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OPTIMIZATION OF THE FUNCTIONAL BEVERAGE FORMULA FROM NIPAH (*Nypa fruticans*) LEAVES USING SIMPLEX LATTICE DESIGN METHOD AND IN VITRO IMMUNOSTIMULATORY ACTIVITY

Fuji Sintia Armi[1,2], Uju[1,3]*, Kustiariyah Tarman[1]
[1]Department of Aquatic Product Technology, IPB University
Lingkar Akademik st. IPB Darmaga Campus, Bogor Indonesia 16680
[2]Department of Fisheries Product Technology, Faculty of Fisheries, Abulyatama University
Blangbintang Lama st., Lampoh Keude, Aceh Indonesia 24415
[3]Surfactant and Bioenergy Research Center (SBRC) IPB University,
IPB Baranangsiang Campus Raya Pajajaran st. No.1, Bogor, Indonesia 16129

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*Correspondence: ujusadi@apps.ipb.ac.id

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Abstract

Nypa fruticans is a source of bioactive compounds with potential for development as a functional beverage. This study aimed to optimize the formulation of a beverage based on nipah leaf extract and evaluate its sensory characteristics and biological activity. Optimization was performed using the simplex lattice design method with the assistance of a design expert, incorporating two factors (nipah leaf extract and water), resulting in eight formulations. The response parameters included hedonic testing (color, aroma, and taste), antioxidant activity (percent inhibition), and cell viability as an initial indicator of immunomodulatory activity (MTT-Assay). The results showed that the optimal formulation was obtained with a composition of 40% nipah leaf extract and 39.4% water, yielding a desirability value of 0.854. All verified response parameters fell within the 95% confidence interval, indicating the model's suitability. The antioxidant activity reached 81.117%, and an increase in cell viability was observed with increasing extract concentration. The product has viscosity characteristics of 21.6 cP, a pH of 4.9, and total soluble solids of 22.15°Brix, and meets the microbiological standards of SNI 3719:2014. The results indicate the potential of this mushroom as a functional beverage with promising biological activity. However, the interpretation of immunostimulatory activity is currently limited to increased cell viability, necessitating further mechanism-based studies to comprehensively confirm immunomodulatory effects.

Keywords: antioxidant, cell viability, MTT-Assay, *Nypa fruticans*

Film Biodegradabel Berbasis Selulosa Kulit Kakao dengan Formulasi Kappa dan Iota-Karagenan

Abstrak

Nypa fruticans merupakan sumber senyawa bioaktif yang berpotensi dikembangkan sebagai minuman fungsional. Penelitian ini bertujuan untuk mengoptimalkan formulasi minuman berbasis ekstrak daun nipah serta mengevaluasi karakteristik sensorik dan aktivitas biologisnya. Optimasi dilakukan menggunakan metode *Simplex lattice design* berbantuan perangkat lunak design expert dengan dua faktor (ekstrak daun nipah dan air) yang menghasilkan 8 formulasi. Parameter respons meliputi uji hedonik (warna, aroma, rasa), aktivitas antioksidan (persen inhibisi), dan viabilitas sel sebagai indikator awal aktivitas imunomodulator (MTT-Assay). Hasil menunjukkan bahwa formulasi optimum diperoleh pada komposisi ekstrak daun nipah 40% dan air 39,4% dengan nilai desirability 0,854. Seluruh parameter respons hasil verifikasi berada dalam rentang confidence interval 95%, menunjukkan kesesuaian model. Aktivitas antioksidan mencapai 81,117%, sedangkan peningkatan viabilitas sel diamati seiring dengan peningkatan konsentrasi ekstrak. Produk memiliki karakteristik viskositas 21,6 cP, pH 4,9, dan total padatan terlarut 22,15°Brix, serta memenuhi standar mikrobiologi SNI 3719: 2014. Hasil menunjukkan potensi sebagai minuman fungsional dengan aktivitas biologis yang menjanjikan, namun interpretasi aktivitas imunostimulan masih terbatas pada peningkatan viabilitas sel sehingga diperlukan kajian lanjutan berbasis mekanisme molekuler untuk mengonfirmasi efek imunomodulator secara komprehensif.

Kata kunci: antioksidan, MTT-Assay, *Nypa fruticans*, viabilitas sel

INTRODUCTION

The nipah plant (*Nypa fruticans*) is a group of palms that grow in Indonesia's mangrove forest areas, particularly in tidal areas. The distribution of mangrove forests in Indonesia covers 3,364,076 ha, with 700,000 ha dominated by nipah plants (Kementerian Lingkungan Hidup dan Kehutanan, 2021). This makes Indonesia the largest habitat for nipah palms, surpassing Papua New Guinea and the Philippines. Traditionally, nipah palms have been utilized for roofing, handicrafts, firewood, and traditional medicine, such as overcoming stomach pain, diabetes, mouth ulcers, and toothaches (Muthmainnah & Sribianti, 2016). However, the potential of the nipah plant extends far beyond these conventional uses.

Recent studies have highlighted the pharmacological benefits of nipah plants, particularly their immunostimulant properties (Oktaviani, 2022). Nipah leaves exhibit strong antioxidant activity, protecting the body from free radicals that contribute to various diseases (Jiang *et al*., 2021). Natural free radicals in the human body are free oxygen molecules obtained from the oxidation process in the mitochondria. In contrast, free radicals from outside the body are often triggered by toxic substances such as cigarette smoke, ultraviolet irradiation, radical-triggering substances in food, and other pollutants (Setiyowati, 2020; Oktaviani, 2022). Consuming foods rich in antioxidants, such as nipah leaves, can help mitigate these effects and strengthen the immune system (Zulianti *et al.,* 2023).

Among the parts of the nipah plant, the leaves have garnered particular attention because of their superior antioxidant content compared to the fruit (Khairi *et al*., 2020). For instance, methanolic extracts of nipah leaves exhibited an IC_{50} value of 17.72 ppm, significantly lower than the fruit's IC_{50} of 48.02 ppm, indicating higher antioxidant activity (Putri *et al*., 2013). Armi *et al*. (2025) reported an IC_{50} value of 26.89 μg/mL for the nipah leaf extract. Additionally, Yahaya *et al*. (2021) noted that ascorbic acid in nipah exhibited the highest antioxidant activity, with an IC_{50} value of 21.29±0.74 μg/mL. This indicates that the nipah leaves possess very high antioxidant content.

