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Formulation and evaluation of thin film nanoemultion from rose flower ethanol extract (rosa hybrida hort.) and antidiabetes mellitus type 2 activity test using in vitro enzyme

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ABSTRACT

Diabetes mellitus is a chronic disease with a rising global prevalence. Natural antidiabetic agents derived from plant bioactive compounds offer a promising therapeutic alternative. Rose flowers (*Rosa hybrida Hort*) contain flavonoids such as kaempferol, known for their antidiabetic activity. However, poor solubility and bioavailability limit their therapeutic potential. This study aimed to develop and characterize a nanoemulsion of rose flower extract to enhance the stability and bioavailability of its active compounds. The extract was obtained by maceration using 96% ethanol and analyzed using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Nanoemulsion formulations were developed using various types and concentrations of nonionic surfactants. Characterization included particle size, polydispersity index (PDI), zeta potential, and morphology. The optimal nanoemulsion showed a particle size <300 nm, PDI <0.5, and zeta potential around ±20 mV, indicating good stability. Transmission Electron Microscopy (TEM) revealed uniformly distributed spherical globules. LC-MS/MS confirmed the presence of kaempferol as the main active compound. The formulated nanoemulsion was successfully incorporated into an oral thin film, exhibiting favorable characteristics such as appropriate particle size, PDI, zeta potential, morphology, and satisfactory evaluation results including organoleptic properties, moisture content, pH, weight uniformity, film thickness, folding endurance, and disintegration time.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic disease with a prevalence that continues to increase globally. In 2021, approximately 537 million adults (20-79 years) were living with diabetes, and this number is expected to increase to 643 million in 2030 and 783 million in 2045 (IDF Diabetes Atlas 10th Edition) (Kendek, Haskas, & Abrar, 2023)(Sriwiyati, Wibisono, Permata, & Nur, 2024). In Indonesia, the prevalence of diabetes based on blood sugar examination increased from 6.9% in 2013 to 8.5% in 2018. DM is characterized by hyperglycemia, where fasting blood glucose levels are >126 mg/dl and plasma glucose 2 hours after TTGO is >200 mg/dl, while prediabetes is characterized by fasting blood glucose levels of 100-125 mg/dl and plasma glucose 2 hours after TTGO 140-199 mg/dl (Salsabila, 2023)(Tjiptaningrum & Ayu, 2021).

Diabetes mellitus (DM) is a chronic disease with a prevalence that continues to increase globally (Arania, Triwahyuni, Esfandiari, & Nugraha, 2021)(Ardiani, Permatasari, & Sugiatmi, 2021). However, its low bioavailability is an obstacle to its therapeutic effectiveness. Kaempferol has limited bioavailability due to its low solubility in water, so it is often used in nanoparticle formulations to increase its bioavailability (Samriani, 2022)(Atiqah, 2017).

Nanotechnology, especially in the form of nanoemulsions, has several advantages, including superior thermodynamic stability, a transparent or translucent appearance, and slower deposition. This condition is influenced by the reduction of the gravitational effect due to the smaller particle mass, accompanied by a significant increase in surface area (Aini, 2021)(Rahmaniyah, 2018). This triggers a stronger repulsive interaction between particles and supports Brownian motion. In addition, nanoemulsions show high solubilization capabilities that can accelerate drug absorption (Hanista et al., 2023)(Alawiyah, 2020). In addition, the incorporation of nanoemulsions into oral thin films (OTFs) is an innovative solution to increase the bioavailability of active compounds in diabetes therapy. OTFs offer advantages in faster and more efficient drug release through the oral mucosa (Athallah et al., 2024). This dosage form is known to be more comfortable and well accepted by patients. Various studies have shown that thin films have advantages such as prolonging the duration of action, reducing the frequency of dosing, and increasing therapeutic efficiency. Thin film technology also offers additional benefits such as minimizing drug side effects and reducing metabolism caused by proteolytic enzyme activity (Ningsih et al., 2024)(Pratiwi et al., 2023). Thus, further research is needed to develop nanotechnology-based formulations to improve the effectiveness of natural-based diabetes therapy such as red roses (DIENILAH, 2022)(Rachmadani, 2022).

