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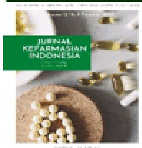

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Nutraceutical Drink as Immune Booster Made from Red Ginger Combined with Lime and Honey to Prevent Viral Infection

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ABSTRACT

Nutraceutical drinks are increasingly being chosen by the public as alternative medicine or as supportive therapy for disease prevention, offering minimal side effects with long-term use. This trend has gained momentum, mainly due to the rising incidence of COVID-19 and the emergence of highly transmissible variants. The combination of red ginger, lime juice, and honey will produce a healthy drink preparation containing antioxidants with vitamin C levels as an immune booster. This study aims to formulate a nutraceutical drink made from red ginger ((*Zingiber officinale* var. *rubrum*) combined with lime (*Citrus aurantifolia*) and honey also determine the antioxidant activity and the highest vitamin C levels. Physical stability tests include organoleptic, homogeneity, pH, specific gravity, viscosity, and accelerated stability tests using freeze-thaw cycling method. The antioxidant activity test using DPPH (1,1-diphenyl-2-picrylhydrazyl) method and analysis of vitamin C levels using the UV-Vis spectrophotometry method. The formulation was made with variations of lime juice FI (1%), FII (2%), and FIII (4%). The results of physical stability of the three formulations are not much different but based on organoleptic, FI has the best taste which is not too spicy and we are sure that most people will like it. Meanwhile, the highest antioxidant activity was possessed by FIII with an IC50 value of 11.21 ppm, which is classified as very strong and has the highest vitamin C content of 9103.88 µg/mL.

***Keywords*:** Antioxidant; Honey; Lime; Nutraceutical; Vitamin C

INTRODUCTION

The COVID-19 pandemic has heightened public interest in alternative medicine and nutraceuticals1., with Indonesia's rich biodiversity offering promising sources like ginger, cinnamon, and Moringa oleifera[2,3]. Despite their potential, regulatory challenges and uncertainties surrounding their efficacy against COVID-19 variants remain[4,5]. While vitamin C and zinc are widely promoted to boost immunity, their effectiveness against the virus is not fully established[6]. Healthcare professionals are key in guiding patients through the benefits and risks of these treatments[7].

Red ginger (*Zingiber officinale* var. rubrum), rich in bioactive compounds such as gingerols, shogaols, and essential oils has emerged as a potential antiviral agent against SARS-CoV-2[8]. Studies indicate that red ginger possesses strong antibacterial, antioxidant, and anti-inflammatory properties, and traditional red ginger drinks may reduce COVID-19 severity[9]. Computational studies have also predicted that ginger compounds could inhibit key inflammatory receptors involved in severe COVID-19 cases[10]. However, further research is necessary to confirm its antiviral efficacy[11].

Lime (Citrus aurantifolia) is a versatile fruit known for its high flavonoid, terpenoid, and essential oil content, contributing to its antibacterial, antioxidant, and anti-inflammatory properties[12,13]. Honey, with its proven antimicrobial[14], and immunomodulatory effects[15], further complements these ingredients in promoting health and well-being[16].

Research on antioxidant herbal drinks has demonstrated the potency of red ginger, with variations in antioxidant activity attributed to formulation methods[17]. Combining red ginger, lime juice, and honey presents a promising nutraceutical option for managing oxidative stress, improving metabolic profiles, and addressing conditions such as diabetes, hyperuricemia, and cholesterol[18, 19].

Despite the promising potential of these ingredients, however, no research has specifically determined the optimal formulation of red ginger extract, lime juice, and honey that maximizes antioxidant and vitamin C levels.

This study aims to develop a nutraceutical beverage combining red ginger, lime juice, and honey, focusing on its potential to boost immunity and offer health benefits, especially for individuals vulnerable to COVID-19, including those with diabetes and obesity. Such a formulation supports the broader effort to utilize Indonesia's local resources for health product innovation.