Given their antioxidant potential, nipah leaves can be developed into functional beverages that combine health benefits with sensory appeal. Functional beverages, a subset of functional foods, must provide bioavailable nutrients and deliver a pleasant sensory experience, including flavor and texture (Susanto & Kristiningrum, 2021). To the best of our knowledge, the optimization of functional beverage formulas from nipah leaf extract has not been investigated. The use of nipah leaves is primarily focused on the pharmaceutical field. Several studies have demonstrated their various bioactive potentials, including antihypertensive (Oktaria & Marpaung, 2023), antibacterial (Az-Zhahra *et al*., 2020), and anticancer properties (Noviana *et al*., 2022). Additionally, nipah leaves have been reported to lower blood sugar levels in male Wistar rats (Aryani *et al*., 2023) and to show potential as an antihyperuricemic agent (Raharjo *et al*., 2024). This study addresses this gap by utilizing the simplex lattice design method, which is a robust and efficient experimental approach for optimizing complex formulations (Bezerra *et al*., 2020). The simplex lattice design is an optimization method used to determine the optimal formulation of a mixture of ingredients, where the sum of the proportions of all ingredients must equal 1 (100%). By employing this method, we ensured that the resulting product was not only health-promoting but also aligned with consumer preferences for sensory satisfaction, thereby increasing its market acceptability (Bezerra *et al*., 2020).

Research on the formulation optimization of functional beverages containing nipah leaf extract has not yet been conducted. Formulation and optimization are complex processes that require numerous trials and a large number of samples. The use of appropriate experimental methods can simplify the process and yield valid results with minimal time and effort (Bezerra *et al*., 2020). Previous studies have demonstrated that a 3-component mixture design can help optimize functional beverage formulations for various types of beverages, such as functional beverages made from temu lawak powder, red ginger, and palm sugar (Sabariman *et al*., 2021), rosella flower extract, stevia leaves, and chia seeds (Batubara *et al.*, 2020), lime extract (Assalam *et al.*, 2023), functional moringa leaf beverages (Kahfi *et al.*, 2021), chlorella milk (Michelle & Widyaningrum, 2023), and beverages made from black mulberry and canistel (Irfan *et al.*, 2022). The results indicate potential as a functional beverage with promising biological activity; however, the interpretation of immunostimulatory activity is currently limited to increased cell viability, necessitating further mechanism-based studies to comprehensively confirm immunomodulatory effects. This study aims to optimize the formulation of a beverage based on nipah leaf extract and to evaluate its sensory characteristics and biological activity.

MATERIALS AND METHODS

Nipah Leaf Water Extraction

The extraction process of nipah leaves begins with preparation. Nipah leaves were separated between the leaf bones and leaves. Furthermore, the leaves were cut into small pieces measuring approximately 2 cm, cleaned, and boiled with water in a mass ratio of 1:2 (leaves and water) using a 21-liter SUS 304 stainless steel pot from Royal Star Indonesia. The extraction process was performed at 80°C for 5 min (Kahfi *et al*., 2021). The extraction results were filtered using a 28.5 cm stainless steel bowl strainer from Kona's Kitchen Indonesia and placed in bottles.

Experimental Design

A simplex lattice design experiment was conducted using Design Expert 13 software. The beverage formula was optimized by optimizing the composition of the main ingredients. The components used as process variables were nipah leaf extract and water. The independent variables for the mixture design were bounded as follows: Nipah leaf extract (w/v) ranged from a lower limit of 10% to an upper limit of 40%, and water (v/v) was incorporated within a concentration range of 39.3% to 69.4%.

Furthermore, the proportions of nipah leaf extract and water were determined using the simplex lattice design method, resulting in eight variations of the formula. The formulation used in this design is presented in Table 1.

Nipah Leaf Functional Beverage Formulation

The extraction results were then formulated with functional beverage fillers, namely carrageenan 0,1 (as a thickener) purchased from the Indoplant online store in Yogyakarta, honey 15% (as a sweetener) purchased from the Madu Tava Official Jakarta online store under the Madu Tava brand, Na benzoate 1% (food preservative) purchased from the Koepoe Koepoe Official Shop online store under the Koepoe Koepoe brand, lime juice 1.5% (as resultant), and ginger and lemongrass 1.5 (as flavor) obtained from the Dramaga Market in Bogor (Kahfi *et al*., 2021).

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Table 1 Nipah leaf beverage formulation

Material (%)	Formulation							
	F1	F2	F3	F4	F5	F6	F7	F8
Nipah leaf extract	25.0	10.0	17.5	40.0	32.5	10.0	40.0	25.0
Water	54.4	69.4	61.9	39.4	46.9	69.4	39.4	54.4
Carrageenan	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Honey	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Na benzoate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ginger	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Lemongrass	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Lime	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Sensory Test

The hedonic test uses human sensory evaluation to measure product acceptability as a component of a quality management system. A total of 25 semi-trained panelists evaluated the coded samples without knowing the treatments applied. The samples were assessed based on their aroma, taste, and color. According to SNI 3719:2014, the evaluation was conducted using a 5-point scale (1=strongly dislike, 2=dislike, 3=somewhat dislike, 4=like, and 5=strongly like) (BSN, 2014). This sensory test was conducted by semi-trained panelists and validated using ANOVA.

Physical, Chemical and Microbiological Characteristics of Nipah Leaf Extract Beverages

The beverage characteristics, including acidity level, dissolved solids, plate count, coliform presence, *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and mold/yeast, were analyzed in accordance with the following standards: SNI 3719:2014 (acidity level (pH) measured using a WalkLAB TI 9000 pH meter from Trans Instruments, Singapore, dissolved solids are measured using an ATAGO N-1E hand refractometer Brix 0-32% made in Japan, viscosity measured using a Brookfield viscometer Brookfield brand, RV DV-E230 SN E9419 type, made in USA, and total plate count) (BSN, 2014), SNI ISO 4831:2012 (*coliform* test) (BSN, 2012a), SNI ISO 7251:2012 (*Escherichia coli* test) (BSN, 2026), ISO 6579-1:2017/AMD 1:2020 (*Salmonella*) (ISO, 2020), ISO 6888-1:2021/AMD 1:2023 (*Staphylococcus aureus*) (ISO, 2021), and SNI ISO 21527-1:2012 (*mold/yeast* test) (BSN, 2012b).

Antioxidant Activity

A total of 0.1 mL of the sample solution was placed in a test tube, followed by the addition of 0.9 ml ethanol. The mixture was vortexed, and 2 mL of 1.1-diphenyl-2-picrylhydrazyl (DPPH) solution was then added. The test tube was vortexed again and incubated for 30 min in the dark. Absorbance was measured using a Spectrostar Omega spectrophotometer (BMG Labtech) at a wavelength of 517 nm (Fauzi *et al.*, 2019). The antioxidant activity of a sample, as measured by the extent of DPPH radical scavenging, was determined by calculating the percentage of DPPH scavenging inhibition using the following formula:

$$\%Inhibition = \frac{\text{Blank abs-Sample abs}}{\text{Blank abs}} \times 100$$

Blank abs. = absorbance of 50 μM DPPH
Sample abs. = absorbance of test sample

Immunostimulant Test

The test of immunostimulant activity of Nipah leaf beverage on human lymphocyte cell proliferation using the MTT assay method was carried out based on the approval of the Health Research Ethics Commission of the Poltekkes Kemenkes Aceh (Number

DP. 04.03/12. 7/140/2024), with procedures including separation of single-nucleated blood cells using the fill method, incubation of lymphocytes at 37°C with 5% CO_2, addition of samples, and measurement of cell viability using optical density BIO RAD Type iMark optical density made in Japan at a wavelength at λ 595 nm, where viability was calculated based on the percentage of inhibition. All stages were carried out with RPMI 1640 or D-MEM culture media enriched with FBS and antibiotics (Nirmalasari *et al.*, 2013).