RESEARCH METHOD

Tool

The tools used in this study include 12 and 18 mesh sieves, mortar and mortar, oven, analytical balance (Mettler Toledo), chamber, glassware commonly used in laboratories, filter paper, dropper pipettes, spatulas, stopwatches, including glassware (Pyrex®), hot plate (Oxone®), magnetic stirrer (IKA® C-mag HS 10), sonicator probe (Lvymen® System CY-500), Particle Size Analyzer (PSA) (Malvern instruments Ltd), microcentrifuge MPW-55, UV Spectrophotometer (Shimadzu UV-1800), Zetasizer (Zetasizer Nano ZS; Malvern), Fourier Transform Infra-Red (FTIR) (Agilent Technologies Cary 630 FTIR), spray bottle, analytical balance (Mettler Toledo), minispin (Eppendorf, Germany), Transmission Electron Microscopy (TEM, JEOL JEM 1400, Japan), and Toledo pH meter, ELISA, microplate, rotary evaporator for drying extracts, micropipettes, and test tubes.

Material

Red rose flower simplicia (Rosa hybrida Hort) obtained from Parongpong, West Bandung, distilled water, methanol PA (PT. Merapi Utama Pharma), enzymes: α-glucosidase, α-amylase, and surfactants Chremopore and Plantacare® (Evonik Industries AG) or lauryl glucoside.

Material Preparation and Determination

Red roses (Rosa hybrida Hort.) used for the study were obtained from the Parongpong plantation, Lembang. Then determination was carried out in the Jatinangor herbarium, Plant Taxonomy Laboratory, Department of Biology, FMIPA, Padjajaran University.

Making Rose Flower Extract (Rosa hybrida Hort.)

The obtained roses were wet sorted, cleaned and then dried using an oven at a temperature of 50°C. The drying results were then dry sorted and the rose petals were ground. Furthermore, maceration was carried out on 800 grams of rose flower simplicia powder using 20L of 96% ethanol then put into each macerator and the ethanol was replaced every day for 3 consecutive days. The extraction results were then evaporated using a rotary evaporator and a thick extract was obtained.

Phytochemical Screening of Extracts

a) Flavonoid Test, weighed as much as 0.1 grams of extract then added 0.1 mg of Mg powder, 0.4 mL of amyl alcohol and 4 mL of 96% ethanol. Positive results in this test are indicated by the formation of a brick red or purple color.
b) Alkaloid test, weighed as much as 2 grams of extract then added 10 mL of chloroform then filtered. The filtrate results were added with 3 drops of dragendorf reagent. Positive results in this test are indicated by the formation of brick red or orange red.
c) Saponin Test, weighed 0.1 grams of extract, then added 10 mL of distilled water and shaken for 30 seconds. Positive results in this test are indicated by the formation of stable foam.
d) Quinone Test, weighed 0.1 grams of extract, then added 1N NaOH. Positive results in this test are indicated by the formation of a red color.
e) Tannin Test, weigh the extract as much as 0.1 grams then add 2 mL of 1% FeCl. The formation of a blackish green or blue color change indicates positive tannin.
f) Steroid/Triterpenoid Test, the extract was weighed as much as 0.1 grams, then 2 drops of anhydrous acetic acid and 1 drop of concentrated H2SO4 were added. The formation of a green color indicates positive steroids, while the formation of a purple or red color indicates positive triterpenoids (Ningsih et al., 2018).

Nanoemulsion Making

The preparation of nanoemulsion begins by mixing the oil phase in the form of sunflower oil and thick extract then heated on a hotplate at a temperature of 50°C. Then a mixture of the water phase is made by mixing surfactants and co-surfactants which are heated on a hotplate at a temperature of 50°C. Furthermore, the water phase mixture is added to the oil phase and stirred using a magnetic stirrer at a temperature of 50°C with a stirring speed of 750 rpm for 30 minutes (Jafar et al., 2017).

Characterization of Nanoemulsion

Characterization of red rose extract nanoemulsion was carried out using several parameters such as polydisperity index, particle size and zeta potential. Measurements were carried out using a Particle Size Analyzer (Malvern ZSP) at room temperature of 25°C by taking 1 mL of nanoemulsion sample then adding distilled water up to 10 mL, then the sample was inserted into the analysis cuvette.

Thin film formulation

The preparation of Thin Film was carried out using the Solvent Casting method. In the initial stage, gelatin was dissolved in distilled water with a magnetic stirrer. Suclarose and nipagin were dissolved separately in distilled water at a temperature of 60℃. After that, this solution is mixed and stirred together with rose flower extract nanoemulsion (Riswanto et al., 2023). after mixing evenly the mixture is left for 10 minutes to remove air bubbles, the mixture is poured into a flat mold and dried by storing it at room temperature for 2x24 hours and the top of the mold is covered by plastic warp. After drying the film is carefully removed from the mold and then cut into 2x3 cm sizes (Marzuki, 2019).