METHODS

Material

The tools utilized in this study include a variety of laboratory equipment and instruments: 100-mesh sieves, test tubes, microscope slides, Erlenmeyer flasks, evaporating dishes, analytical balances (CHQ-DJ series) DJ303A with a capacity of 300 g x 0.001 g, stirring rods (Pyrex), spatulas (Pyrex), porcelain dishes (Pyrex), 60 ml clear glass bottles, hot plates, water bath (Memmert), pH meters (ATC), preparation glassware (Iwaki), pycnometers (Iwaki), Brookfield viscometer, and a UV-Vis spectrophotometer (Spectroquant Pharo 300).

The study utilized the following ingredients: red ginger powder extract (PT. Palapa Muda Perkasa), lime juice (sourced from Kebayoran Lama Market), honey powder (PT. Haldin Pacific Semesta), sucrose (PT. Palapa Muda Perkasa), sodium benzoate (Gloria Interchem), standard ascorbic acid (Sigma Aldrich), and DPPH (Sigma Aldrich).

Formulation of nutraceutical beverage preparations

The nutraceutical drink is formulated using 3% red ginger extract for all samples, labeled FI, FII, and FIII. The concentration of lime juice varies between formulations: 1% in FI, 2% in FII, and 4% in FIII. The process begins by boiling water, followed by adding a mixture of powdered honey and sucrose in equal amounts to all formulations. The mixture is stirred until completely dissolved, then boiled at a temperature of 80ºC for 5 minutes to ensure proper mixing. The prepared formulation is transferred into a dark, airtight glass bottle to protect it from light and prevent degradation. The stability of beverage storage was tested under three conditions: room temperature (25±2ºC), cold storage (4±2ºC), and high temperature (40±2ºC).

Physical evaluation of nutraceutical drink preparations

Organoleptic test

Organoleptic testing, a critical food and beverage industry method, relies on human sensory evaluation to assess product quality through sight, smell, taste, touch, and hearing[20]. Both trained and untrained panels are used to measure these sensory responses[21]. As a scientific discipline, sensory evaluation employs statistically robust techniques such as

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quantitative descriptive analysis[22], preference ranking, and hedonic scales to ensure reliable and consistent results[21]. Despite its importance, traditional organoleptic testing methods face challenges in procedure design and analysis, which can be addressed through improved methodologies[20]. Sensory evaluation can also be structured through ontologies, linking food properties to sensory perceptions. While there are limitations, human evaluators remain crucial for maintaining sensory quality in products.

pH level test

The pH value was measured using a digital pH meter, ensuring accurate and consistent readings throughout the analysis.

Homogeneity test

The homogeneity test assesses whether the active substance and the main ingredients are uniformly distributed throughout the liquid preparation. Additionally, this test is conducted to detect the presence of any undissolved particles or sediment that could indicate incomplete mixing within the formulation.

Specific gravity test

Specific gravity is calculated by comparing the mass of a nutraceutical drink (Wsample) with water mass (Wwater) at the same temperature a nd volume measured using a pycnometer. The formula used is as follows.

$$Specific\ gravity = \frac{Wsample}{Wwater}$$

Viscosity test

Viscosity and flow properties were measured using a Brookfield Viscometer with an appropriate spindle number for the formulation.

Accelerated stability test

The stability of the beverage formulations was assessed using freeze-thaw cycling. Samples were subjected to temperatures of 4°C and 40°C for 24 hours each, completing six cycles. Observations were made for organoleptic properties, homogeneity, and pH.

Antioxidant activity test

The antioxidant activity of the nutraceutical beverages was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and a UV-Vis spectrophotometer. A 1 mL beverage sample was diluted to 50 µg/mL with methanol in a volumetric flask. Serial dilutions (2, 4, 6, 8, 10, and 12 µg/mL) were prepared and brought to volume with methanol. Each solution was then mixed with 2 mL of a 50 µg/mL DPPH solution and left to react for 30 minutes at room temperature.

