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Formulation & Evaluation Rosella Suspension (*Hibiscus sabdariffa* L.) as Immunomodulator with Variation of Carboxymethylcellulose Sodium Concentration

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Abstract

Rosella flower (Hibiscus sabdariffa L.) has proven in previous studies through clinical trials and preclinical trials to be safe as an immunostimulant. Suspension preparations have the advantage of being faster to absorb than solid preparations and are easy to administer to patients who have difficulty swallowing, especially children. The purpose of this study was to determine the stability of suspension preparation by varying the concentration of Carboxymethylcellulose (CMC) Sodium as a suspending agent. The suspension preparations are divided into 4 formulas: Formula 0 (without CMC Sodium), Formula 1 (CMC Sodium 1.5%), Formula 2 (CMC Sodium 1.75%), Formula 3 (CMC Sodium 2%). The results of the organoleptic test showed that the dosage form Fo was liquid, F1 and F2 were slightly thick liquid, and F3 was thick. Density test results in Fo 1.09g/mL, F1, F2, and F3 1.10g/mL. Viscosity test on Fo 20.8 cps, F1 128.6 cps, F2 268.6 cps and F3 475.6 cps. The pH test of all formulas is in the acidic range. Sedimentation test Fo = 1, F1 and F2 = 0.97 and F3 = 0.99 or close to 1. Redispersion time test of all preparations <30 seconds. The optimal formula is based on the evaluation of suspension rosella preparations, namely F1 and F2, with Na CMC concentrations of 1.5% and 1.75%.

Keywords: CMC sodium; Rosella flower (Hibiscus sabdariffa L); Immunomodulator; Suspension

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INTRODUCTION

Indonesia has 20,000 types of medicinal plants, of which 1,000 types have been recorded, and 300 types of medicinal plants have only been utilised as a form of traditional medicine. Medicinal plants are efficacious in healing or preventing disease.[1] The substances that can affect or increase the body's immune system are called immunomodulatory agents.[2] The use of herbal plants as immunomodulatory agents has high interest because people feel safer.[3] Indonesian people generally believe that disease prevention can be done by consuming health-enhancing drinks from

rosella petals because they can increase the body's immune system.[4]

The efficacy of rosella flowers is inseparable from the chemical composition in rosella flowers. The most dominant content of secondary metabolic compounds in red rosella is the presence of anthocyanin compounds that form flavonoids that act as antioxidants. Rosella flavonoids consist of flavonols and anthocyanin pigments.[5] Flavonoids and polyphenols can improve the immune system through an increase in Interleukin-12 (IL-12) and lymphocyte proliferation. IL-12 compounds stimulate the production of IFN-γ by NK cells, while IFN-γ has a role in activating macrophages.[6]

This proves that giving rosella powder in capsule form to healthy volunteers can increase interleukin (IL), which is part of the immune system, especially IL-10, which plays a role in optimising the antibody system.[7] The results of preclinical and clinical trials by giving rosella flower powder at a dose of 500 mg a day for 30 days to healthy volunteers proved to increase the percentage of CD_4 cells, so it is proven that the antioxidant elements in rosella flowers are anthocyanins, quercetin, L-ascorbic acid, and protocatechuic acid which can work as immunostimulants.[7] In previous studies, powder preparations in capsules have been made for rosella flower preparations as immunomodulators.

Therefore, researchers want to make powder preparations in solution or suspension for rosella flower powder preparations as immunomodulators. Suspension preparations have the advantage of being faster to absorb than solid preparations and are easy to administer to patients who have difficulty swallowing, especially children.[8] Suspensions are liquid preparations containing insoluble solid particles dispersed in a liquid phase.[9] The use of suspending agents must be appropriate and cannot interact with drugs or active substances.[10] Carboxymethyl Cellulose Sodium (CMC Sodium) in food preparations, especially pharmaceuticals, is usually used as a suspending agent and stabilising agent.[11] In previous studies, suspension preparations using CMC sodium with variations of 0.5%, 1%, and 1.5% produce suspension preparations that can improve the evaluation results of the viscosity test and density test.[12] So, the author wants to determine the effect of the concentration of CMC sodium on the physical and chemical characteristics of rosella flower suspension preparations as immunomodulatory products by

varying the concentration of CMC sodium at concentrations of 1.5%, 1.75%, and 2%.

