

FORMULATION AND IMMUNOMODULATORY BIOACTIVITY TEST OF NANOPARTICLE SYRUP OF ETHANOL EXTRACT OF SUNKAI LEAVES (*Peronema canescens* Jack)

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ABSTRACT

Sungkai plants are beneficial immunomodulators because of the presence of flavanoid compounds that increase oxygen radicals and antibody production. Pharmaceutical preparations function as immunomodulators, typically in the form of syrups, tablets, capsules, suspensions, and emulsions. Until now, there has been no nanoparticle syrup on the market, even though nanoparticle preparations have the advantage of modifying particle size, controlling the release of active ingredients to reach the active side, protecting drugs from degradation, reducing toxicity, and minimizing side effects. The purpose of this study was to determine whether the syrup of nanoparticles in the ethanol extract of Sungkai leaves acts as an immunomodulator and the stability of the preparation during storage. The methods used were experimental in the laboratory with extraction using the maceration method. The extract obtained was formulated into a nanoparticle solution using the ionic gelation method, then formulated into FO syrup (Base syrup) and F1 syrup (Nanoparticles of ethanol extract of sungkai leaves) and then carried out transmittance tests, particle size analyzer tests, immunomodulatory tests and stability tests. The yield of the extract was 17.5%. The raw material examination of all the materials was in accordance with the literature. The particle size of the nanoparticle solution was 40 nm, that of F0 syrup was 670 nm, and that of F1 syrup was 964 nm. All syrup preparations fell into the nanoparticle size category of 10–1,000 nm. The leukocyte count of the negative control treatment (syrup base) was 5,011 cells/mm³, the positive control (stimuno suspension) was 6,105 cells/mm³, and that of the nanoparticle syrup was 8,166 cells/mm³. The stability test of The preparation was stable during storage. It can be concluded that the sungkai leaf ethanol extract nanoparticle syrup has the best immunomodulatory activity and is stable during storage.

Keywords: Extract, Immunomodulator, Nanoparticle Syrup, Sungkai Plant.

INTRODUCTION

In recent years, coronavirus disease (COVID-19) has become a global health problem; therefore, people are consuming supplements and herbal medicines to boost the immune system (Sholihah and Joko Santoso, 2021). Based on several researches which have been conducted, natural ingredients that have activity as immunomodulators are tempuyung leaves (Sukmayadi *et al.*, 2014), meniran (Aldi, Ogiana and Handayani, 2018), waru leaves (Sihombing and Octora, 2019) and sungkai leaves (*Peronema canescens* Jack) (Rahman *et al.*, 2021). The sungkai plant (*Peronema canescens* Jack) is a typical Jambi plant (Rahman *et al.*, 2021). Some of these plants are known to contain secondary metabolites that act as immunomodulators in test animals.

Sungkai plants contain secondary metabolites, such as alkaloids, phenolics, flavonoids, steroids, tannins, terpenoids, and saponins (Latief, Tarigan, *et al.*, 2021; Rahman *et al.*, 2021). Sungkai plants are empirically used as antipyretic, antimalarial, antibacterial, antiplasmodium, antihyperuricemic, antidiabetic, and immunomodulatory treatments (Latief, Sari, *et al.*, 2021;

Latief, Tarigan, *et al.*, 2021; Rahman *et al.*, 2021). Sungkai plants can be useful as immunomodulators because they contain flavanoid compounds that work by increasing oxygen and nitrogen radicals and antibody production by downregulating inhibitory receptors and enhancing activating receptors (Rahman *et al.*, 2021). A Sungkai leaf decoction at a concentration of 20% was used to boost endurance (Rahman *et al.*, 2021). Sungkai leaf extract at a concentration of 0.567 mg/KgBB can increase the number of leukocytes in the blood, which increases immunity (Putranto, 2014). boost immunity is commonly used from boiled water or has been formulated into pharmaceutical preparations.

Pharmaceutical preparations that function as immunomodulators are usually in the form of syrups, tablets, capsules, suspensions, and emulsions (Food and Drug Administration of the Republic of Indonesia, 2020); however, syrup preparations are usually used by children and adults of all ages. Until recently, there was no nanoparticle syrup on the market, although nanoparticle preparations have the advantage of modifying particle size, controlling the release of active ingredients to reach the active side, protecting drugs from degradation, reducing toxicity, and minimizing side effects (A. Yuwanda, Rahmawati and Farmasita, 2021). Previous research conducted by Sari *et al.* (2021) formulated syrup from lime peel, which was proven to have activity as an immunomodulator (increasing endurance). Ariani *et al.*, (2013) has attempted to make a nanoparticle syrup preparation from rosela flowers but it has not formed a nanoparticle preparation but a microparticle syrup preparation.

Based on the literature, researchers are interested in conducting research on the formulation and bioactivity test of immunomodulatory syrup preparation of nanoparticles of ethanol extract of sungkai leaves (*Peronema canescens* Jack).

The purpose of this study was to prepare a nanoparticle syrup preparation from ethanol extracts of sungkai leaves with chitosan and NaTPP, and to determine whether the nanoparticle syrup preparation of ethanol extract of sungkai leaves has bioactivity as an immunomodulator.

METHOD

The research method used was experimental, which began with the collection of materials, making simplisia, extraction, phytochemical screening test, formulation of extract nanoparticle solution, PSA test of extract nanoparticles, formulation of syrup from nanoparticles of ethanol extract of Sungkai leaves, PSA test of Sungkai leaf ethanol extract nanoparticle syrup, immunomodulatory activity test, and preparation stability test.