The percentage of free radical scavenging activity of the health drink formulation was calculated using the following formula:

$$\text{Percentage of inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

Acontrol: the absorbance of the DPPH solution without the sample
Asample: the absorbance of the DPPH solution with the sample.

Analysis of vitamin C levels in nutraceutical drink preparations

Vitamin C levels were quantified using UV-Vis spectrophotometry. A 100 ppm ascorbic acid standard solution was prepared by dissolving 10 mg of ascorbic acid in 100 mL of distilled water. Serial dilutions were made to achieve concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, and 30 ppm. The absorbance of these solutions was measured between 200-300 nm, with the highest absorption observed at 245 nm.

For the drink samples (FI, FII, FIII), 1 mL of each sample was filtered, transferred to a 100 mL volumetric flask, diluted with distilled water, and homogenized using a sonicator. For further analysis, 1 mL of each stock solution was placed into separate 5 mL volumetric flasks and diluted to the mark with distilled water to achieve the required concentration.

RESULTS AND DISCUSSION

Nutraceutical drink formulation from red ginger combination of lime and hone

Table 1. Formulation of nutraceutical drink preparations

Material	Formulation			Function
	FI	FII	FIII	
Red Ginger Extract	3	3	3	Active Substance
Honey Powder	16	16	16	Active Substance
Lime Juice	1	2	4	Active Substance
Sucrose	5	5	5	Sweetener
Sodium Benzoate	0.1	0.1	0.1	Preservative
Aquadest	105	104	102	Solvent

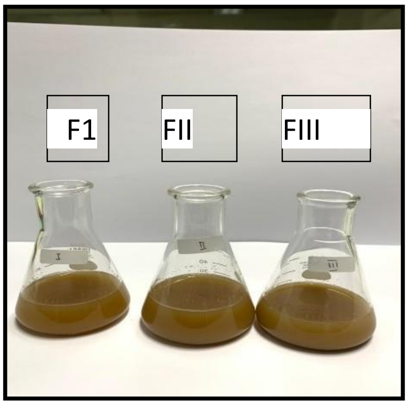

Figure 1. Results of nutraceutical drink

Three nutraceutical beverage formulations were developed using red ginger, lime, and honey. To determine the optimal lime juice concentration, the formulations varied in lime content: 1% in Formulation I, 2% in Formulation II, and 4% in Formulation III.

Physical evaluation of nutraceutical drink preparations

Organoleptic test

The organoleptic test results are presented in Table 2. Based on the observations from Table 2, the organoleptic tests conducted over four weeks at varying storage conditions—room temperature (25 ± 2ºC), cold temperature (4 ± 2ºC), and hot temperature (40 ± 2ºC)—on nutraceutical drink preparations made from red ginger, lime, and honey, with three different lime juice concentrations, reveal consistent characteristics.

The preparations maintained their distinctive red ginger aroma, liquid form, and slightly spicy, sweet-sour taste throughout the storage period, with no significant changes observed. However, by the 4th week, notable color changes were detected, influenced by both temperature and storage conditions. Higher concentrations of lime juice resulted in a more intense color, which could be attributed to the oxidation of vitamin C in the lime juice[23]. Harahap (2016) similarly found that in the production of red ginger powder with added pineapple skin extract, there was a significant impact on the color, likely due to the caramelization process during instant powder preparation, which produced a consistent brown hue[24].

Mohd-Hanif et al. (2016) observed that UVC irradiation of lime juice affected various physicochemical properties, with changes more prominent at higher dosages [25].

Non-enzymatic browning changes in lemon juice can also be caused by oxidation of ascorbic acid, with the intensity of browning being influenced by pH and the presence of amino acids[26]. These studies collectively demonstrate that colour change in orange juice and related products is a complex process influenced by multiple factors, including processing methods, additives, as well as storage condition. This suggests that the color changes observed in our study may be linked to the oxidation of lime juice's vitamin C content.