Data Analysis

The fit of the response data was tested using linear, quadratic, cubic, and special cubic models. Model fit tests, response surface plot visualization, and determination of the optimal equation were performed using Design Expert software version 13. ANOVA with a p-value<0.005 and a non-significant lack of fit (p>0.005) were used to evaluate the significance of each model and its equation. A desirability function was used for the optimization. The results of the data analysis are presented in graphical and descriptive formats.

RESULT AND DISCUSSION

Characterization of Nipah Leaf Extract

Nipah leaf extract was obtained by boiling for 5 min at 80°C, producing a yellow-brown color due to the release of pigments and bioactive compounds such as phenolics and flavonoids. This heating process also triggers a browning reaction similar to caramelization in nipah sap (Apirattananusorn, 2021; Moonrungsee *et al.,* 2022). Nipah leaf extract is known to have antioxidant activity, which acts as an immunostimulant by counteracting free radicals and strengthening the immune response. Antioxidants help reduce oxidative stress, which can trigger chronic inflammation and impair immune function (Bhol *et al.*, 2024; Biswas *et al.*, 2024). Antioxidant activity analysis was conducted using the DPPH method, which showed an inhibition percentage of 82.6%, indicating that it is classified as a high antioxidant. Nipah leaf extract is also known to exhibit immunostimulatory activity. The higher the DPPH inhibition percentage, the stronger the antioxidant activity, as more free radicals are neutralized by the antioxidant compounds in the sample. This is confirmed by another study on nipah leaf extract, which showed a similar inhibition percentage (82.4%) and was classified as a very strong antioxidant (Hasanah *et al.*, 2025), further supporting the potential of nipah leaf extract as an immunostimulant, as demonstrated in Nile tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila* (Oktaviani *et al.*, 2022).

The lymphocyte cell proliferation test results on nipah leaf extract showed fluctuating viability values. A concentration of 400 showed the highest viability value, while a concentration of 200 showed the lowest viability (Figure 1). The relationship between lipids and the immune system can cause fluctuations in viability. Lipids can influence immune system modulation by altering immune responses, such as cytokine production, cell proliferation, NK cell activity, phagocytosis, and other mechanisms (Pablo

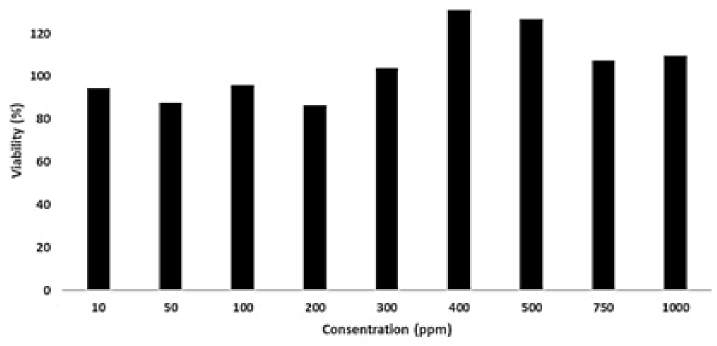

Figure 1 Cell viability in nipah leaf beverage

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& Cienfuegos, 2000). Cell viability values represent the number of cells that remain viable after exposure to substances (Perez *et al.*, 2017).

Formula Optimization

Formula optimization is the best formula produced in determining the most effective combination of variables that affect product quality. Optimization is used to estimate the answer to a function of response variables generated from experimental design to produce an optimal formula (Elcistia & Zulkarnain, 2018)

Appropriate model selection results

ANOVA in the *Design Expert* software shows the validity of the predicted R^2 and adjusted R-squared if the difference is less than 0.2 (Said & Sarbon, 2022). R-squared (R^2) measures the strength of the relationship between variables; the closer it is to 1, the stronger (Dzulhijjah *et al.*, 2024). Antioxidant and hedonic tests showed a significant model with a p-value<0.05, and consistent predictions (Shwartz dan Dagan 2007). The results of the model are presented in Table 2.

The experimental points according to the experimental design were the proportion of Nipah leaf extract to water, and the results of the analysis of the responses are presented in Table 3.

Optimization Parameters

The following optimization parameters were tested on functional nipah leaf beverages to assess the effect of each factor on the antioxidant content and sensory properties of the product.

Table 2 Model results of design expert on selected responses

Parameters	Mathematical model	P-value	R^2	Adj R^2 model	Pred R^2 model
Hedonic color	Linear	0.0004	0.8934	0.8756	0.8366
Hedonic aroma	Linear	0.0033	0.7874	0.7520	0.6306
Hedonic taste	Linear	0.0388	0.5365	0.4593	0.2402
Antioxidant activity	Quadratic	0.0161	0.9660	0.9524	0.9253

Adj=adjusted; Pred=predicted

Table 3 Experimental design trial points and response values at each trial point

Formulation	Experiment point	Description	Material composition		Hedonic value			Antioxidant activity* (%inhibition)
			Nypa leaf extract	Water	Color	Aroma	Taste	
1	Center	Replicate	25	54.4	3.6	3.4	3.4	70.126
2	Vertex	Replicate	10	69.4	3.3	3.4	3.4	66.856
3	AxialCB	NA	17.5	61.9	3.4	3.4	3.5	70.473
4	Vertex	NA	40	39.4	3.7	3.7	3.7	79.995
5	AxialCB	NA	32.5	46.9	3.6	3.5	3.4	74.769
6	Vertex	NA	10	69.4	3.3	3.4	3.4	67.867
7	Vertex	Replicate	40	39.4	3.8	3.8	3.8	81.117
8	Center	NA	25	54.4	3.4	3.6	3.6	69.799
Mean					3.512	3.525	3.525	72.625
Standard deviation					0.1763	0.147	0.147	5.072