METHODS

This experimental research was conducted by making rosella flower suspension preparations (Hibiscus sabdariffa L.) as an immunomodulator, using variations of sodium carboxymethylcellulose (CMC Sodium) as a suspending agent. In this study, 4 formulas were developed: formula 0, which did not contain CMC Sodium; formula 1, which contained 1.5% CMC Sodium; formula 2, which contained 1.75% CMC Sodium; and formula 3, which included 2% CMC Sodium. After making the preparation, the suspension preparation was evaluated with several parameters, involving an organoleptic test (evaluation of sensory properties of the preparation, specifically shape, colour, taste, and odour), pH measurement, density analysis, viscosity testing, sedimentation assessment, redispersion time evaluation and stability testing for 28 days.

Tools

Henherr BL-H2 analytical balance (China), Hanna Instrument pH meter (America), brookfield viscometer, mesh No.40, and other glassware.

Materials

Rosella flower simplisia (*Hibiscus sabdariffa* L.) (Ananta Hidroponik & Florikultura Sidaraja, Kuningan, Jawa Barat), CMC sodium (PT. Dipa Prasada Husada, Tasikmalaya), propylene glycol (PT. Dipa Prasada Husada, Tasikmalaya), methyl paraben (PT. Dipa Prasada Husada, Tasikmalaya), sorbitol (PT. Dipa Prasada Husada, Tasikmalaya) and distilled water (PT. Ikapharmindo Putramas, Jakarta).

Preparation of Powdered Rosella Flower (*Hibiscus sabdariffa L.*)

Rosella flower symplisia as much as 1 kg is pollinated using a blender or other pollinating device until smooth, then sieved using a 40 mesh sieve.[13]

Qualitative Flavonoid Content Test of Rosella Flower (*Hibiscus sabdariffa* L.)

The test of flavonoid content was carried out using the Wiltsatter test. As much as 1 mL of 0.5 g rosella flower powder solution was added to 0.1 g of magnesium powder (Mg) and dripped with 2-4 drops of concentrated HCl, and then the solution was shaken. The formation of an orange colour indicates the presence of flavonoids flavonol and flavone groups.[14]

Formulation of Rosella Flower Suspension (Hibiscus sabdariffa L.)

Table 1. Formulation of Rosella Flower Suspension (*Hibiscus sabdariffa* L.)

Components	Concentrations (%)			
	F0	F1	F2	F3
Roselle flower powder	3,5 g	3,5 g	3,5 g	3,5 g
CMC sodium	-	1.5	1.75	2
Propylene glycol	3	3	3	3
Sorbitol	30	30	30	30
Methyl paraben	0.5	0.5	0.5	0.5
Distilled water	Ad 100 mL	Ad 100 mL	Ad 100 mL	Ad 100 mL

Preparation of Suspension

Initially, dissolve CMC Sodium using hot water (50^{0}C) according to the specified formula (F1 1.5 g, F2 1.75 g, F3 2 g) until mucilage is formed (mixture 1), then put 3.5 g rosella flower powder into the mortar, add propylene glycol 3 g as a wetting agent until homogeneous (mixture 2). Gradually add mixture 1 into the mortar. After that, transfer the combined mixtures into a beaker glass and homogenise by stirring for 20 minutes. Sorbitol as much as 30 ml and 0,5 g of methyl paraben is slowly added into mixture 2 while stirring until homogeneous. Once all components are mixed, then add water until the suspension volume reaches 100 mL. Finally, the suspension is put in a 100 mL bottle, and markers between formulas are provided.[15]

Organoleptic Test

An organoleptic test is a direct examination of the suspension preparation of rosella flowers (Hibiscus sabdariffa L.) by observing changes in shape, colour, taste, and odour.[16]

Density Test

The density test is carried out by measuring with a pycnometer. A clean empty pycnometer was weighed at room temperature (W grams). The pycnometer was filled with water and weighed again (W2 grams). Then, the water is removed, and the pycnometer is cleaned. Then, the pycnometer is filled with the sample and weighed (W3 grams).[17] A good density

which is ≥ 1 g/mL.[16] Using the following formula:[18]

$$\rho = \frac{W3 - W1}{W2 - W1} \text{ x density of water (g/mL)}$$

Description:
ρ = density of suspension
W1 = empty pycnometer weight
W2 = pycnometer weight + distilled water
W3 = pycnometer weight + suspension