Overall, the organoleptic evaluations indicate that all formulations exhibited significant color changes by the 4th week of storage, regardless of the temperature. This suggests that despite the addition of preservatives, temperature and storage duration play critical roles in affecting the visual quality of the beverage. To mitigate these effects, the inclusion of antioxidants in the formulation could be beneficial in preventing oxidative color degradation.

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Table 2. Organoleptic observation results of nutraceutical drink preparations

Week	Organoleptic	FI	FII	FIII
1	Smell	Ginger	Ginger	Ginger
	Form	Liquid	Liquid	Liquid
	Flavor	Slightly Spicy Sweet Sour	Sweet and Sour Spicy	Very Spicy Sweet Sour
	Color	*Tawny Olive TCX Light Chocolate (16-0953)*	*Tawny Olive TCX Light Chocolate (16-0953)*	*Tawny Olive TCX Light Chocolate (16-0953)*
2	Smell	Ginger	Ginger	Ginger
	Form	Liquid	Liquid	Liquid
	Flavor	Slightly Spicy Sweet Sour	Sweet and Sour Spicy	Very Spicy Sweet Sour
	Color	*Tawny Olive TCX Light Chocolate (16-0953)*	*Tawny Olive TCX Light Chocolate (16-0953)*	*Tawny Olive TCX Light Chocolate (16-0953)*
3	Smell	Ginger	Ginger	Ginger
	Form	Liquid	Liquid	Liquid
	Flavor	Slightly Spicy Sweet Sour	Sweet and Sour Spicy	Very Spicy Sweet Sour
	Color	*Tawny Olive TCX Light Chocolate (16-0953)*	*Tawny Olive TCX Light Chocolate (16-0953)*	*Tawny Olive TCX Light Chocolate (16-0953)*
4	Smell	Ginger	Ginger	Ginger
	Form	Liquid	Liquid	Liquid
	Flavor	Slightly Spicy Sweet Sour	Sweet and Sour Spicy	Very Spicy Sweet Sour
	Color	*Tawny Olive TCX Light Chocolate (16-0953)*	*Tawny Olive TCX Light Chocolate (16-0953)*	*Tawny Olive TCX Light Chocolate (16-0953)*

pH level test

The results of the pH Levels in room temperature are presented in Table 3 and Figure 2. Based on Table 3 and Figure 2, the pH measurements of the combination drink preparations with three variations in lime juice concentrations, stored at room temperature (25 ± 2°C) over a 4-week period, indicate a gradual decrease in pH from week 1 to week 4. The average pH values recorded were 2.865 for Formulation I (FI), 2.713 for Formulation II (FII), and 2.679 for Formulation III (FIII).

Table 3. pH Levels in room temperature

F	pH level				
	W1	W2	W3	W4	Average
FI	3.284	3.024	2.845	2.307	2.865
FII	3.264	2.734	2.567	2.288	2.713
FIII	3.248	2.734	2.492	2.245	2.679

F: Formulation
W1:Week1, W2:Week2, W3:Week3, W4:Week4

The results of the pH Levels in Low temperature are presented in Table 4 and Figure 3

Table 4. pH Levels in Low temperature

F	pH test				
	W1	W2	W3	W4	Average
FI	3.284	3.037	2.986	2.861	3.042
FII	3.264	2.969	2.874	2.575	2.920
FIII	3.248	2.602	2.437	2.184	2.617

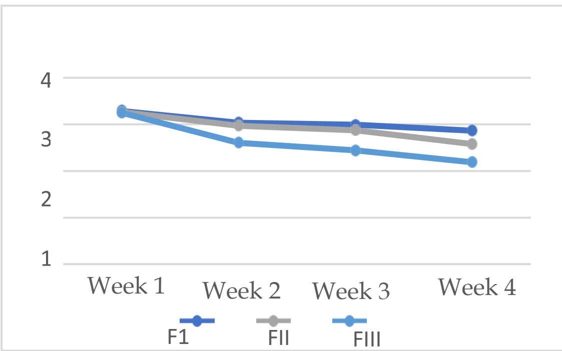

Figure 2. pH level curve of nutraceutical beverage preparation at room temperature