*The inhibition percentage is the average of 3 replicates

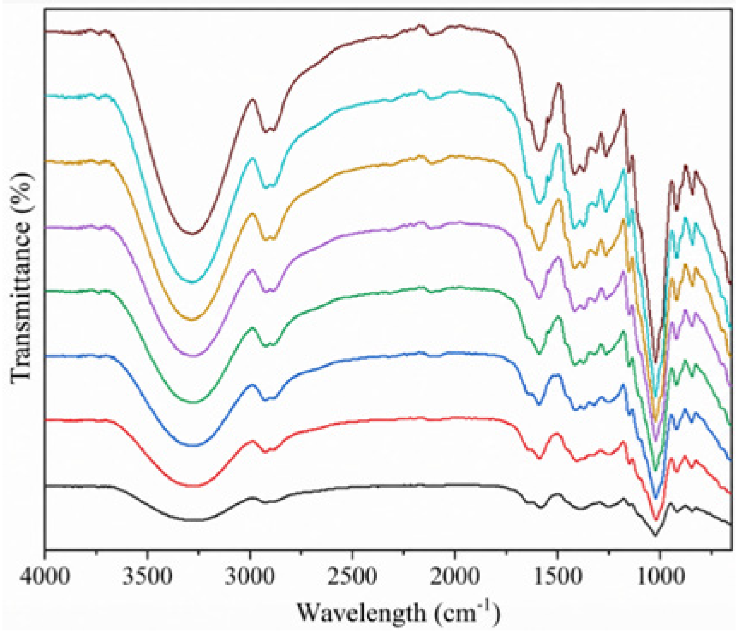

Figure 2 FTIR spectra of biodegradable films prepared from cocoa pod husk cellulose, κ-, and ι-carrageenan (T1: ▬, T2: ▬, T3: ▬, T4: ▬, T5: ▬, T6: ▬, T7: ▬, T8: ▬)

Sensory Test

Formulas 4 and 7 produce a more intense color, while formula 2 and 6 produce a lighter color. The color is caused by compounds such as flavonoids and tannins in the leaves, which function as natural colorants. When the leaves are boiled, these compounds dissolve in water, producing a brownish-yellow color (Azamatkyzy *et al*., 2024). Formulas 4 and 7 (40% nipah leaf extract) received the highest hedonic scores of 3.8, while Formulas 2 and 6 (10% nipah leaf extract) had the lowest scores of 3.3.

Analysis of variance (ANOVA) results using Design Expert 13 at the 5% significance level for color hedonic value showed that the resulting linear model had a *p-value* of 0.0004, well below the 0.05 threshold (Figure 2). The mathematical equation for the hedonic color value response is Y=3.73472A+3.29028B.

The highest aroma hedonic value was for formula 7 (3.8), and the lowest aroma hedonic values were for formulas 2, 3, and 6 (3.4). This emphasizes the effect of nipah leaf extract on aroma, which is influenced by volatile aldehyde compounds in the leaves, such as C6-aldehyde, C6-alcohol, (2E)-hexenal, and (3Z)-hexenol (Jaraee *et al*., 2023). The results of the analysis of variance show that the resulting model is linear and produces a p-value smaller than 0.05, which is 0.0033 (Figure 2). The mathematical equation for the hedonic value response of flavor was Y=3.70417A+3.7083B. The aroma of the nipah leaf functional beverage is influenced by the volatile aldehyde compounds in the leaves, such as C6-aldehyde, C6-alcohol, (2E)-hexenal, and (3Z)-hexenol (Jaraee *et al*., 2023). According to Vincenti *et al.* (2019), volatile compounds from leaves, such as aldehydes and C6-alcohols, including (2E)-hexenal and (3Z)-hexenol, significantly contribute to the fresh green aroma of food products, enhancing their sensory characteristics and making them more appealing to consumers, thereby influencing their preference for beverage flavors.

The highest taste hedonic test result was for formula 7, at 3.8. The analysis of variance results revealed that the model was linear and had a p-value of 0.0388 (Figure 2). The mathematical equation for the hedonic taste response is Y=3.66944A+3.38056B. This equation shows that the coefficient A is greater than the coefficient B. This shows that the nipah leaf extract has a significant influence on the hedonic value of taste. The nipah leaf beverage in formula 7, containing 40%

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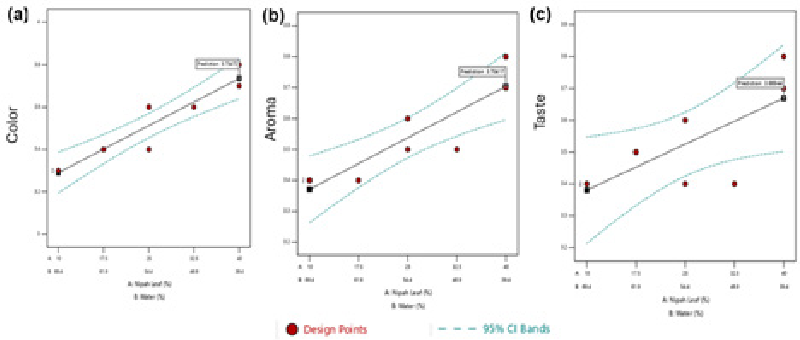

Figure 2 Counter plot chart of sensory test; (a) Color, (b) Aroma, (c) Taste)

nipah leaf extract and 39.4% water, showed good acceptance with a high hedonic score. Formula 7 was chosen because the distinctive flavor of nipah leaves combined with honey, lemongrass, ginger, and lime produced a taste that the panelists liked. The addition of honey, lemongrass, ginger, and lime also contributed to the functional flavor of the nipah leaves. According to Tias *et al.* (2022), honey not only functions as a sweetener, but also enriches the taste of the beverage while acting as an alternative sweetener that contributes to increased antioxidant activity,6 Santoso *et al.* (2019) mentioned that lime acts as a flavoring agent that enriches the overall taste of the beverage, making it more attractive, and Santoso *et al.* (2019) stated that ginger, with its aromatic properties, can reduce bitterness and enhance the sensory experience. The addition of lemongrass significantly improved flavor attributes, creating a balanced flavor profile when combined with other ingredients (Putra *et al.*, 2023).

Based on the results of the analysis of variance (ANOVA) at a 5% significance level (95% confidence interval), the p-values for all parameters were less than 0.05. This indicates that the differences observed among the treatments were significant. Therefore, it can be concluded that these various treatments have a significant effect on the panelists' preference ratings for all tested parameters, namely, color, aroma, and taste.

Antioxidant Activity

The results of the antioxidant activity test on nipah leaf functional beverages are expressed in percent inhibition. The highest inhibition percentage was found in the seventh formula (81.117) and the lowest in the second formula (66.856). This is consistent with Hasanah (2025), who reported that methanolic extracts of *Nypa fruticans* leaves exhibited DPPH inhibition values ranging from 51.31% to 82.4% across different extraction methods (maceration, percolation, and soxhletation) and sample concentrations, with all extracts classified as having very strong antioxidant activities. These percent inhibition values closely resemble the range obtained in the present study, further confirming the strong antioxidant potential of nipah leaves as a natural source of phenolic and flavonoid compounds.

