0.53 ± 0.07	-37.13 ± 5.43
PCX F3	11.4	11.4	-	1	-	100 mL	115.70 ± 21.64	0.54 ± 0.11	-28.18 ± 7.59
PCX F4	11.4	11.4	-	0.5	1.75	100 mL	385.75 ± 228.32	0.77 ± 0.06	-22.32 ± 8.62
PCX F5	11.4	11.4	-	0.75	1.75	100 mL	460.05 ± 48.72	0.86 ± 0.05	-18.67 ± 1.84
PCX F6	11.4	11.4	-	1	1.75	100 mL	487.35 ± 18.60	0.75 ± 0.13	-18.97 ± 5.26
PCX F7	11.4	11.4	-	1.25	1.75	100 mL	694.20 ± 328.95	0.76 ± 0.34	-19.39 ± 8.42
PCX F8	11.4	11.4	-	1.5	1.75	100 mL	678.50 ± 354.12	0.87 ± 0.19	-21.99 ± 5.97
PCX F9	11.4	11.4	0.5	-	1.75	100 mL	1993.00 ± 551.54	0.97 ± 0.04	-16.04 ± 1.44
PCX F10	11.4	11.4	0.75	-	1.75	100 mL	882.10 ± 801.72	0.94 ± 0.08	-19.56 ± 3.34
PCX F11	11.4	11.4	1	-	1.75	100 mL	1311.01 ± 1835.64	0.64 ± 0.51	-5.81 ± 9.32
PCX F12	11.4	11.4	1.25	-	1.75	100 mL	1439.00 ± 601.04	1.00 ± 0.00	-19.70 ± 4.12
PCX F13	11.4	11.4	1.5	-	1.75	100 mL	1027.05 ± 558.54	0.87 ± 0.19	-12.15 ± 1.78

Note: SDB: Binahong Leaf Simplisia, SDS: Bay Leaf Simplisia, PLA: Plantacare, TWE 80: Tween 80

The characterization results of the nanoparticle herbal medicine combination of binahong leaves and bay leaves in Table 3 show that the preparation without HPMC has better characteristics. This is indicated by a particle size of <500 nm, a PDI value of <0.5, and a zeta potential of approximately ±30 mV. These parameters are considered the main indicators of nanoparticle stability, as stated in previous studies (Jafar et al., 2022; Jafar, et al., 2025).

These findings indicate that the addition of HPMC to this formulation does not necessarily improve the physical characteristics of the nanoparticles. Under certain formulation conditions, the addition of HPMC does not always improve the physical characteristics of the nanoparticles. This finding aligns with reports (Touqeer et al., 2022), which shows that increasing the concentration of HPMC to the optimum limit can reduce particle size, while concentrations exceeding the optimum point actually tend to increase particle size. This condition is thought to be related to the formation of a thicker stabilizing layer on the particle surface and increased system viscosity, so that the diffusion process during precipitation is hampered.

Particle Size

Based on the characterization results, the particle size of each formula ranged from 115.70 ± 21.64 nm to 1993.00 ± 551.54 nm. The smallest particle size was obtained by PCX F3, while the largest particle size was obtained by PCX F9. Small particle size can increase the contact surface area, improve solubility, and support dose uniformity (Rahmi et al., 2025).

Polydiversity Index

The polydispersity index (PDI) values range from 0.39 ± 0.06 to 1.00 ± 0.00. The lowest value was obtained in PCX F1 and the highest in PCX F12. A low PDI indicates a more homogeneous particle size distribution, while a high value indicates heterogeneity and potential interglobule clumping (Jafar, Pebriawati, Latifah, & Pratama, 2025).

Zeta Potential

All formulas showed negative zeta potential, which ranged from -5.81 ± 9.32 mV to -37.13 ± 5.43 mV. Absolute zeta potential values ≥30 mV are generally associated with good colloidal stability (Németh et al., 2022; Ramaye et al., 2021). Thus, PCX F2 is thought to have the best electrostatic stability, while formulas with values close to zero, such as PCX F11, tend to be less stable (Németh et al., 2022).