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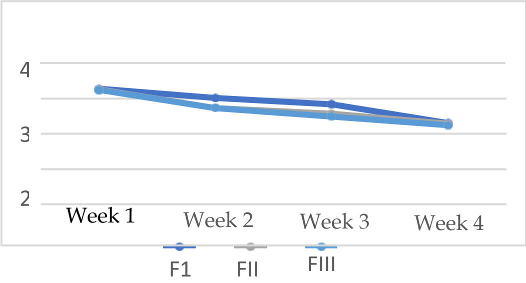

Figure 3 pH Level curve for nutraceutical beverage preparations at low temperatures

In Table 4 and Figure 3, pH measurements of nutraceutical beverages stored at low temperatures (4 ± 2°C) over four weeks show a consistent decrease in pH from week 1 to week 4. The average pH values were 3.042 for Formulation I (FI), 2.920 for Formulation II (FII), and 2.617 for Formulation III (FIII).

Table 5. pH Levels in Hot Temperature

F	pH test				
	W1	**W2**	**W3**	**W4**	**Average**
FI	3.284	3.007	2.734	2.922	2.986
FII	3.264	2.610	2.452	2.571	2.724
FIII	3.248	2.571	2.386	2.132	2.584

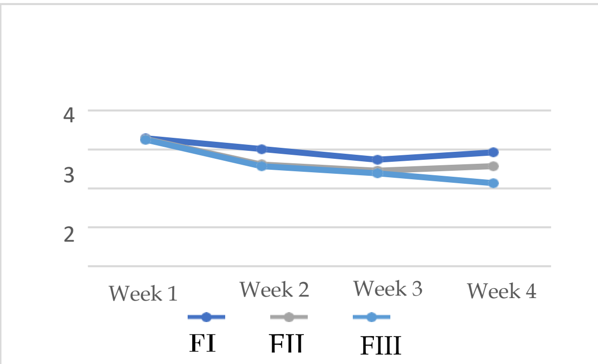

Figure 4. pH level curve of nutraceutical beverage preparation at a hot temperature

Based on Table 5 and Figure 4 show that at hot temperatures (40 ± 2°C), pH decreased for FIII from week 1 to week 4. For FI and FII, pH decreased until the 3rd week, then slightly increased in the 4th week. The average pH values recorded were 2.986 for FI, 2.724 for FII, and 2.584 for FIII. Overall, storage at all temperatures resulted in minor pH changes, with values consistently between 2 and 3.

Research by Valentin GF (2018) reported that red ginger and lime juice typically have pH values between 4 and 5.4[27]. Their study found that a red ginger herbal drink had a pH of 5.57, which is within the acceptable range of 4 to 7.5[28]. Similarly, Hamidi F, Effendi R, and Hamzah F (2016) observed that adding 15% lime juice significantly impacted the pH, taste, color, aroma, and overall acceptability of under-fruit syrup[29]. High lime concentrations introduced a sour taste and lowered the pH, while lower concentrations had minimal effects, preserving anthocyanin stability [29].

In our study, the pH values of the three formulations, which ranged between 2 and 3, were consistent with previous findings showing that lime juice lowers pH. Increased lime juice concentrations resulted in more acidic pH levels. However, these values fall below the recommended pH range, making the beverages excessively acidic. While highly acidic beverages with a pH below 4 may contribute to weight loss[30], they also pose a risk of dental caries, enamel erosion[31-33] and gastric issues[30], necessitating careful consideration of acidity in product formulations.