Tools and Materials

The tools used were vacuum rotary evaporator (*Buchi*®), particle size analyzer (*Beckman Coulter*), magnetic stirrer (C- MAG HS 7), haemocytometer (*Improved Neubauer*®), Viscometer Ostwald (*Iwaki*®), pH meter (*Hanna*®), analytical scales, maceration bottle, waterbath (6 Hole Electric®), sonicator and glassware found in the laboratory.

The materials used were ethanol extract of sungkai leaves (*Peronema canescens* Jack), 70% ethanol, NaTPP (*Arrow Fine Chemicals*), distilled water, chitosan (*Sigma Aldrich*), tween 80 0.5%, nipagin (*Ueno Fine Chemicals Industry*), sucrose (*Jawamanis Rafinasi*), propylene glycol (*Dow Chemical Pacific*), nipasol (*Rasula Pharmaceuticals*), Na-CMC 0.5%, glacial acetic acid, alcohol, turk solution, physiological NaCl 0.9%, buffer solution (*Hanna*®) FeCl₃ 1%, HCL pa, Mg and wagner reagent, stimuno suspension solution and mice (*swiss webster*) test animals.

Research Procedures

Sample Collection and Determination

Sungkai plants were obtained from Tebo Regency, Jambi Province, and then analyzed at the UNPAD Plant Taxonomy Laboratory to ensure the accuracy of the plants used.

Extract Preparation

The samples used were the sungkai leaves. Sungkai leaves were thoroughly washed using running water, weighed as much as 1.5 kg of sungkai leaves and then chopped. Clean leaves were then dried. The dry symplisia obtained was soaked (macerated) in 70% ethanol in a black bottle until all of it had been soaked in the solvent for 3×24 hours then the soak was stirred every day. Afterwards, it was filtered to obtain the filtrate, which was evaporated with the solvent using a rotary vacuum evaporator at a temperature of 50 °C.

The viscous extract of sungkai leaves was calculated using the following formula (Depkes, 2008; Maulida *et al.*, 2020):

$$\text{Percentage of Extract Yield} = \frac{\text{Weight of extract obtained}}{\text{Weight of simplisia}} \times 100\%$$

Inspection of Raw Materials

The examination of the raw materials was carried out using organoleptic and solubility tests. The results of the examination were compared with the literature in the Indonesian Pharmacopoeia edition V standard book and Handbook of Pharmaceutical Excipients.

Phytochemical Screening Tests

1. Flavonoid Test

A total of 200 mg of extract was added to ethanol and heated in a test tube. Then transferred to a drip plate added 0.2 g Mg powder and concentrated HCl were added to a drip plate. The formation of a red or orange color indicated that the sample contained flavonoids. (Harborne, 1996; Maharani, Mukaromah and Farabi, 2014). (Maharani, Mukaromah and Farabi, 2014)

2. Alkaloid Test

A 10 mg sample of extract was added to 10 ml of 2% HCL, heated for 2 minutes while stirring, and then filtered. The filtrate was divided into two parts, each of which reacted with Mayer and Dragendrof reagents. The presence of alkaloid compounds was indicated by the formation of white and orange precipitates. (Harborne, 1996; Maharani, Mukaromah and Farabi, 2014).

3. Tannin Test

A 0.5 gram sample of the extract was placed in a test tube heated with 20 ml of distilled water. After filtration, a few drops of 0.1% FeCl₃ were added until a color change occurred. Positive results if the appearance of a green color (Harborne, 1996; Maharani, Mukaromah, and Farabi, 2014).

4. Saponin Test

A 0.5 gram sample of the extract was added to 5 ml of distilled water and shaken vigorously. In the sample tube, the formation of froth as high as 1 cm is positive for saponins (Harborne, 1996; Maharani, Mukaromah, and Farabi, 2014).

Preparation of Nanoparticles of Ethanol Extract of Sungkai Leaf (*Peronema canescens* Jack) by Ionic Gelation Method

Table I. Nanoparticle Formula of Sungkai Leaf Ethanol Extract (*Peronema canescens* Jack)

No	Materials	Formulas	
		F1	F2
1.	Extract (g)	1,5	1
2.	Ethanol (mL)	-	70
3.	Water (mL)	-	30
4.	Chitosan 0.1 % (mL)	36	100
5.	NaTPP 0,2 % (mL)	18	100
6.	Tween 80 0,5% (mL)	6	36

*The finest Sungkai leaf ethanol extract nanoparticle formula was then formulated into a nanoparticle syrup preparation.

Preparation of sungkai leaf ethanol extract nanoparticles, in which sungkai leaf ethanol extract (formula of 2 extracts dissolved in ethanol and water using a magnetic stirrer at 1500 rpm for 1 hour and sonicated for 2 hours at 45 °C), was mixed with chitosan solution (concentration 0.1%) dropwise using a magnetic stirrer at 1500 rpm for 2 hours. NaTPP solution (concentration 0.2%) was added dropwise using a magnetic stirrer at 1500 rpm for 2 hours then tween 80 (concentration 0.5%) was added dropwise using a magnetic stirrer at 1500 rpm for 2 hours until a nanoparticle solution was formed, which was then sonicated for 8 hours at 45 °C. (Ariani *et al.*, 2013; Samudra *et al.*, 2021).