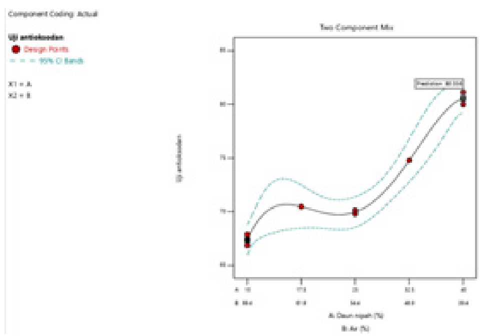

Figure 3 Counter plot graph of antioxidant activity

Analysis of Variance (ANOVA) results from design expert 13 show that the *p-value* for antioxidant activity is significant, more diminutive than 0.05 (0.0161). The resulting model was quadratic. The mathematical equation for the antioxidant activity response was Y=80.43A+67.74B-13.38AB.

Optimum Formula

Determination of optimum rormula

The response parameters in this study showed a significant ANOVA value of <0.05. A significant model of the response indicated an insignificant lack of fit. If the lack of fit value (F-value) is <0.05, it indicates a significant lack of fit, while if it is>0.05, it indicates an insignificant lack of fit. This significance level affects the fit between the parameter test data and the proposed model (Shangguan *et al*., 2023). The desirability values of the eight formulas optimized for several parameters ranged from 0.000 to 0.852.

Optimum model validation

The optimum formula selected was 40% nipah leaf extract concentration and 39.4% water, with the highest total desirability value of 0.854. All response data from the validation of the optimum formula in the laboratory are by the model predictions and are still within the prediction confidence range (α= 0.05). All response data from the validation of the optimum formula in the laboratory were by the model predictions and within the prediction confidence range (α= 0.05) (Table 4).

Characterization of Nipah Leaf Functional Beverages

The physical parameters of the nipa leaf functional beverage were pH, total soluble solids, and viscosity.

pH analysis

The resulting pH value of the Nipa leaf beverage was 4.9, which is acidic. Kinanti and Amelia (2023) reported that the pH values of functional okra beverages ranged from 4.88–5.42. These pH values indicate that the beverage has a significant level of acidity. One of the main factors that cause acidity in this beverage is the presence of lime as one of the additional ingredients. According to Assalam *et al.* (2023), lime has a low pH because it contains high concentrations of citric acid.

Dissolved solids

The total soluble solids content in the Nipah leaf beverage was recorded at 22.15°Brix, indicating a fairly high concentration of solids, which affected the beverage's sensory characteristics. Research shows that high total soluble solids values can increase consumer acceptance, as they affect essential attributes, such as flavor and viscosity. One of the main factors affecting the total soluble solids levels in this beverage is the addition of 15% honey, which significantly increases the solids content through natural sugar components such as fructose and glucose (Wahyunita *et al.,* 2023).

Viscosity

The viscosity of the nipa leaf beverage was recorded at 21.6 cP, which is a typical value resulting from the combination of carrageenan, ginger, and lemongrass. Carrageenan, particularly the iota type, plays the major role in improving emulsion stability and preventing sedimentation, thereby increasing viscosity, since one of the main functions of stabilizers is to stabilize the emulsion and prevent phase separation. Al-Baarri *et al.* (2019) confirmed that the addition of iota carrageenan increases emulsion stability in ginger-based beverages

Table 4 Optimization criteria, prediction, and validation results of optimum formula response

Respons	Goals	Model prediction	95% confident interval	Validation result
Color	Maximize	3.7±0.1	3.6±3.8	3.8±0.1
Aroma	Maximize	3.7±0.1	3.6±3.8	3.7±0.1
Taste	Maximize	3.7±0.1	3.5±3.9	3.7±0.1
Antioxidant activity	Maximize	81±0.1	79±81.9	81±0.1

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by inhibiting particle sedimentation, with higher viscosity corresponding to less phase separation. In addition, an increase in carrageenan concentration directly increases viscosity (Kiranawati *et al.*, 2022).

Immunostimulant Activity of Nipah Leaf Beverage

The immunostimulant activity of the nipah leaf beverage was evaluated using the MTT assay to evaluate lymphocyte cell proliferation. The test was performed at concentrations ranging from 10 to 1,000 µg/mL. The results of the immunostimulant activity test are shown in Figure 4.

Based on Figure 4, the optimal concentrations for lymphocyte cell growth were 10, 50, 100, and 200, while concentrations above 200, namely 300, 400, 500, 750, and 1,000, showed fluctuating values. Fluctuations in viability values in immunostimulant tests are usually caused by variations in the concentrations used, where there are concentrations that can be optimal, but there are also concentrations that are toxic to cells, so that under certain concentration conditions, the cell can die or grow unstable. Pablo and Cienfuegos (2000) stated that the reason for fluctuations in viability values is the relationship between lipids and the immune system. Lipids have the ability to affect the modulation of the immune system by altering immune responses, such as cytokine production, cell proliferation, NK cell activity, phagocytosis, and other mechanisms. Cell viability values represent the number of cells that remain alive after exposure to a given agent (Perez *et al.*, 2017). In this test, the cell control used showed a viability value of 100%, which is a reference for the optimal condition of cells without any treatment. Viability values that reached or exceeded 100% indicated that the cells remained alive, whereas values below 100% indicated cell death (Subaryono *et al.*, 2017). These results indicate that the Nipah leaf functional beverage can increase lymphocyte cell proliferation, suggesting its potential as an immunostimulating agent. This finding is in line with the research of Laklaeng & Kwanhian, (2020), which states that *Nypa fruticans* increases antibody production and phagocytic activity in mice, thus indicating its potential as a functional beverage with immunomodulatory properties.

Microorganism Analysis

The Indonesian Food and Drug Authority (BPOM) mandates microbiological testing of nipah leaf-based functional beverages to ensure product quality. Microbiological damage can be caused by spoilage and pathogenic microbes (Cahyani & Widodo, 2023). The microbiological standard refers to the Indonesian National Standard (SNI) No. 3719:2014 food microbiological criteria for fruit juice. This nipah leaf beverage product meets the requirements related to microbes through safe processing and microbiological standards (BSN, 2014). The laboratory test results of the nipah leaf functional beverage are presented in Table 5.