Viscosity Test

Suspension preparations before and after accelerated storage were measured for viscosity. Viscosity measurements were carried out using a Brookfield viscometer spindle number 61 with a speed of 6 rpm.[19] The viscosity value of a good suspension is in the range of 38 cPs - 396 cPs.[20]

Sedimentation Test

Rosella flower suspension preparation is put into a measuring cup as much as 100 ml and then stored and kept away from interference for 4 weeks. Measure the initial volume (Vo) before settling and measure the final volume (V) after settling.[21] A good sedimentation degree has a sedimentation degree (F) close to 1 or F = 1.[22] Calculate the sedimentation volume using the formula:

$$F = \frac{V0}{V}$$

Description:
F = degree of sedimentation
Vo = final sedimentation volume
V = initial volume before sedimentation

Redispersion Time Test

The redispersion time test is performed after performing the sedimentation test. The preparation bottle was shaken and then allowed to settle. After that, rotate the bottle 1800 and return it to its original position. Record the time it takes for the suspension to disperse in its original form.[23] A good redispersion time is no more than 30 seconds.[24]

Chemical Evaluation of Suspensions

The pH measurement test is carried out using a calibrated pH meter. The suspension is put into a beaker glass, and then the pH meter spindle is inserted into the suspension. The pH value of the suspension can be seen in the number indicated on the pH meter-monitor.[19] The pH requirement for suspension preparations is 4-7.[20]

Stability Test of Suspensions

The stability test was conducted in an accelerated stability test by conducting storage for one month on days 0, 7, 14, 21, and 28, with a storage temperature of 25°-30°C or room temperature.[25]

RESULTS AND DISCUSSION

The research was conducted by Azizah Syahrana & Darmawan to determine the effect of rosella flower powder capsules on interleukin 10 (IL-10) on 21 healthy volunteers. Interleukins (IL) are part of the immune system called cytokines that activate the immune system. IL-10 is an anti-inflammatory cytokine, functioning to inhibit the production of several other types of cytokines (TNF-α, IL-1, IFN-γ, chemokines, and IL-12) and inhibits macrophage function in assisting T cell activation. Elevated IL-10 is known to be an important mediator in defense responses. IL-10 activity occurs because it inhibits TNF-α production and influences B cells to produce antibodies.[26]

This research was also reinforced by research preclinical and clinical trials, such as giving rosella flower powder at a dose of 500mg/day for 30 days to healthy volunteers, which has been proven to increase the percentage of CD4 cells. CD4 is a marker on the surface of human and animal white blood cells, especially lymphocytes. CD4 cells have a role in the regulation of immune system maintenance and control various immune responses by preventing or limiting immune responses called regulatory T cells (TR). An immunostimulator can activate the immune system in various ways, such as increasing the number of T-cell, NK-cell, and macrophage activities and releasing interferons and interleukins. The increase in CD4 cell percentage in this study can be triggered by the content of phenol and flavonoid compounds in Rosella flower powder, which act as antigens or mitogens.[7]

Flavonoids and polyphenols can increase the immune system through an increase in Interleukin-12 (IL-12) and lymphocyte proliferation. IL-12 compounds stimulate the production of IFN-γ by NK cells, while IFN-γ has a role in activating macrophages.[6]

The identification of flavonoid content qualitatively in rosella flower powder (*Hibicus sabdariffa* L.) was carried out at the Chemistry Laboratory of STIKes Muhammadiyah Kuningan. The test results can be seen in Table 2, which shows that rosella flower powder is detected to

contain secondary metabolites in the form of flavonoids.