Morphology

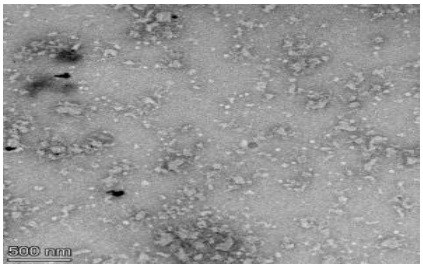

Figure 1. Morphological test results

The results of morphological observations show that the particles formed are irregular. This is in line with previous research, namely(Abraham et al., 2021)which is related to PlantCrystal and produces irregular particle morphology.

Dialysis Membrane

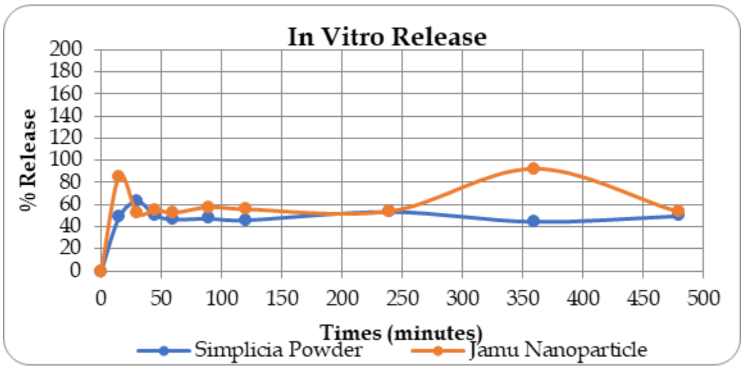

Figure 2. Release of combination simplicia powder and combination nanoparticle herbal medicine at pH 6.8

Based on the graph, the herbal formulation in nanoparticle form showed a higher release percentage, especially at the beginning of the observation period, at 85.54%, compared to the simple drug, which only reached 48.96%. This finding is in line with research. (Suhandi et al., 2023), who reported that nanoparticle preparations in the form of nanostructured lipid carriers (NLC) of α-mangostin produced a much higher release of active substances compared to the powder form, thereby significantly increasing solubility. Thus, nanoparticle herbal medicine formulations are thought to be able to improve dissolution characteristics compared to simple herbal preparations.

Table 4. Blank herbal thin oral formula

Code	Gelatin (%)	Nipagin (%)	Sucralose (%)	Aquadest (add)
OTHK F1	11	0.18	0.5	25 mL
OTHK F2	12	0.18	0.5	25 mL
OTHK F3	13	0.18	0.5	25 mL
OTHK F4	14	0.18	0.5	25 mL

OTHK F5	15	0.18	0.5	25 mL

Table 5. Characterization results of oral thin film blank

Testing	Formula					Condition
	F1	F2	F3	F4	F5	
Organoleptic	Thin sheet Clear transparent The distinctive smell of gelatin Sweet	Thin sheet Clear transparent The distinctive smell of gelatin Sweet	Thin sheet Clear transparent The distinctive smell of gelatin Sweet	Thin sheet Clear transparent The distinctive smell of gelatin Sweet	Thin sheet Clear transparent The distinctive smell of gelatin Sweet	
Water content	19.56%	16.66%	19.51%	16.66%	14.84%	<20%
pH Film	6.85	6.83	6.12	6.4	6.89	5.5 - 7.9
Fold Resistance	191	219	230	241	255	>200 times
Time Destroyed	13.24 seconds	20.34 seconds	27.29 seconds	30.11 seconds	62.92 seconds	≤ 60 seconds
Weight Uniformity (Mean Deviation)	0.93%	1.41%	0.37%	0.84%	2.14%	≤ 5%
Film Thickness	0.1 mm	0.1 mm	0.1 mm	0.1 mm	0.1 mm	≤0.35 mm

Based on the characterization results, all blank oral thin herbal formulas generally met the main physical requirements. F2 was selected as the best formula due to its most balanced physical characteristics, including low water content, appropriate pH, good crease resistance, acceptable weight uniformity, and a relatively fast disintegration time compared to the other formulas.