Characterization of Rose Extract Thin Film Nanoemulsion
Particle Size And Polydispersity Index

Measurements were taken using the Malvern Particle Size Analyzer (PSA). The sample was dissolved in distilled water until it reached a volume of 10 mL and then inserted into a cuvette. The cuvette was then inserted into the PSA to analyze the particle size (Mujiyanti, Surianthy, & Junaidi, 2019).

Zeta Potential

Zeta potential measurements were performed using a Malvern ZSP Zetaizer (UK) Particle Size Analyzer (PSA). The sample was diluted using distilled water until it reached a volume of 10 mL. The sample was then diluted and then inserted into a disposable cuvette covered with a dip cell electrode. The cuvette was then inserted into the PSA to determine the zeta potential value (Prihantini, Zulfa, Prastiwi, & Yulianti, 2020).

TEM Morphology

Morphological observations of the samples were carried out using transmission Electron Microscopy (TEM). The samples were first dissolved in deionized water to a volume of 20 ml and stirred until homogeneous. This homogeneous solution was dropped onto a 400 mesh test grid, then 10 ml of uranyl acetate solution was added. The remaining solution that was not needed was removed using filter paper and the grid was left to dry for 30 minutes. After it was completely dry, the grid was inserted into the TEM to be photographed, so that the morphological structure of the sample could be observed (Pebiansyah & Yuliana, 2021).

Characterization of Thin Film Preparations
Organoleptic

Organoleptic testing is an evaluation method to assess the physical characteristics of thin films including aroma, taste, shape and color (Ode et al., 2021).

Water content (%)

A total of 2 grams of edible film was weighed and placed in a container of known mass. Furthermore, the sample was dried using an oven at a temperature of 105°C for 3 hours. After the drying process, the sample was cooled in a desiccator for 15 minutes, then weighed. This process was repeated until a stable weight difference was obtained, with a tolerance of approximately ±0.2 grams (Ballo, Nge, Rafael, & Bullu, 2022).

pH

The film is placed in a petri dish, then dripped with sufficient aquadest. After that, the pH value is observed and recorded when the pH meter electrode touches the surface of the film. The pH range in thin film preparations is between 6.2 and 7.4, which is in accordance with the natural pH of the oral cavity(Susanti, Endah, Nofriyaldi, Indri, & Adlina, 2024).

Film Thickness

Film thickness is measured using a calibrated digital caliper or digital screw micrometer. Measurements are made at five different points, four at the corners and one in the middle of the film. The number of samples used is at least 5 films and the results obtained are averaged (Arsana, Wiryanta, Wiguna, & Gunawan, 2021).

Fold Resistance

Film flexibility is determined by repeatedly folding the film at the same place at an angle of 180° until the film breaks. A film is said to have good flexibility if it has a folding value of more than 200 times (Ambarwati et al., 2024a).

Uniformity of weight

The uniformity of the film weight was tested by weighing six films of each formulation in one weighing. After that the average weight was calculated to determine the uniformity of the film weight (Ambarwati et al., 2024b).

Time Destroyed

The disintegration time of the film was determined visually in a petri dish containing 10 ml of phosphate buffer pH 6.8 at 37°C, where the container is shaken every 20 seconds. The film disintegration time is the time when the film begins to break or disintegrate (Nurdianti et al., 2021).

RESULTS AND DISCUSSIONS

A thick rose extract of .. grams was obtained from the maceration of 800 grams of red rose flower simplicia. The results of the Phytochemical Screening stated that the rose flower extract contained alkaloids, flavonoids, quinones, tannins, saponins, and steroids/triterpenoids with the results of the phytochemical screening described in.

Table 1. Phytochemistry states that rose flower extract contains alkaloids, flavonoids, quinones, tannins, saponins, and steroids/triterpenoids with phytochemical screening results

Compound	Results	Information
Alkaloid	+	A brick red precipitate is formed
Flavonoid	+	An amyl alcohol layer is formed
Quinone	+	Red color formed
Tannin	+	A white precipitate forms
Saponins	+	A stable foam is formed
Steroids/Triterpenoids	+	Red-purple/green-blue color formed

With the presence of flavonoids in the extract, the extract can be formulated into a nanoemulsion for antidiabetes.