Preparation of Sungkai Leaf Ethanol Extract Nanoparticle Syrup

Table II. Formula Design of Sungkai Leaf Ethanol Extract Nanoparticle Syrup @ 100 mL (*Peronema canescens* Jack)

No	Materials	Formulas	
		F0	F1
1.	Extract nanoparticle solution (mL)	-	29,56
2.	Sucrose (g)	10	10
3.	Propylene glycol (g)	1	1
4.	Nipagin (g)	0,2	0,2
5.	Nipasol (g)	0,1	0,1
6.	Aquadest ad (mL)	100	100

Preparation of sungkai leaf ethanol extract nanoparticle syrup, which was used to prepare a solution of sungkai leaf ethanol extract nanoparticles (solution 1). Sucrose was dissolved in hot water until it dissolved (solution 2). Nipagin and nipasol were dissolved in propylene glycol and water (Solution 3). Solutions 1 and 2 were mixed and stirred using a magnetic stirrer at 1500 rpm for 60 minutes until homogeneous, and solution 3 was added and stirred using a magnetic stirrer at 1500 rpm for 60 minutes. The mixture was stirred using a magnetic stirrer and heated to 700 mL in a beaker glass (Ariani, Syukur, and Mighfar, 2013). The syrup was prepared in a total volume of 700 mL per formula.

Transmittance Test

The percent transmittance was measured to determine the clarity of the syrup preparation; in this measurement, the sample was placed in a cuvette. The solution was measured for percent transmittance using a UV-Vis spectrophotometer and was used as a blank during testing (Rahmadevi, Hartesi, and Wulandari, 2020).

Particle Size Analyzer Test

This test was carried out by sending the samples to the Padjajaran University Central Lab. By submitting a sample of 25 ml. A particle size analyzer (PSA) was used for this analysis. The basic principle of this tool involves shooting the sample with a laser beam and scattering the light. Light scattering was detected at certain angles. The droplet measurement results were expressed as the diameter of the droplet contained in the dispersion medium. (Rahmadevi, Hartesi and Wulandari, 2020).

Immunomodulatory Activity Test

1. Animal Test

The test animals used in this study were 27 male *Swiss Webster* mice with a body weight of 20-30 grams.

2. The Treatment of Mice with the Carbon Clearance Method

Table III. Mice Behavior

No	Mice	Treatment
1.	Group 1	Control - (Formula 0)
2.	Group 2	Control + (Stimuno suspension)
3.	Group 3	Given a syrup solution of extract nanoparticles

Each group was given treatment, namely group 1 was given formula 0 syrup preparation, group 2 was given stimuno preparation, group 3 was given syrup preparation from nanoparticles of ethanol extract of sungkai leaves orally once a day for 7 days, on the 8th day an immunomodulatory test was carried out using the carbon clearance method. The tail of the mice was moistened with ethanol using cotton so that the blood vessels of the tails of the mice were dilated, followed by an intravenous injection of 0.2 mL / 20 gBB of Chinese ink suspension in the tail of the mice. then 15 minutes after the injection, the tip of the tail was cut and the blood was collected in a drip plate. (Putranto, 2014; H. Rahman *et al.*, 2016).

3. Calculating Total Leukocyte Cell Count

The total number of leukocytes was calculated using an Improved Neubauer type hemocytometer by means of heparin-treated blood sucked with a leukocyte pipette until the number 0.5 and adding Turk solution until the number 11, shaking for 3 minutes, putting into the counting chamber of the hemocytometer, letting it stand for 2 minutes, observing under a microscope, and counting the number of leukocytes. (H. Rahman *et al.*, 2016; Rezi *et al.*, 2021).

Syrup Stability Test

The stability test of the sungkai leaf extract nanoparticle syrup was carried out for 28 days at three different temperatures (4 °C, room temperature, and 40 °C), and each test was observed at (H0, H7, H14, H21, and H28) which include:

1. Organoleptical Test

Organoleptic tests were carried out by observing the color, odor, and clarity three times. A good syrup has a distinctive taste, odor, color of the extract used, and clarity (Ermawati and Wahdaniah, 2021).

2. Homogeneity Test

A homogeneity test was carried out on 50 ml of the syrup preparation in a container. The containers were cornered and observed for homogeneity. The test was replicated three times, and a good syrup was stable, homogeneous, non-turbid, and free from contamination and microbial growth (Hidayati Styawan and Khotimah, 2020).

3. pH Test

pH testing is an important parameter because the stable pH value of the preparation indicates an even distribution of the basic ingredients in the preparation. pH testing was performed using a pH stick or pH meter. 2 milliliters of each sample were placed into a test tube. Furthermore, the pH meter was calibrated first with a pH 4 and pH 7 buffer solution and then dipped the pH stick in a test tube containing the syrup preparation. A good pH for syrup preparations is between 4-7 (Wijayanty *et al.* 2015).

4. Specific gravity measurement

Specific gravity measurements were performed to determine the specific gravity of syrup. The specific gravity of a good syrup is >1.3 g/mL. Specific gravity measurements were performed by weighing a clean and dry empty pycnometer. Distilled water was placed into the pycnometer and weighed, followed by a cleaned and dried pycnometer. Syrup is put into a pycnometer, then weighed to determine the volume of the pycnometer. The specific gravity of the syrup can be measured using the following calculation (Firmansyah and Sandistira, 2021):

$$\text{Specific gravity } (\rho) = \frac{A2-A}{A1-A} \times \text{Water specific gravity (1 g/mL)}$$

Notes :

ρ = specific gravity (g/mL)

A = empty pycnometer

A1 = pycnometer containing water

A2 = pycnometer containing sample

5. Pour-Time Test

The pour-time test was performed by pouring the syrup at a 45 °tilt. The test was conducted three times with the time required to reach a certain volume recorded. Standard values for syrup pouring time are 2-3 seconds ([Hidayati, Styawan and Khotimah, 2020](#))

6. Viscosity Test

The viscosity measurements were performed using an Ostwald viscometer. A Sstwald viscometer was used because the samples to be tested are not viscous or dilute and are included in Newton's law. A clean and dry Ostwald viscometer was used, and the sample was pipetted into a viscometer with a pipette. The liquid was then suctioned using a pushball until it passed the two limits. Prepare a stopwatch, loosen the liquid to the first limit, and then begin the calculation. ([Ambari, 2019; Firmansyah and Sandistira, 2021](#)).