Table 6. Oral thin herbal formula based on jamu nanoparticles

Material	Formula (%)
Nanoparticle Combination Herbal Medicine (mg)	Eqv 11.4 mg/slice
Gelatin (%)	12
Nipagin (%)	0.18
Sucralose (%)	0.5
Aquadest ad	25 mL

Table 7. Characterization results of oral thin herbal based on nanoparticle jamu

Testing	Results	Condition
Organoleptic	Thin sheet Dark yellowish green Sweet	
Water content	17.84%	<20%
pH Film	5.84	5.5-7.9
Fold Resistance	220	>200 times
Time Destroyed	40.75 seconds	≤ 60 seconds
Weight Uniformity (Mean Deviation)	4.53%	≤ 5%
Film Thickness	0.1 mm	≤0.35 mm

Organoleptic

Organoleptic test results showed that the herbal nanoparticle-based oral thin preparation was thin, dark green-yellowish in color, and had a sweet taste. Organoleptic characteristics are important in the development of herbal oral thins, as a pleasant taste can improve patient compliance (Jacob et al., 2023).

Water Content

Water content testing was conducted to determine the water content in the thin film preparation. The results showed a water content of 17.84%, thus still meeting the requirements of

<20% (Tanjung et al., 2021). Water content that is too low can cause the film to be easily damaged, while water content that is too high can make the surface of the film sticky.(Shin & Han, 2024).

pH Film

The pH value of the herbal oral thin was 5.84. This value indicates that the preparation meets the required pH range of 5.5–7.9.(S et al., 2023). pH compatibility with the physiological conditions of the oral mucosa is important to minimize the risk of irritation and increase comfort of use (Jacob et al., 2023).

Film Thickness

The thickness of the film obtained was 0.1 mm, so it met the maximum thickness requirement of 0.35 mm with a standard deviation below (ADA, 2024). Low film thickness can improve patient comfort and influence the rate of drug release and absorption. Furthermore, consistency of thickness is important to ensure uniformity of content in each dosage unit (Jacob et al., 2023).

Folding Resistance

Fold resistance tests are performed to assess the flexibility of the film and its susceptibility to damage due to folding (Gustana et al., 2025). The test results show a folding resistance value of 220 times, Whichshows that the film has good flexibility and has met the requirements, namely more than 200 folds (Ambarwati et al., 2024).

Weight Uniformity

The results of the weight uniformity test showed a variation of 4.53%, thus fulfilling the requirements, namely ≤ 5% (S et al., 2023). This parameter is used to ensure that each film contains a consistent amount of drug without significant deviations (Gustana et al., 2025).

Time Destroyed

The film disintegration time of 40.75 seconds indicates that the preparation has met the requirements, namely no more than 60 seconds (Gustana et al., 2025). Fast disintegration time is an important characteristic of herbal oral thins because it supports the rapid release of active ingredients in the oral cavity (Jacob et al., 2023).

CONCLUSION

A nanoparticle herbal medicine combination of binahong leaves and bay leaves was successfully formulated using PlantCrystal technology with characteristics that meet nanoparticle parameters. The best formula was obtained in PCX F3 with a particle size of 115.70 nm, PDI 0.54, and zeta potential of −28.18 mV which indicated good system stability. The release test results showed that the nanoparticle herbal medicine formulation provided a higher release of active substances (85.54%) compared to the simplicia powder (48.96%). The formulation was also successfully developed into an oral thin herbal preparation that showed good physical characteristics including organoleptic, pH, water content, weight uniformity, film thickness, folding resistance, and disintegration time that met the requirements.

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