Phytochemical Screening Results of Red Rose Flower Extract (Rosa hybrida Hort.)

Phytochemical screening of red rose flower extract showed the presence of various bioactive compounds, including alkaloids, flavonoids, quinones, tannins, saponins, and steroids/triterpenoids. These results indicate that red rose flower extract has significant pharmacological potential, as supported by several previous studies. Alkaloids were detected by the formation of a brick red precipitate after the addition of Dragendorff's reagent (Khan et al., 2023). Flavonoids were identified by the formation of an amyl alcohol layer. Flavonoids are phenolic compounds that have strong antioxidant activity with the ability to capture free radicals and protect cells from oxidative damage (Patel et al., 2022). Studies have shown that flavonoids from flower extracts have significant anti-inflammatory effects. The formation of a red color indicates the presence of quinones in the extract (Ali et al., 2023). Tannins were detected by the formation of a white precipitate with FeCl3 solution. Tannins are polyphenolic compounds that act as astringents and have antimicrobial and anti-inflammatory properties (Ghosh et al., 2023).

The formation of stable foam confirms the presence of saponins in the extract. Saponins are known for their hemolytic properties and potential as immunomodulators (Kim et al., 2023). Steroids/triterpenoids are confirmed by the formation of red-purple/green-blue colors. Steroids and triterpenoids have various pharmacological activities, including anti-inflammatory and anticancer (Nguyen et al., 2023). These compounds play a role in stabilizing cell membranes and modulating inflammatory pathways. These screening results indicate that red rose flower extract contains secondary metabolites that can contribute to its therapeutic effects. These bioactive compounds can be further developed in pharmaceutical formulations, including in nanoemulsion-based delivery systems.

Characterization Results of Nanoemulsion of Red Rose Flower Extract (Rosa hybrida Hort.)

Table 2. Nanoemulsion formula and characterization results of red rose flower extract nanoemulsion

Code	Formulation					Particle size (nm)	PdI	Zeta Potential (mV)
	Oil Phase		Surfactant Phase		Surfactant Co			
	EXT (%)	SFO (%)	CHRE (%)	PLA (%)	PEG 400 (%)			
F1	0.5	10	6.37	-	10	239.95	0.358	-12.2
F2	0.5	5.5	28,185	-	10	70,585	0.401	-14.6
F3	0.5	1	50	-	10	22.9	0.2125	-3,515
F4	0.5	3.25	39,092	-	10	28,275	0.4685	-6,045
F5	0.5	7.75	17,277	-	10	137.8	0.337	-17.3
F6	0.5	10	-	13.5	10	357.3	0.26	-17.15
F7	0.5	5.5	-	31.75	10	303.6	0.5755	-32.6
F8	0.5	1	-	50	10	84.99	0.322	-41.15
F9	0.5	3.25	-	40,875	10	346.7	0.801	-37.55
F10	0.5	7.75	-	22,625	10	241.65	0.1425	-32

Note: EXT: Red rose flower extract, SFO: Sun Flower Oil, CHRE: Chremophor, PLA: Plantacare

The results of the characterization of red rose extract nanoemulsions in Table 2 show that all formulas have particle sizes below 1000 nm, polydispersity index (PdI) <0.5, and several formulas have a zeta potential of more than ±30 mV. These parameters are the main indicators of nanoemulsion stability, as stated in various previous studies (Jafar et al., 2021).

Particle Size and Polydispersity Index

The particle size of the nanoemulsions obtained ranged from 22.9 nm to 357.3 nm. The formula with the smallest particle size was F3 (22.9 nm), while the formula with the largest particle size was F6 (357.3 nm). Particles with a size of <200 nm generally showed increased bioavailability and system stability, as reported in previous studies (Kawish et al., 2017). Small particle size can increase the contact surface area, accelerate the release of active ingredients, and improve cellular penetration (Jafar et al., 2021).

Zeta Potential

Zeta potential indicates the electrostatic stability of nanoemulsions. Values greater than ±30 mV indicate good colloidal stability due to the repulsive force between particles, which prevents aggregation (Nurdianti et al., 2021). From the table, the formulas that meet this criterion are F7 (-32.6 mV), F8 (-41.15 mV), F9 (-37.55 mV), and F10 (-32 mV). A more negative zeta potential value indicates better colloidal stability due to increased electrostatic repulsive forces between particles (Ambarwati et al., 2024). Formulations with zeta potential values lower than ±30 mV, such as F1 (-12.2 mV) and F4 (-6.045 mV), indicate the possibility of long-term particle aggregation.