The viscosity of the test preparation was calculated using the following formula ([Firmansyah and Sandistira, 2021](#)) :

$$\text{Viscosity } \frac{\eta_2}{\eta_1} = \frac{t_2 \rho_2}{t_1 \rho_1}$$

Notes :

η_1 = viscosity of comparison liquid

η_2 = viscosity of testing sample liquid

ρ_1 = sample specific gravity

ρ_2 = water specific gravity: 0.89 cP

t_1 = sample flow time

t_2 = water flow time

Data Analysis

The data were obtained from the calculation of the number of leukocytes processed by statistical analysis using SPSS, namely, by using one-way ANOVA. Based on the number of leukocytes obtained, normality and homogeneity tests were carried out with a sig value > 0.05, indicating that the data were normally distributed. Then, an ANOVA test was performed, which is useful for testing differences in the average data of more than 2 groups with the condition that the sig value. > 0.05 if the sig value. <0.05, then proceed with further tests, namely the Duncan test, to determine which group data were different on average, and the preparation stability evaluation data were analyzed descriptively.

RESULTS AND DISCUSSION

1. Plant Determination

Determination of sungkai plants was performed at the Plant Taxonomy Laboratory of Padjajaran University. The results confirmed that the plants used in this study were *Peronema canescens* Jack species.

2. Extract preparation

The extraction was performed using the maceration method. Maceration results were obtained in the form of a thick greenish dark brown extract with a distinctive odor and a yield of 17.5%.

Table IV. Extract Preparation

Fresh leaves	Simplisia	Condensed extract	Extract yield
1.500 g	595 g	104,3 g	17,5 %

3. Raw Material Inspection

The raw materials used for nanoparticle syrup preparation were examined, including organoleptics and solubility. Examination of raw materials based on the Indonesian Pharmacopoeia and Handbook of Pharmaceutical Excipients.

4. Phytochemical Screening Test

The results of phytochemical screening aimed to determine the secondary metabolites present in the ethanol extract of sungkai leaves.

Table V. Phytochemical Screening Results

No	Secondary metabolites	Results	Description
1.	Alkaloid (Dragendrof)	(+)	Orange-colored precipitate
2.	Alkaloid (Mayer)	(+)	White-colored precipitate
3.	Flavonoid	(+)	Orange-colored
4.	Saponin	(+)	Forms a stable foam >1 cm in height
5.	Tannin	(+)	Green-colored

Notes : (+) Contain secondary metabolites

(-) Contains no secondary metabolites

5. Nanoparticle Solution of Sungkai Leaf Ethanol Extract

The best sungkai leaf ethanol extract nanoparticle formula is formula 2, longer stirring and sonication times produce a solution with a % transmittance of 88% and a particle size of 40 nm. These results are in accordance with the research conducted by [Prihantini et al. \(2020\)](#) and [Taurina et al. \(2017\)](#), in which the longer the stirring and sonication time, the smaller the resulting particle size. The solution was then formulated into nanoparticle syrup.

6. Sungkai Leaf Ethanol Extract Nanoparticle Syrup

The sungkai leaf ethanol extract syrup was made in 2 formulas, namely formula 0 (syrup base) and formula 1 (sungkai leaf ethanol extract syrup).

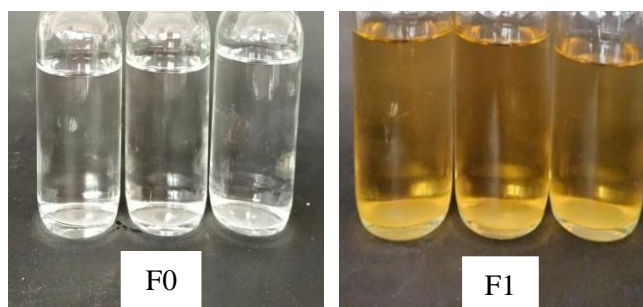


Figure 1. Syrup preparation

Notes :

F0 : Syrup base

F1 : Sungkai leaf ethanol extract syrup

7. Transmittance test

The results of the examination of % transmittance of nanoparticle syrup preparation of ethanol extract of sungkai leaves can be seen in [Figure 2](#), and the percent transmittance

examination was carried out with the aim of clarifying the syrup preparation. Formulas with a % transmittance of 90–100% indicate that the formula has a clear and transparent visual appearance. The high transmittance indicates that the preparation has a smaller particle size. The % transmittance test results indicate that the addition of sungkai leaf ethanol extract to the syrup preparation formula reduces the % transmittance value, and with a decrease in the % transmittance value, the particle size increases.