Based on microbiological tests, functional beverages from nipah leaves are safe for consumption. The test results showed *E. coli*, *Salmonella sp*., and *coliform* values of 0, and mold and yeast less than 10, which

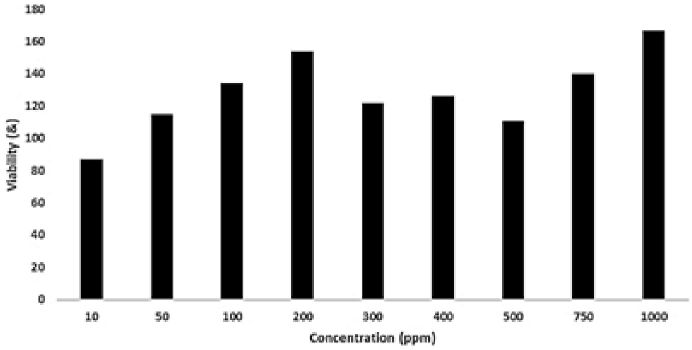

Figure 4 Cell viability in the nipah leaf beverage

Table 5 Results of microorganism analysis on nipah leaf beverage

Parameters	Result	Standard
E. coli (MPN/mL)	0	<3
Salmonella sp. (25mL)	Negative	Negative/25mL
Mold/yeast (colony/mL)	<10	Max. 1×10^2
ALT (colony/mL)	2.65x101	1×10^4
Coliform (MPN/mL)	0	Max. 20

*Indonesia National Standard (SNI) No. 3719:2014 (BSN, 2014)

met the maximum limit of 1 × 102. The total plate count (TPC) value was 2.45×10^1, which is well below the stipulated requirement of 1×104. This indicates no contamination of TPC, *E. Coli, Salmonella sp., coliform, mold,* and *yeast* in the beverage.

CONCLUSION

This study successfully determined the optimum formula for a nipa leaf functional beverage using the simplex lattice design. The optimal formula consisted of 40% nipa leaf extract and 39.4% water, with a desirability value of 0.854. Verification confirmed that all response parameters—color, aroma, and taste hedonics, as well as antioxidant activity—fell within the 95% confidence interval predicted by the model, validating the simplex lattice design approach for this product formulation. The optimum formula also showed potential as an immunostimulant, as indicated by increased lymphocyte viability in the in vitro assay. These results confirm that the research objectives were achieved: an optimum, sensorially acceptable, antioxidant-rich nipa leaf beverage formula with preliminary evidence of immunomodulatory potential was developed.

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REFERENCE

Al-Baarri, A. N., Legowo, A. M., Pramono, Y. B., Rizqiati, H., Pratama, Y., Masykuri, Abduh, S. B. M., Nurwantoro, Septyaningrum, A., Saraswati, R. O., Puspitoasih, A. D., Lestari, F. P., & Widayat. (2019). Stability analysis of ginger (*Zingiber officinale*) emulsion affected by iota carrageenan [Conference session]. *IOP Conference Series: Earth and Environmental Science*, 309(1), 1-5. https://doi.org/10.1088/1755-1315/309/1/012038

Apirattananusorn, S. (2021). Effect of heating processes on physical and chemical properties of syrup from sap of nipa palm (*Nypa fruticans* Wurmb.). *Sugar Tech*, 23(4), 907–914. https://doi.org/10.1007/s12355-021-00950-2

Armi, F. S., Khairi, I., Hasanah, U., & Zuraidah, S. (2025). Formulation of functional beverage with antioxidant properties using nypa leaves (*Nypa fruticans*) [Conference session]. *BIO Web of Conferences*, 02012, 1–7. https://doi.org/10.1051/bioconf/202517602012

Aryani, F. P., Tajudin, T., & Setiyabudi, L. (2023). Uji aktivitas antioksidan infusa daun nipah (*Nypa fruticans* wurmb) dalam menurunkan kadar gula darah pada tikus jantan putih Wistar. *Sains Indonesiana*, 1(1), 12–19.

Assalam, S., T, G., Ikrawan, Y., & Nurfalia, I. (2023). Optimalisasi formula minuman olahan jeruk nipis (*Citrus aurantifolia*) dengan parameter karakteristik produk. *Jurnal Penelitian Pertanian Terapan*, 23(2), 288–301. https://doi.org/10.25181/jppt.v23i2.2923

Az-Zahra, F. R., Sari, N. L. W., Saputry, R., Nugroho, G. D., Sunarto, Pribadi, T., Setyawan, A. D. (2021). Review: traditional knowledge of the dayak tribe (borneo) in the use of medicinal plants. *Biodiversitas*. 22(10), 4633– 4647. https://doi.org/10.13057/biodiv/d221057.

Azamatkyzy, A., Zheldybayeva, A. A., Kyrykbai, K. R., & Kozhkenova, E. K. (2024). Use of natural phenolic dyes derived from plants as safe dyes in food production. *Bulletin of Shakarim University. Technical Sciences*, 2(14), 122–128. https://doi.org/10.53360/2788-7995-2024-2(14)-16

[BSN] Badan Standardisasi Nasional. (2014). SNI 3719:2014. Minuman sari buah. Jakarta: Badan Standardisasi Nasional.

[BSN] Badan Standardisasi Nasional. (2012a). SNI ISO 4831:2012. Mikrobiologi bahan pangan dan pakan – metode horizontal untuk deteksi dan enumerasi koliform – teknik angka paling mungkin. Jakarta: Badan Standardisasi Nasional.

[BSN] Badan Standardisasi Nasional. (2012b). SNI ISO 21527-1:2012. Mikrobiologi bahan pangan dan pakan – metode horizontal untuk enumerasi kapang dan khamir – bagian 1: teknik penghitungan koloni pada produk dengan aktivitas air lebih besar dari 0.95. Jakarta: Badan Standardisasi Nasional.

[BSN] Badan Standardisasi Nasional. (2026). SNI ISO 7251:2005 Amd.1:2023. Mikrobiologi bahan pangan dan pakan – Metode horizontal untuk deteksi dan enumerasi *Escherichia coli* terduga – teknik angka paling mungkin (APM) amendemen 1: penyertaan pengujian kinerja media biakan dan reagen. Jakarta: Badan Standardisasi Nasional.

Batubara, S. C., Sona, S. H., & Nurkolis, F. (2020). Developing of ready to drink based on rosella flower (*Hibiscus sabdariffa* l .), stevia leaf (stevia rebaudiana bertoni), and chia seed (*Salvia hispanica* l .). *Int. J. Creat. Res. Thoughts*. 8(12), 2282–2298.