Homogeneity test

The results of the Homogeneity test are presented **in Table 6** and **Figure 5.**

Table 6. Homogeneity test of preparations

F	Foam	Homogeneity Test
FI	Liquid	Not Homogeneous
FII	Liquid	Not Homogeneous
FIII	Liquid	Not Homogeneous

A B

Figure 5. Homogeneity Test Results of Preparations

A: The preparation looks coarse and grainy.
B: The preparation looks like sediment.

Based on Table 6 and Figure 5, the results of observations of the homogeneity test of the beverage preparation show that there are coarse grains on the object glass and sediment on the bottle, so it can be concluded that the preparation is not homogeneous. According to research by Forestryana D and Rahman SY (2020), determining the mixing of materials is subjective, where a beverage preparation formulation is declared homogeneous with the parameters of no sediment or coarse grains[34]. Therefore, the preparation must be shaken first before use.

Specific weight test

Table 7. Test results for type of dosage weight

Formulation	Specific gravity (g/mL)
FI	1.36
FII	1.40
FIII	1.45

Based on Table 7, the specific gravity test using a pycnometer shows that the three formulas have specific gravities ranging from 1.36 to 1.45 g/mL. This finding aligns with Zakaria N et al. (2024), who reported that the specific gravity of SPKKJ falls between 1.021 and 1.028 g/mL, which meets the polyherbal syrup's specific gravity requirements of 1.02 to 1.19 g/mL [35]. Besides that, Herdaningsih S and Kartikasari D (2022) and Susanti SFE (2023) indicated that a desirable specific gravity for syrup preparations is greater than 1.2 g/mL [36,37]. Thus, it can be concluded that all three formulas satisfy the required specific gravity standards.

Viscosity test

Based on Table 8, viscosity tests conducted during the first and fourth weeks reveal that all three formulas have viscosity values between 190 and 195 cP, well above the minimum requirement of >1 cP[28]. Notably, Formula III shows the highest viscosity due to its greater lime juice concentration. This aligns with the findings of Verawati et al. (2023), who demonstrated that factors such as white ginger concentration, high-carbohydrate tape, water, and other spices notably impact formulation viscosity[38].

Table 8. Viscosity test of preparations

Formulation	Average viscosity (cP)
FI	190
FII	192
FIII	195

Accelerated stability test

The accelerated stability test results using the Freeze-Thaw method are presented in Table 9. Based on Table 9, the accelerated stability testing of the three ginger formulations using the freeze-thaw method revealed their stability during storage, as evidenced by the lack of significant changes in organoleptic properties, pH, specific gravity, or viscosity. All preparations exhibited a distinctive ginger taste and brown color.

Organoleptically, FIII was characterized by a notably sour taste due to its higher lime juice concentration, aligning with Ronauli SF et al.'s (2021) findings. FI and FII displayed a milder, spicy-sour-sweet taste [39]. While pH levels decreased slightly in all formulas with each cycle, they remained within the acceptable range of 3.00-6.00 (Zakaria N et al., 2024) for polyherbal syrups and 3-8 (general literature) for herbal drinks[35]. This indicates good pH stability[38].

Similarly, specific gravity values exhibited minor decreases but stayed within the specified range, confirming their stability. This aligns with Zakaria N et al.'s (2024) findings for SPKKJ formulations[35].

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Table 9. Accelerated Stability Test Results (Freeze-Thaw Method)