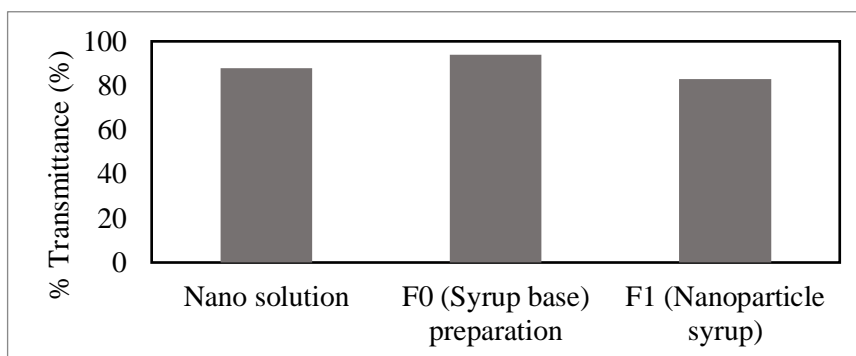


Figure 2. Results of % Transmittance Test

8. Particle Size Analyzer Test

The results of the particle size examination of the sungkai leaf ethanol extract nanoparticle syrup preparation are shown in **Figure 3**, where the particle size increased in each preparation group, between nanoparticle solution, formula 0 (syrup base), and formula 1 (sungkai leaf ethanol extract nanoparticle syrup). Based on these data, a particle size diagram can be obtained for each preparation formula. The particle size of all syrup formulas tested using the PSA tool shows that all preparation particle sizes are included in the nanoparticle size category, which is 10–1,000 nm ([A. Yuwanda, Rahmawati and Farmasita, 2021](#)). Based on the results of the particle size test using PSA, the stirring time and sonication affected the particle size; the longer the stirring and sonication, the smaller the resulting particle size, because more particles were broken into smaller particles. These results are in accordance with research conducted by ([Taurina et al., 2017](#); [Prihantini et al., 2020](#)), who found that the longer the stirring and sonication times, the smaller the resulting particle size.

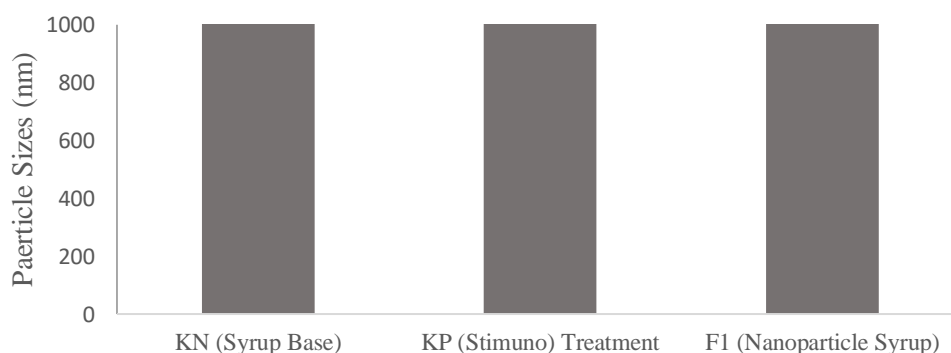


Figure 3. Particle size results

9. Immunomodulatory Test

The results of the examination of the number of leukocytes increased in each treatment group between the negative control group, the positive control group and the group given the nanoparticle syrup solution. Based on this data, a diagram of the average number of leukocytes in each treatment group is shown in **Figure 4**. Immunomodulator testing aims to determine the immunomodulatory effectiveness of a material or preparation. Formula 1 syrup preparation has the best immunomodulatory activity because the

preparation is in the form of nanoparticles so that the therapeutic effect of the preparation increases and contains secondary metabolites that can function as immunomodulators. (A. Yuwanda, Rahmawati and Farmasita, 2021; Rahman *et al.*, 2021).

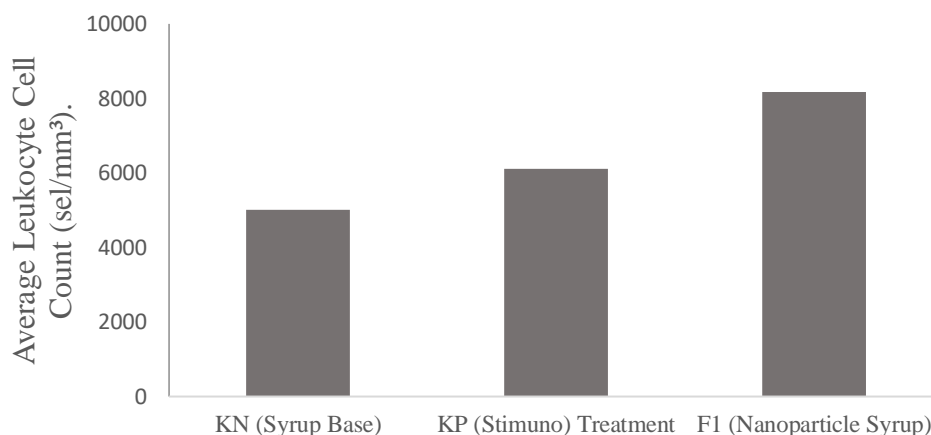


Figure 4. Average Leukocyte Count

10. Stability Test

1) Evaluation of Particle Size of Syrup Preparation

The particle size of the syrup preparation obtained using formula 1 was 964 nm, and formula 0 was 670 nm. The evaluation results showed an increase in size from formula 0 and 1. The change in size from nanometers to micrometers is caused by the acidity level in each formula, where pH variations affect the ionization of chitosan, which in turn affects the bond strength of the encapsulated complex. (A. Yuwanda, Rahmawati and Farmasita, 2021).

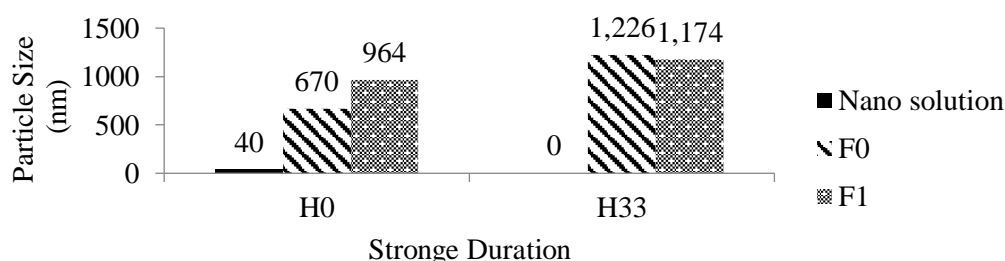


Figure 5. Particle size results

2) Organoleptic and Homogeneity Test

The organoleptic test F1 had the strongest aroma of leaves, while F0 did not have a distinctive odor due to the absence of the nanoparticle solution content of the ethanol extract of sungkai leaves. Moreover, formula F1 has a clear yellow color. When compared to formula F0, which does not have a concentration of the extract nanoparticle solution, it causes no color change. According to previous research, the concentration of extracts can affect the physical properties, organoleptic properties, and color intensity of the preparation (Marina *et al.*, 2021). Thus, it can be concluded that the clear yellow color in formula 1 (F1) was produced by the sungkai leaf extract nanoparticle solution.