Table 2. Results of Flavonoid Identification

Parameter	Result	Description
Flavonoid	(+)	Resulting in orange-yellow color

The flavonoid content test was carried out by dissolving 2 mL of 0,5 g rosella flower powder in 95% alcohol, followed by the addition of 0.1 g of magnesium powder, then added 10 drops of concentrated HCl, shaken gently until a colour change of orange-red to purple red (positive flavonoids) or orange-yellow (flavones, chalcones, aurones).[18] The resulting color change of the preparation with solvent or blank from pale yellow to orange after adding other reagents. Research on flavonoid levels in rosella flower powder has not been conducted, but there are flavonoid levels in rosella flower extracts using ethanol solvents, which produce total flavonoid levels of 3.46 mg QE/g extract.[27]

The identification of flavonoid content in rosella flowers aims to prove that in the rosella flower powder suspension preparation, there are flavonoids that can affect the immune system or act as immunomodulators.[28]

The results of the organoleptic test can be seen in Table 3, showing that there is no change in shape, odour, taste, or colour in the suspension preparation stored for 28 days at room temperature (25° - 30° C).

Table 3. Results of Organoleptic Test

Tests	Time	Formulation			
		F0	F1	F2	F3
Shape	Day 0	Liquid	Liquid slightly thick	Liquid slightly thick	Thick
	Day 7	Liquid	Liquid slightly thick	Liquid slightly thick	Thick
	Day 14	Liquid	Liquid slightly thick	Liquid slightly thick	Thick
	Day 21	Liquid	Liquid slightly thick	Liquid slightly thick	Thick
	Day 28	Liquid	Liquid slightly thick	Liquid slightly thick	Thick
Odour	Day 0	Typical rosella flower	Typical rosella flower	Typical rosella flower	Typical rosella flower
	Day 7	Typical rosella flower	Typical rosella flower	Typical rosella flower	Typical rosella flower
	Day 14	Typical rosella flower	Typical rosella flower	Typical rosella flower	Typical rosella flower
	Day 21	Typical rosella flower	Typical rosella flower	Typical rosella flower	Typical rosella flower
	Day 28	Typical rosella flower	Typical rosella flower	Typical rosella flower	Typical rosella flower
Taste	Day 0	Slightly sweet and sour	Slightly sweet and sour	Slightly sweet and sour	Slightly sweet and sour
	Day 7	Slightly sweet and sour	Slightly sweet and sour	Slightly sweet and sour	Slightly sweet and sour
	Day 14	Slightly sweet and sour	Slightly sweet and sour	Slightly sweet and sour	Slightly sweet and sour
	Day 21	Slightly sweet and sour	Slightly sweet and sour	Slightly sweet and sour	Slightly sweet and sour
	Day 28	Slightly sweet and sour	Slightly sweet and sour	Slightly sweet and sour	Slightly sweet and sour
Colour	Day 0	Blackish brown	Blackish brown	Blackish brown	Blackish brown
	Day 7	Blackish brown	Blackish brown	Blackish brown	Blackish brown
	Day 14	Blackish brown	Blackish brown	Blackish brown	Blackish brown

	Day 21	Blackish brown	Blackish brown	Blackish brown	Blackish brown
	Day 28	Blackish brown	Blackish brown	Blackish brown	Blackish brown

The results of organoleptic observations on the four samples showed no changes before and after storage. It can be seen from the shape of the preparation that it has not changed. The colour remains the same, and the distinctive smell of rosella flowers and a slightly sweet and sour taste are parameters that ensure that the suspension preparation is stable before and after storage for 28 days.[29]

Organoleptic testing of rosella flower suspension preparations (*Hibicus sabdariffa* L.) shows no effect on colour, odour, and taste from increasing the concentration of CMC Sodium which is a white or almost white powder that has no odour and no taste.[26]

The results of the density test can be seen in Table 4, which shows that the average density of the F0 preparation is 1.09 g/mL, and the average density of F1, F2, and F3 is 1.10 g/mL in storage for 28 days at room temperature (25^0 - 30^0 C).