F	T(^{o}C)	R	Flavor	Smell	Color	pH	Spesific gravity(g/mL)	Viscosity
FI	4	1	Slightly Spicy Sweet Sour	Ginger	Brown	3.886	1.38	190
		2	Slightly Spicy Sweet Sour	Ginger	Brown	3.884	1.37	190
		3	Slightly Spicy Sweet Sour	Ginger	Brown	3.884	1.38	190
	40	1	Slightly Spicy Sweet Sour	Ginger	Brown	3.708	1.36	191
		2	Slightly Spicy Sweet Sour	Ginger	Brown	3.704	1.34	191
		3	Slightly Spicy Sweet Sour	Ginger	Brown	3.703	1.36	191
FII	4	1	Sweet and Sour Spicy	Ginger	Brown	3.847	1.42	192
		2	Sweet and Sour Spicy	Ginger	Brown	1.40	192	192
		3	Sweet and Sour Spicy	Ginger	Brown	3.848	1.41	192
	40	1	Sweet and Sour Spicy	Ginger	Brown	3.675	1.41	194
		2	Sweet and Sour Spicy	Ginger	Brown	3.676	1.38	194
		3	Sweet and Sour Spicy	Ginger	Brown	3.672	1.39	194
FIII	4	1	Very Spicy Sweet Sour	Ginger	Brown	3.721	1.46	195
		2	Very Spicy Sweet Sour	Ginger	Brown	3.726	1.45	195
		3	Very Spicy Sweet Sour	Ginger	Brown	3.532	1.45	196
	40	1	Very Spicy Sweet Sour	Ginger	Brown	3.532	1.45	196
		2	Very Spicy Sweet Sour	Ginger	Brown	3.545	1.44	196
		3	Very Spicy Sweet Sour	Ginger	Brown	3.537	1.44	196

F: Formulation, T(^{o}C): Temperaturen(celcius), R: Repeat

Viscosity measurements showed a slight increase in all formulas with each cycle, remaining within the required range. As per Suhendy H (2021), a viscosity value greater than one cP is desirable for red ginger antioxidant herbal drinks[28]. The higher viscosity of FIII is attributed to its higher lime juice content, which is consistent with the understanding that less water leads to thicker preparations (general literature)[38].

While all three formulations meet the physical evaluation and stability requirements, they are not entirely homogeneous. Among them, FI demonstrates the most favorable pH and viscosity values. It is recommended to shake the preparations before use.

Analysis of antioxidant activity of nutraceutical beverage preparations

This study investigated the antioxidant activity of nutraceutical drink preparations containing red ginger extract, lime juice, and honey using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) method.

The DPPH method, a simple and rapid technique requiring minimal samples, measures free radical scavenging activity by monitoring the color change from purple to yellow using a UV-Vis spectrophotometer[40]. A higher degree of color change indicates greater effectiveness in neutralizing free radicals[41]. Vitamin C, a well-established antioxidant with a high free radical scavenging capacity, was used as a positive control. Its free hydroxyl group facilitates the capture of free radicals [42].

Figure 6 presents linear regression equations for each vitamin C type, with corresponding R2 values indicating strong correlations between the independent and dependent variables (R2 values range from 0.992 to 0.9997). The equations are as follows: Vitamin C I: $y = 5.1891x - 0.3387$; Vitamin C II: $y = 4.6708x - 8.257$; Vitamin C III: $y = 4.7404x - 5.7802$; Vitamin C IV: $y = 4.7093x - 2.7763$.

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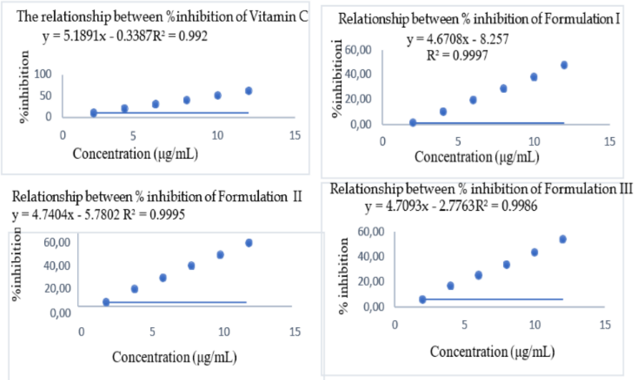

Figure 6. Relationship curve of antioxidant activity: vitamin C, formulation I, and formulation II