The results of the homogeneity test indicated that increasing the amount of extract in the formula had no effect on the homogeneity of the syrup (Hidayati, Styawan and Khotimah, 2020). Syrup preparations F0, F1, and the comparison formula (FP) remained stable and homogeneous because the extracts in the nanoparticle solution and

the ingredients used were mixed and dissolved well to produce a good and homogeneous syrup preparation.

Table VI. Organoleptic Test Results and Homogeneity of Syrup Preparations

Temperature	Observation	Formula	Storage				
			0	7	14	21	28
4° C	Color	F0	Clear	Clear	Clear	Clear	Clear
		F1	Clear yellow	Clear yellow	Clear yellow	Clear yellow	Clear yellow
		FP	Black	Black	Black	Black	Black
	Odor	F0	Odorless	Odorless	Odorless	Odorless	Odorless
		F1	Leaf odor	Leaf odor	Leaf odor	Leaf odor	Leaf odor
		FP	Aromatic odor	Aromatic odor	Aromatic odor	Aromatic odor	Aromatic odor
	Form	F0	Liquid	Liquid	Liquid	Liquid	Liquid
		F1	Liquid	Liquid	Liquid	Liquid	Liquid
		FP	Liquid	Liquid	Liquid	Liquid	Liquid
	Flavor	F0	Sweet	Sweet	Sweet	Sweet	Sweet
		F1	Sweet	Sweet	Sweet	Sweet	Sweet
		FP	Mint	Mint	Mint	Mint	Mint
	Homogeneity	F0	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
		F1	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
		FP	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Room	Color	F0	Clear	Clear	Clear	Clear	Clear
		F1	Clear yellow	Clear yellow	Clear yellow	Clear yellow	Clear yellow
		FP	Black	Black	Black	Black	Black
	Odor	F0	Odorless	Odorless	Odorless	Odorless	Odorless
		F1	Leaf odor	Leaf odor	Leaf odor	Leaf odor	Leaf odor
		FP	Aromatic odor	Aromatic odor	Aromatic odor	Aromatic odor	Aromatic odor
	Form	F0	Liquid	Liquid	Liquid	Liquid	Liquid
		F1	Liquid	Liquid	Liquid	Liquid	Liquid
		FP	Liquid	Liquid	Liquid	Liquid	Liquid
	Flavor	F0	Sweet	Sweet	Sweet	Sweet	Sweet
		F1	Sweet	Sweet	Sweet	Sweet	Sweet
		FP	Mint	Mint	Mint	Mint	Mint
	Homogeneity	F0	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
		F1	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
		FP	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
40° C	Color	F0	Clear	Clear	Clear	Clear	Clear
		F1	Clear yellow	Clear yellow	Clear yellow	Clear yellow	Clear yellow
		FP	Black	Black	Black	Black	Black
	Odor	F0	Odorless	Odorless	Odorless	Odorless	Odorless

	F1	Leaf odor	Leaf odor	Leaf odor	Leaf odor	Leaf odor
	FP	Aromatic odor	Aromatic odor	Aromatic odor	Aromatic odor	Aromatic odor
Form	F0	Liquid	Liquid	Liquid	Liquid	Liquid
	F1	Liquid	Liquid	Liquid	Liquid	Liquid
	FP	Liquid	Liquid	Liquid	Liquid	Liquid
Flavor	F0	Sweet	Sweet	Sweet	Sweet	Sweet
	F1	Sweet	Sweet	Sweet	Sweet	Sweet
	FP	Mint	Mint	Mint	Mint	Mint
Homogeneity	F0	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	F1	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	FP	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous

Notes :

F0 = Syrup preparation formula without sungkai leaf ethanol extract nanoparticle solution

F1 = Syrup preparation formula with sungkai leaf ethanol extract nanoparticle solution

Fp = Comparator syrup preparation

3) pH Test

Based on the results of pH testing, it is known that all preparations are in the range of 4.1-4.6 and there are no significant changes during 28 days of storage at 4°C, room temperature, and 40°C. The pH test results met the good pH requirements of 4-7 (Department of Health, 2014). This shows that the different storage temperatures did not affect the pH of the preparation. The pH test results are shown in [Figures 6, 7, and 8](#), respectively.