Bezerra, M. A., Lemos, V. A., Novaes, C. G., de Jesus, R. M., Filho, H. R. S., Araújo, S. A., & Alves, J. P. S. (2019). Application of mixture design in analytical chemistry. *Microchemical Journal*, 152(1), 1-43. https://doi.org/10.1016/j.microc.2019.104336

Bhol, N. K., Bhanjadeo, M. M., Singh, A. K., Dash, U. C., Ojha, R. R., Majhi, S., Duttaroy, A. K., & Jena, A. B. (2024). The interplay between cytokines, inflammation, and antioxidants: mechanistic insights and therapeutic potentials of various antioxidants and anti-cytokine compounds. *Biomedicine and Pharmacotherapy*, 178, 117-177. https://doi.org/10.1016/j.biopha.2024.117177

Biswas, S., Biswas, S., PadmaPriya, G., Kaur, J., Pramanik, A., Islam, F., Tariq, F., Imran, A., & Shah, M. A. (2024). Role of nutraceuticals in viral infections as immunomodulators: a comprehensive review. *Journal of eFood,* 5(5), 1-16. https://doi.org/10.1002/efd2.70000

Cahyani, W. K. D., & Widodo, D. S. (2023). Uji mikrobiologi dan uji etanol pada minuman sari buah mengkudu di UD X. *Agrointek: Jurnal Teknologi Industri Pertanian*, 17(3), 571–576. https://doi.org/10.21107/agrointek.v17i3.17089

Dzulhijjah, W., Fitriani1, F., S, A., Arahman, N., Bilad, M. R., Rahmah, K., Akbar, E. H., & Raqib, M. (2024). Formulation optimization of bionanocomposite film based on polyvinyl alcohol/glycerol/cellulose nanocrystal from pineapple crown leave fibers using response surface methodology Formulation optimization of bionanocomposite film based on polyvinyl alcoh [Conference session]. *IOP Conf. Series: Earth and Environmental Science*, 1(1), 1-11, doi:10.1088/1755-1315/1290/1/012010

Elcistia, R., & Zulkarnain, A. K. (2018). Optimasi formula sediaan krim o/w kombinasi oksibenzon dan titanium dioksida serta uji aktivitas tabir suryanya secara *in vivo*. *Majalah Farmaseutik*, 14(2), 63–78. https://doi.org/10.22146/

farmaseutik.v14i2.42596

Fauzi, M., Novijanto, N., & Rarasati, D. P. (2019). Karakteristik organoleptik dan fisikokimia kopi jahe celup pada variasi tingkat penyangraian dan konsentrasi bubuk jahe. *Jurnal Agroteknologi*, 13(1), 1-9. https://doi.org/10.19184/j-agt.v13i01.8370

Hasanah, M., Dwi, D., Putra, P., Munarsih, E., & Hilma, H. (2025). Ekstraksi daun nipah (*Nypa frutican*) dengan beberapa metode dan uji aktivitas antioksidan. *Jurnal Ilmiah Bakti Farmasi*, 10(2), 61–67.

Irfan, Y., Taufik, Y., & Sutisna A. N. (2022). Formula optimization of black mulberry and canistel mixed juice using mixture d-optimal from design expert software. *Food Sci. Technol. J.*, 5(2), 81–95. https://doi.org/10.25139/fst.vi.4936.

[ISO] International Organization of Standardization. (2021). 6888-1:2021. Microbiology of the food chain – horizontal method for the enumeration of coagulase-positive staphylococci (*staphylococcus aureus* and other species) – part 1: method using baird-parker agar medium amendment 1. International Organization of Standardization.

[ISO] International Organization of Standardization. (2020). ISO-6579-1:2017/Amd 1:2020. Microbiology of the food chain — horizontal method for the detection, enumeration and serotyping of Salmonella — part 1: detection of *Salmonella spp*. amendment 1: broader range of incubation temperatures, amendment to the status of annex d, and correction of the composition of MSRV and SC. International Organization of Standardization.

Jaraee, J., Adeni, D. S. A., Bilung, L. M., & Azmin, P. A. (2023). Physicochemical and microbiological assessment of *Nypa fruticans* sap collected in Sarawak, Malaysia. *Food Research*, 6(1), 44–50. https://doi.org/10.26656/fr.2017.6(S4).004

Jiang, L., Zhang, G., Li, Y., Shi, G., & Li, M. (2021). Potential application of plant-based functional foods in the development of immune boosters. *Frontiers in Pharmacology*, 12(1), 1–18. https://doi.org/10.3389/fphar.2021.637782

Kahfi, M. A., Sutisna, A. N., Ainia, H., & Cecep, A. R. (2021). Using design expert d-optimal for formula optimization of functional drink that enriched with moringa leaf extract (*Moringa oleifera*) [Conference session]. *IOP Conference Series: Earth and Environmental Science,* 759(1), 1-10. https://doi.org/10.1088/1755-1315/759/1/012002

[KLHK] Kementerian Lingkungan Hidup dan Kehutanan. (2021). Peta mangrove nasional. Kementerian Lingkungan Hidup dan Kehutanan.

Khairi, I., Bahri, S., Ukhty, N., Rozi, A., & Nasution, M. A. (2020). Potensi pemanfaatan nipah (*Nypa fruticans*) sebagai pangan fungsional dan farmasetika. *Jurnal Laot Ilmu Kelautan*, 2(2), 60-69. https://doi.org/10.35308/jlaot.v2i2.3146

Kinanti, A. S., & Amelia, J. R. (2023). Karakteristik mutu minuman fungsional okra hijau dengan penambahan sukralosa dan ekstrak kayu manis. *Jurnal Teknologi Pangan dan Kesehatan*, 5(1), 16–25. https://doi.org/10.36441/jtepakes.v5i1.1797

Kiranawati, T. M., Mariana, R. R., & Efrinasari, D. N. (2022). Pengaruh rasio karagenan dan lemon terhadap aktivitas antioksidan dan sifat fisik *jelly drink* bunga telang. *Jurnal Agroindustri*, 12, 29–38. https://ejournal.unib.ac.id/index.php/agroindustri

Laklaeng, S. N., & Kwanhian, W. (2020). Immunomodulation effect of *Nypa fruticans* palm vinegar. *Walailak Journal of Science and Technology*, 17(11), 1200–1210. https://doi.org/10.48048/wjst.2020.10719

Michelle, N. Y. V., & Widyaningrum D. (2023). Optimization of *Chlorella* milk beverage formulation using D-optimal mixture design [Conference session]. *IOP Conf. Ser. Earth Environ. Sci.* 1169(1). https://doi.org/10.1088/1755-1315/1169/1/012086

Moonrungsee, N., Jakmunee, J., Peamaroon, N., Boonmee, A., Kasemsuk, T., Seeda, S., & Suwancharoen, S. (2022). Phytochemical and xanthine oxidase inhibitory activity in *Nypa fruticans Wurmb*. fruit extracts. *Trends in Sciences*, 19(4), 2583. https://doi.org/10.48048/tis.2022.2583

Muthmainnah, & sribianti. (2016). Nilai manfaat ekonomi tanaman nipah (*Nypa fruticans*) Desa Lakkang Kecamatan Tallo Kota Makassar. *Jurnal Hutan Tropis*, 4(2), 140–144. http://dx.doi.org/10.20527/jht.v4i2.3600

Nirmalasari, N., Ulfah, M., & Sasmito, E. (2013). Etanol kelopak bunga rosella (*Hibiscus sabdariffa L.*) terhadap proliferasi sel limfosit mencit galur swiss secara in vitro beserta identifikasi kandungan. *Jurnal Ilmu Farmasi Dan Farmasi Klinik*, 10(1), 23–30. https://doi.org/10.31942/jiffk.v10i1.1379

Noviana, E., Johannes, E., & Sjafaraenan. (2022). Bioactivity of *Nypa fruticans* leaves as a candidate for anticancer compounds against MCF-7 breast cancer cells. *International Journal of Applied Biology*, 6(1), 54–62. https://doi.org/10.20956/ijab.v6i1.19091

Oktaria, D., & Marpaung, M. P. (2023). Penetapan kadar flavonoid total dan aktivitas antioksidan ekstrak akar nipah (*Nypa fruticans wurmb*) dengan metode spektrofotometri UV-Vis. *Lantanida Journal*, 11(1), 36. https://doi.org/10.22373/lj.v11i1.16087

Oktaviani, S. M. (2022). Efektivitas ekstrak daun nipah (*Nypa fruticans Wurmb.*) sebagai imunostimulan pada ikan nila (*Oreochromis niloticus*). [*Skripsi*]. Universitas Sriwijaya.