Table 4. Results of Density Test

Time	**Formulation (g/mL)**				**Standard**
	F0	**F1**	**F2**	**F3**	
Day 0	1.07	1.10	1.10	1.10	≥ 1 g/mL
Day 7	1.09	1.10	1.09	1.11	
Day 14	1.09	1.10	1.10	1.11	
Day 21	1.10	1.11	1.11	1.10	
Day 28	1.10	1.10	1.11	1.10	
Average	1.09	1.10	1.10	1.10	

The purpose of the density test is to determine the density of the suspension preparation and to determine the viscosity value of the preparation because density is one of the factors that can affect the viscosity of the suspension preparation.[17] When the viscosity of the suspension increases, the settling speed will decrease so that the precipitate will form more slowly, but the pourability will decrease, thus disturbing consumer convenience.[15] The density value is directly proportional to the viscosity according to the formula V = k x d x t. If the density is higher, the viscosity value of a preparation is larger.[6] The amount of water also influences the density of the preparation; the less water is given, the solubility of the preparation will decrease, which increases the density of the preparation.[6] The density of suspension preparations with water as a solvent must have a specific gravity ≥ 1 g/mL because water has a density of 1 g/mL. The results of the density test show that Fo, F1, F2 and F3 have complied with the standard of a good density suspension preparation, which is ≥ 1 g/mL [32].[30]

The results of the viscosity weight test can be seen in Table 5, showing the viscosity value of rosella flower suspension preparations with Fo and F3, which do not meet the suspension preparation standards, while F1 and F2 comply with the viscosity standards of suspension preparations. The preparation was stored for 28 days at room temperature (25° - 30°C).

Table 5. Results of the Viscosity Test

Time	Formulation (cPs)				Standard
	Fo	F1	F2	F3	
Day 0	33	1.10	343	602	38 - 396 cPs
Day 7	163	143	317	570	
Day 14	21	129	295	447	
Day 21	12.99	111	207	417.9	
Day 28	9	96.9	181	341	
Average	20.8	128.6	268.6	475.6	

The viscosity value of a good suspension is in the range of 38 cPs - 396 cPs.[15] The research states that increasing the concentration of Na CMC (0.5%, 1%, and 1.5%) has an effect on increasing the viscosity value, but the results of the viscosity test evaluation at these concentrations still comply with the standard.[20]

Viscosity is influenced by the suspending agent. The larger the concentration of the suspending agent, the higher the viscosity value of the suspension preparation.[31] The difference in concentration of CMC Sodium in the formulation of rosella flower suspension produces different viscosity values in each preparation. The higher the concentration of CMC Sodium that is added, the greater the viscosity value. Therefore, the addition of CMC Sodium needs to be considered in order to produce a good viscosity value and meet the specified standards.[32]

The viscosity of the suspension affects the stability and pourability of the preparation. As the viscosity of the suspension increases, the settling speed decreases, leading to slower formation of precipitation. However, this increased viscosity also results in reduced pourability, thus negatively impacting consumer convenience.[15]

The results of the sedimentation test can be seen in Table 6, showing the average degree of sedimentation (F) in preparations F0, F1, F2, and F3, namely 1, 0.97, 0.97, and 0.99 in storage for 28 days in room temperature (25° - 30° C).

Table 6. Results of the Sedimentation Test

Time	Formulation				Standard
	F0	F1	F2	F3	
Day 0	1	1	1	1	F = 1 or close to 1
Day 7	1	0.98	0.99	1	
Day 14	1	0.97	0.97	0.99	
Day 21	1	0.96	0.96	0.98	
Day 28	1	0.94	0.95	0.97	
Average	1	0.97	0.97	0.99	

The sedimentation test is carried out to determine the ratio of precipitation that occurs during the storage period.[33] A good sedimentation degree has a sedimentation degree (F) close to 1 or F = 1.[34] Suspensions with a low degree of sedimentation will form a deflocculation system, which is a system that forms solid deposits at the bottom of the container (cake). These deposits are difficult to redisperse. A good degree of sedimentation forms a flocculation system that settles faster so that there is a separation between sediment and supranatant.[24]

In the rosella flower suspension preparation, Fo has a value of F = 1 because Fo does not have CMC Sodium content in the formula. CMC Sodium is hydrophilic, which will absorb water, which causes swelling. Water that previously moved freely outside the granule cannot move freely anymore as a result of an increase in viscosity [31]. In rosella flower suspension preparations containing CMC Sodium in the formula, F3 with the highest concentration of CMC Sodium has a sedimentation degree close to 0 (specifically 0.99) and also demonstrates the highest viscosity value. The relationship indicates that higher viscosity corresponds to an F value of 1.[24]

The results of the redispersion time test can be seen in Table 7. The redispersion time test is conducted after performing the sedimentation test, which shows the redispersion time of preparation Fo 5 seconds, F1 11,8 seconds, F2 13,6 seconds, and F3 17,8 seconds. This indicates that the redispersion time of the rosella flower suspension preparation is in accordance with the requirements for the redispersion time of the suspension preparation, which is < 30 seconds.[24] The preparation was stored for 28 days at room temperature (25^0 - 30^0 C).