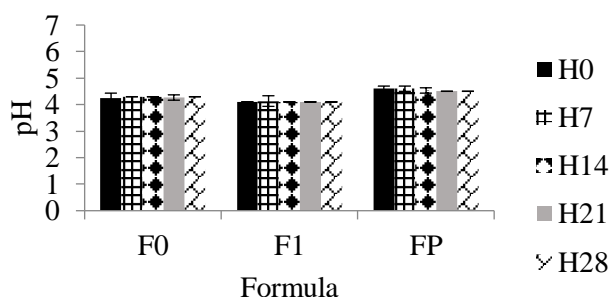


Figure 6. pH values of F0, F1 and FP Temperature 4°C

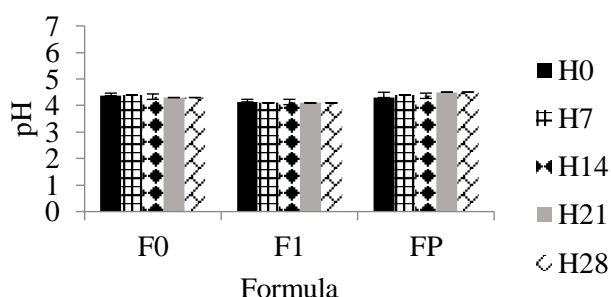


Figure 7. pH values of F0, F1 and FP Room Temperature

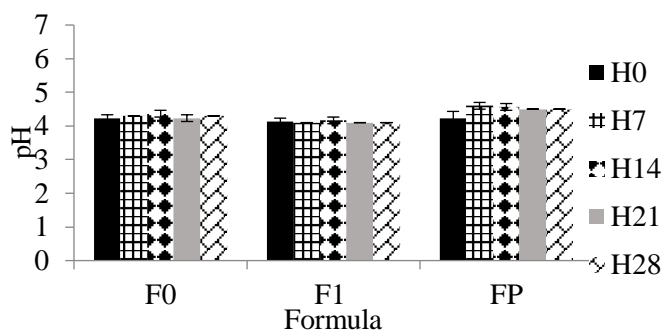


Figure 8. pH values of F0, F1 and FP Temperature 40°C

Notes :

F0 = Syrup preparation formula without sungkai leaf ethanol extract nanoparticle solution

F1 = Syrup preparation formula with sungkai leaf ethanol extract nanoparticle solution

Fp = Comparator syrup preparation

4) Specific gravity test

Based on the results of the specific gravity test, it is known that all preparations are in the range of 0.99-1.26 g/mL and do not experience significant changes during 28 days of storage at 4, room, and 40 °C. All preparations have a good specific gravity value in accordance with the requirements for the specific gravity of syrup preparations, namely > 1.3 g/mL (Rustiani, Anitania, and Effendi, 2021). The results of the specific gravity tests are shown in Figures 9, 10, and 11.

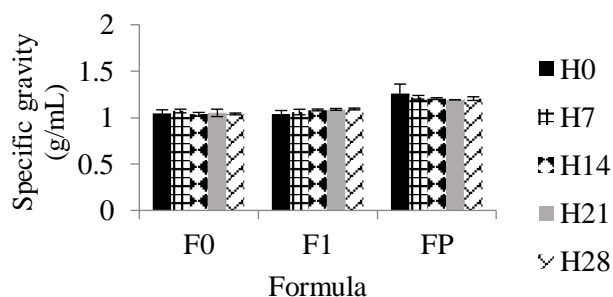


Figure 9. Specific gravity values of F0, F1 and FP Temperature 4°C

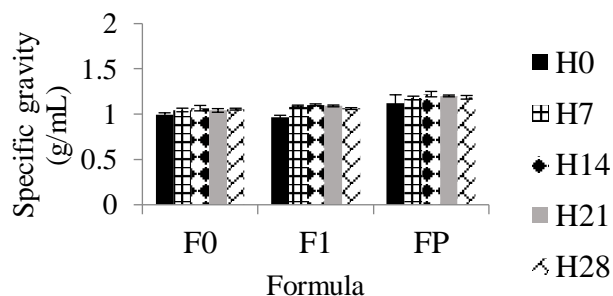


Figure 10. Specific gravity values of F0, F1 and FP Room Temperature

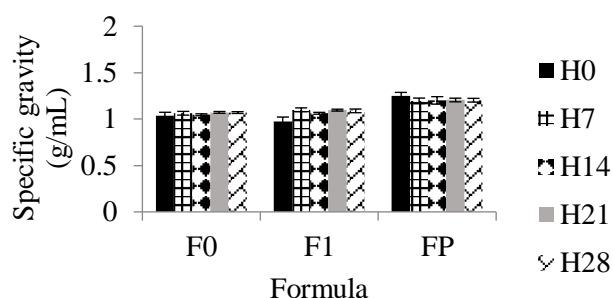


Figure 11. Specific gravity values of F0, F1 and FP Temperature 40°C

Notes :

F0 = Syrup preparation formula without sungkai leaf ethanol extract nanoparticle solution

F1 = Syrup preparation formula with sungkai leaf ethanol extract nanoparticle solution

Fp = Comparator syrup preparation

5) Pour-Time Test

Based on the results of the pouring time test, it is known that all preparations were in the range of 2.09-3.79 seconds and did not experience significant changes during 28 days of storage at 4 °C, room temperature, and 40 °C. All preparations were in the range of good pouring times in accordance with the pouring time requirements for syrup preparations (2-3 seconds). This test was closely related to the viscosity and specific gravity of the preparation. Thus, the higher the viscosity and specific gravity of a preparation, the longer is the pouring time required (Hidayati, Styawan and Khotimah, 2020). The comparison formula (FP) has a longer pouring time than formulas 0 (F0) and formula 1 (F1). This is due to the higher viscosity and specific gravity of the comparison formula compared to those of formulas 0 (F0) and 1 (F1). The addition of extracts to a formula can increase the viscosity of the preparation. The results of the pouring-time tests are shown in Figures 12, 13, and 14.