Morphology

The resulting morphology is spherical. This is in accordance with previous research related to nanoemulsions producing spherical morphological results (Chaiyana et al., 2020).

Thin Film Evaluation Results

Table 3. Formula thin film nanoemulsion red rose flower extract (rosa hybrida hort.)

Material	Formula (%)	Function
Rose flower extract nanoemulsion	0.79	Active substance
Gelatin	15	Film forming polymer
Nipagin	0.18	Preservative
Sucralose	0.5	Sweetener
Aquades ad	30 ml	Solvent

Table 4. Evaluation results of thin film nanoemulsion preparations of rose flower extract

Parameter	Observation result
Organoleptic	Flexible, thin, brown, slightly sweet, distinctive aroma
Water content (%)	13.33±0.09
pH	6.49±0.40
Uniformity of Weight (mg)	62.94±6.24
Film Thickness (mm)	0.05±0
Fold Resistance	230.78±4.35
Time Destroyed(s)	93.67±7.77

Organoleptic

Thin film preparations of rose flower extract must show appropriate organoleptic qualities such as color, taste and aroma (Hemavathy et al., 2022). The thin film preparation made has flexible properties, has a thin thickness, is brown in color, has a slightly sweet taste, and has a distinctive aroma.

Water content

The water content test is an analysis carried out to measure the amount of water content in a thin film sample (Tanjung et al., 2021). The water content obtained from the thin film of rose flower extract nanoemulsion is 13.33%. This result meets the requirements of SNI 06-3735-1995, namely that the thin film preparation contains a water content of <16%.

pH

The results of the pH test on the thin film preparation were 6.49, which shows that the thin film preparation does not irritate the oral mucosa (Fitri et al., 2025) and in accordance with the pH of the oral cavity, which is 6.2-7.4. The pH value of the Thin film preparation plays an important role in maintaining patient comfort and safety. A pH that is too acidic has the potential to cause caries damage to the teeth, while a pH that is too alkaline can cause a burning sensation or irritation in the oral cavity (Darusman, Ramadhan, Lantika, et al., 2023).

Uniformity of Weight

The result of the weight uniformity test of the thin film preparation is 62.94 mg. Weight uniformity is generally determined to ensure that each film contains a consistent amount of drug without significant deviation. Weight inconsistency may indicate an uneven distribution of the active substance in the thin film which may affect the dose homogeneity and therapeutic effectiveness of the preparation.

Film Thickness

The evaluation results of the thickness of the rose extract nanoemulsion film were 0.05 mm with a low standard deviation. These results have met the requirements, namely a film measuring 2x3 cm^2 has a maximum thickness of 0.35 mm and a standard deviation value below 5% (Darusman et al., 2023). Gelatin as a polymer forming material in film making affects the thickness of the edible film. Increasing the concentration of gelatin in the formulation will result in a greater film thickness (Arsita Dewi & Mulya, 2019).

Folding Resistance

The folding endurance test aims to assess the level of film flexibility, where the value obtained reflects the extent to which the film is susceptible to damage due to folding. The lower the folding endurance value, the more fragile the film is (Darusman et al., 2023). The folding endurance of the rose flower extract nanoemulsion thin film showed a result of 230 folds. These results meet the film flexibility requirements, namely >200 folds (Ambarwati et al., 2024a).

Time Destroyed

Thin film preparations are required to have the ability to dissolve quickly when placed on the surface of the tongue so that the drug can be dissolved and absorbed to provide a working effect (Fitri et al., 2025). the disintegration time produced in the thin film preparation was 93.67 seconds, where the results exceeded the specified requirements, namely less than 60 seconds (Nurdianti et al., 2021c). This can happen because the film thickness affects the disintegration time of the thin film preparation where a small film thickness will have a fast disintegration time and vice versa.

CONCLUSION

The nanoemulsion formula of red rose flower extract can be formulated using sunflower oil lipid, chremofor and plantacare surfactants and PEG 400 co-surfactant. The most stable formula based on the criteria of particle size <100 nm, PdI <0.5, and zeta potential more than ±30 mV is F8, with a particle size of 84.99 nm, PdI 0.0322, and zeta potential -41.15 mV. This formula has the best potential in pharmaceutical applications due to its high stability and homogeneous particle size distribution.

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