Table 7. Results of the Redispersion Time Test

Formulation	Time (Day 28)	Standard
F0	5 seconds	< 30 seconds
F1	11,8 seconds	
F2	13,6 seconds	
F3	17,8 seconds	

The redispersion test is carried out to determine the sedimentation time during the storage period so that it can be redispersed if precipitation occurs during the storage period.[25] The ability of the suspension to keep the active substance evenly distributed is measured by the ability of the suspension to redisperse the settled suspension.[22] A good redispersion time is no more than 30 seconds.[24] The results of the redispersion test time for rosella flower suspension preparations in F0, F1, F2, and F3 resulted in a time < 30 seconds, indicating that the redispersion results of the suspension preparation were good.

The redispersibility of the suspension preparation is influenced by the viscosity of the preparation. The higher the viscosity value of the preparation, the lower the redispersibility of the preparation.[11] The redispersibility of the preparation is also influenced by the particles formed by the system in the suspension preparation. If the suspension preparation system occurs cacking, it will not be easy to redisperse. If the system forms flocs, the preparation will be easily dispersed again homogeneously.[16]

Table 8. Results of pH Test

Time	Formulation				Standard
	F0	F1	F2	F3	
Day 0	2.92	3.09	3.18	3.26	4 – 7
Day 7	2.91	2.97	3.10	3.00	
Day 14	2.44	2.82	3.07	3.00	
Day 21	2.39	2.87	3.07	2.91	
Day 28	2.28	2.73	2.94	2.97	
Average	2.6	2.9	3.08	3.03	

The pH of the suspension preparation of rosella flowers in F0, F1, F2, and F3 is acidic, so it does not meet the requirements of the suspension preparation. This is because rosella petals have high amounts of vitamin C and contain sukisnat acid and oxalic acid, which are the dominant organic acids in rosella flowers. Rosella petals also have a higher vitamin C content compared to oranges and mangoes.[35] Based on a journal review, vitamin C has immunomodulatory activity with mechanisms of action as immunostimulants and immunosuppressants.[19] Rosella flower suspension preparations are acidic because rosella flowers contain vitamin C, which is a water-soluble vitamin, so they do not have a specific time to consume vitamin C. However, vitamin C can increase the acidity of the stomach, causing stomach pain. Therefore, the right time to consume vitamin C should be after eating.[36]

To overcome the acidic pH of the preparation, an exposing agent can be added to the dosage formulation to stabilise the pH of the preparation in rosella flower suspension. The exposure agent that can be used is a combination of citric acid and sodium citrate because it can stabilise the pH of the preparation within the range of 4 – 5.[37]

CONCLUSIONS

The effect of adding various concentrations of CMC Sodium to the rosella flower suspension preparation

(*Hibicus sabdariffa* L.) has an influence on the evaluation of the suspension preparation. The higher the addition of CMC Sodium concentration, it will affect the evaluation results in the organoleptic test, specific gravity test, viscosity test, sedimentation test, and redispersion time test of rosella flower suspension preparation *(Hibicus sabdariff*a L.). The stability test shows a good formula based on the evaluation of suspension preparations, namely F1 and F2, with CMC Sodium concentrations of 1.5% and 1.75%.

CONFLICT OF INTEREST

Rosella flower (Hibicus sabdariffa L.) preparations need to continue to be developed with a variety of innovations, not only in capsule and suspension dosage forms. In the preparation of rosella flower suspension preparations (Hibicus sabdariffa L.), the next dosage formulation needs to add a dispersant to cover the acid from rosella flowers in order to achieve pH stability in the suspension preparation, as well as the need to make preparations based on the number of evaluations carried out on each formula so as not to affect the evaluation results of one preparation with another in order to achieve the expected results.

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