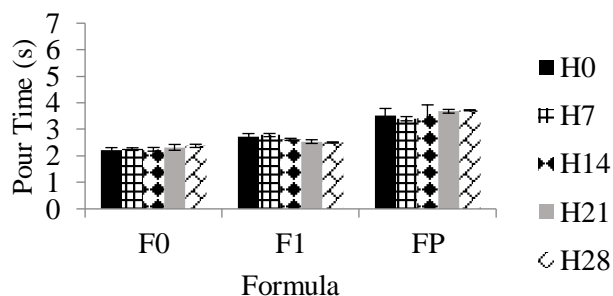


Figure 12. Test Value Pour Time of F0, F1 and FP Temperature 4°C

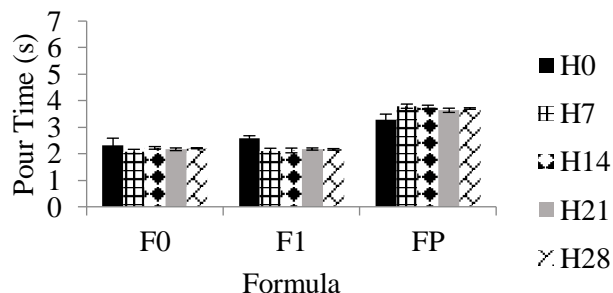


Figure 13. Test Value Pour Time of F0, F1 and FP Room Temperature

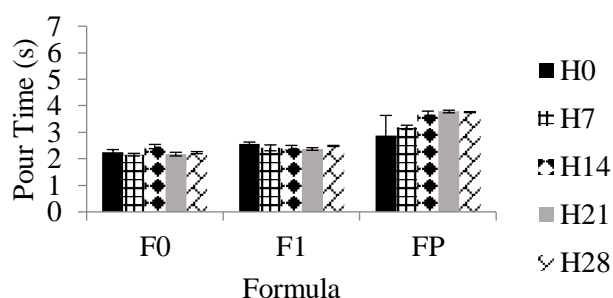


Figure 14. Test Value Pour Time of F0, F1 and FP Temperature 40°C

Notes :

F0 = Syrup preparation formula without sungkai leaf ethanol extract nanoparticle solution

F1 = Syrup preparation formula with sungkai leaf ethanol extract nanoparticle solution

Fp = Comparator syrup preparation

6) Uji Viskositas

Based on the viscosity testing results, it is known that all preparations are in the range of 0.83-4.28 cps. During the 28-day storage at 4°C, all preparations experienced an increase in viscosity value from 2.21-2.38 cps (F0), 1.50-2.30 cps (F1) and 3.52-3.72 cps (FP). This is influenced by the freezing point of the solvent used (water), where water has a freezing point of 0 °C, which is close to the storage temperature of 4 °C.

However, storage at room temperature and 40 °C did not result in any significant temperature changes. This is because the room temperature (15°C-30°) and 40 °C are far from the freezing point of the solvent used. Therefore, it can be concluded that temperature and freezing point affect the viscosity of the preparation (Apriyanti and Fithriyah, 2013). The results of the viscosity tests are shown in Figures 15, 16, and 17.

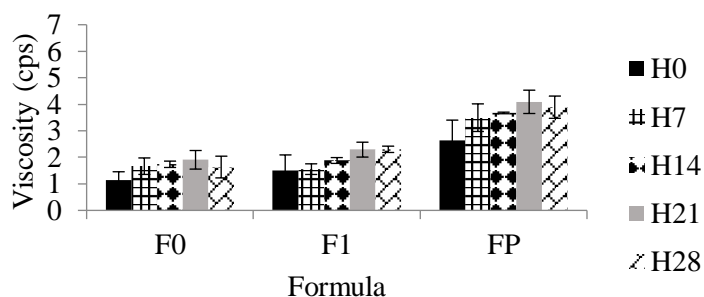


Figure 15. Viscosity Test Values of F0, F1 and FP Temperature 4°C

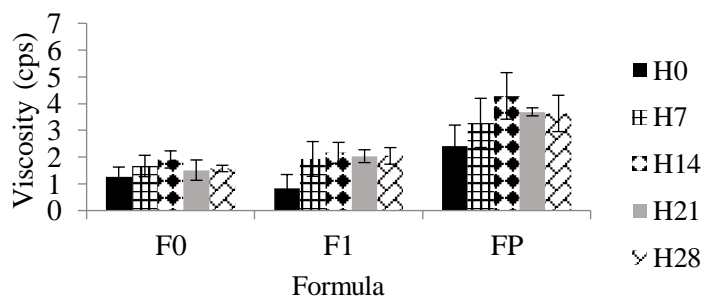


Figure 16. Viscosity Test Values of F0, F1 and FP Room Temperature

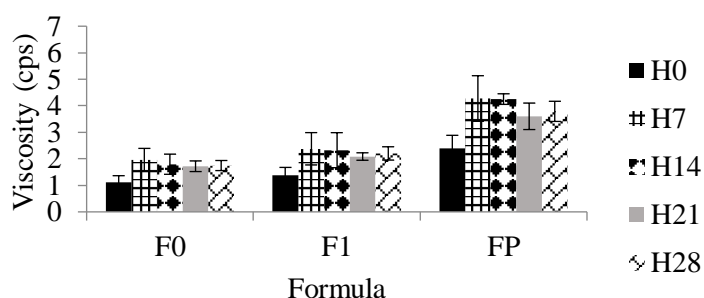


Figure 17. Viscosity Test Value of F0, F1 and FP Temperature 40°C

Notes :

F0 = Syrup preparation formula without sungkai leaf ethanol extract nanoparticle solution

F1 = Syrup preparation formula with sungkai leaf ethanol extract nanoparticle solution

Fp = Comparator syrup preparation

CONCLUSIONS

According to the research conducted, it can be concluded that the ethanol extract of sungkai leaves can be formulated into nanoparticle syrup with a particle size of 964 nm using chitosan and NaTPP. The syrup preparation formula that had the best immunomodulatory activity was formula 1 (sungkai leaf ethanol extract nanoparticle syrup), which increased the number of leukocytes by 8,166 cells/mm³. Sungkai leaf ethanol extract nanoparticle syrup is physically and chemically stable at 4 °C, room temperature, and 40 °C for storage but has not been able to maintain the nanometer size.

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