Pablo, M. A. D., & Cienfuegos, G. Á. (2000). Modulatory effects of dietary lipids on immune system functions. *Immunology and Cell Biology*, 78(1), 31–39. https://doi.org/10.1046/j.1440-1711.2000.00875.x

Perez, M. G., Fourcade, L., Mateescu, M. A., & Paquin, J. (2017). Neutral Red versus MTT assay of cell viability in the presence of copper compounds. *Analytical Biochemistry*, 535, 43–46. https://doi.org/10.1016/j.ab.2017.07.027

Putra, I. G. A. M., Wrasiati, L. P., & Yuarini, D. A. A. (2023). Effect of adding lemongrass stalks on characteristics of herbal drink lemongrass - palm sugar as a functional food. *Journal of Functional Food and Nutraceutical*, 4(2), 103–110. https://doi.org/10.33555/jffn.v4i2.102

Putri, I. J.F. , Fauziyah, & Elfita. (2013). Aktivitas antioksidan daun dan biji buah nipah (*Nypa fruiticans*) asal pesisir Banyuasin Sumatera Selatan dengan metode DPPH. *Maskapai Jurnal*, 5(1), 16–21. http://masparijournal.blogspot.com

Raharjo, D., Dwi S, A., & Ardiyantoro, B. (2024). Antihiperuresemia flavonoid dari ekstrak etanol pelepah daun nipah (*Nypa fruticans.Wurmb*). *Jurnal Ilmiah Ibnu Sina (JIIS): Ilmu Farmasi Dan Kesehatan*, 8(3), 108–119. https://doi.org/10.36387/jiis.v8i3.1681

Sabariman, M., Sandrasari, D. A., Azni, I. N., Permata, T. D. (2021). Aplikasi metode *mixture design* pada formulasi minuman fungsional serbuk temulawak, jahe merah dan gula merah. *J. Teknol. Pangan*, 3(1), 41–48. https://doi.org/10.36441/jtepakes.v3i1.533

Said, N. S., & Sarbon, N. M. (2022). Physical and mechanical characteristics of gelatin-based films as a potential food packaging material: A review. *Membranes*, 12(442), 1-26. https://doi.org/10.3390/membranes12050442

Santoso, D., Yamin, M., & Makhrus, M. (2019). Penyuluhan tentang mitigasi bencana tsunami berbasis hutan mangrove di Desa Ketapang Raya Kecamatan Keruak Lombok Timur. *Jurnal Pengabdian Magister Pendidikan IPA*, 2(1), 12-16. https://doi.org/10.29303/jpmpi.v1i2.242

Setiyowati, P. A. I. (2020). Efek pericarpium manggis (*Garcinia mangostana* L.) terhadap protein spermatozoa epididimal mencit setelah dipapar 2-methoxyethanol. *Biology Education Science dan Technology*, 3(2), 69–77. https://doi.org/10.30743/best.v3i2.2809

Shangguan, Y., Ni, J., Jiang, L., Hu, Y., He, C., Ma, Y., Wu, G., & Xiong, H. (2023). Response surface methodology-optimized extraction of flavonoids from pomelo peels and isolation of naringin with antioxidant activities by Sephadex LH20 gel chromatography. *Current Research in Food Science*, 7(2), 1-8. https://doi.org/10.1016/j.crfs.2023.100610

Shwartz, V., & Dagan, I. (2018). Paraphrase to explicate: revealing implicit noun-compound relations [Conference session]. *Proceedings of the 56th Annual Meeting of the Association for Computational Linguistics.* 1200–1211.

Subaryono, Perangiangin, R., Suhartono, M. T., & Zakaria, F. R. (2017). Aktivitas imunomodulator oligosakarida alginat (OSA) yang dihasilkan dari alginat asal *Sargassum crassifolium*. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 20(1), 63–73. https://doi.org/10.17844/jphpi.v20i1.16434

Susanto, D. A., & Kristiningrum, E. (2021). Pengembangan standar nasional indonesia (SNI) definisi pangan fungsional. *Jurnal Standardisasi*, 23(1), 53. https://doi.org/10.31153/js.v23i1.851

Tias, F., Sabta Aji, A., Salfarino, R., & Aprilia, V. (2022). Antioxidant activity test on sungkai leaf (*Peronema canescens jack*) steeping drink with the addition of honey. *Journal of Global Nutrition*, 2(2), 151–158.

Vincenti, S., Mariani, M., Alberti, J. C., Jacopini, S., Caraffa, V. B. B. D., Berti, L., & Maury, J. (2019). Biocatalytic synthesis of natural green leaf volatiles using the lipoxygenase metabolic pathway. *Catalysts*, 9(873), 1-35. https://doi.org/10.3390/catal9100873

Wahyunita, N. S., Rahmi, S. L., & Gusriani, I. (2023). Karakteristik minuman fungsional ekstrak daun kersen (*Mutingia calabura l.*) dan madu. *Mediagro*, 19(2), 126–139. https://doi.org/10.31942/mediagro.v19i2.8398

Yahaya, S. F. R., Samsuddin, N., Mamat, S., Hod, R., Abdullah, N. Z., Rahman, N. A. A., & So'ad, S. Z. M. (2021). Determination of antioxidant compounds, proximate compositions and assessment of free radical scavenging activities of nypa fruticans wurmb. sap. *Pertanika Journal of Science and Technology*, 29(3), 2061–207 https://doi.org/10.47836/pjst.29.3.30.

Zulianti, D., Gita, P., Safitri, A., & Chasanah, U. (2023). Efektifitas imunomostimulant ekstrak etanolik umbi talas jepang (*Colocasia esculenta var.* antiquorum) sebagai peningkat sistem imun melalui uji *in-vivo. Buletin Anatomi Dan Fisiologi*, 8(1), 20–28. https://doi.org/10.14710/baf.8.1.2023.20-28