Table 10. Results of antioxidant activity analysis of various preparations

F	IC_{50} value (ppm)	Category
FI	12.47	vey strong
FII	11.77	vey strong
FIII	11.21	vey strong

The results of this study indicate that the antioxidant activity of the three formulations is classified as very strong, with an IC_{50} value below 50 ppm. Among the three formulas, FIII exhibited the most potent antioxidant activity with an IC_{50} value of 11.21 ppm. This superior performance may be attributed to its higher lime juice content, known for its rich composition of alkaloids, flavonoids, tannins, and phenols, which contribute to free radical inhibition. In addition to their antioxidant properties, these formulas likely possess immunomodulatory effects [43]. Lime peel extract, for instance, has been shown to enhance macrophage activity and phagocytic capacity. Honey, with its phenol, peroxide, and non-peroxide compounds, is also known for its broad-spectrum antibacterial and immunomodulatory activities[44].

Recent studies have explored the antioxidant activity of various plant extracts, particularly from the Zingiberaceae family. Nurhidayati Harun (2022) found that a combination of ginger, turmeric, galangal, and *Kaempferia galanga* L extracts exhibited potent antioxidant activity, with an IC_{50} value of 23.5 ppm for their most effective formula[45]. Red ginger (*Zingiber officinale* var. rubrum) has shown promising results, with Jesica Merry (2024) reporting IC_{50} values of 7.30 and 11.90 for digestion maceration and maceration extraction methods, respectively[46]. D. Aulifa et al. (2022) developed effervescent granules from red ginger extract, demonstrating moderate antioxidant activity with an IC_{50} of 283.28ppm[18]. Kristiani et al. (2024) investigated polyherbal combinations, finding that formulations containing *Zingiber officinale*, *Cymbopogon citratus*, and *Phyllanthus reticulatus* exhibited strong antioxidant capacity with IC_{50} values below 50 µg/ml for specific combinations[47]. Furthermore, Herlina et al. (2022) found lime-infused water to possess potent antioxidant properties (IC_{50} = 24.39 ppm)[48].

Analysis of vitamin C levels in nutraceutical drink preparations

Vitamin C levels were quantified using UV-Vis spectrophotometry at a wavelength of 245 nm. This approach aligns with previous research by Sudiarta et al. (2021), who analyzed vitamin C in packaged supplement drinks using UV-Vis spectrophotometry with distilled water. Their study found a maximum absorbance at 243 nm and a highest vitamin C concentration of 6646.28 mg/L with an 89.66% suitability value[49].

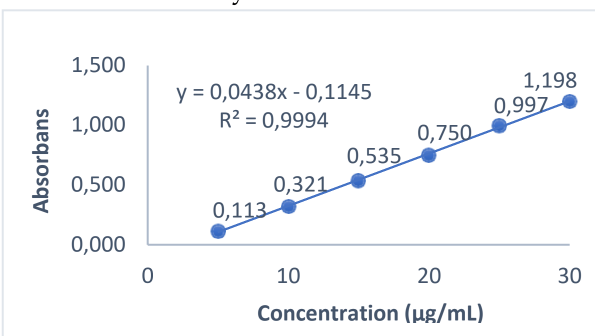

Figure 10. Standard curve of raw vitamin C

The sequential vitamin C absorbance values (0.113, 0.321, 0.535, 0.750, 0.997) were plotted and fitted to a linear regression equation: $y = 0.0438x - 0.1145$ ($R^2 = 0.9994$). This strong correlation indicates a linear relationship between absorbance and vitamin C concentration.

Table 11. Results of vitamin C level analysis in preparations

F	R	[A]	ppm value	average	Vit C level	
					µg/mL	in 5mg/mL
FI	1	0,345	10,49	10,49	5245,43	26,23
	2	0,345	10,49			
F2	1	0,365	10,95	10,95	5479,45	27,4
	2	0,366	10,97			
F3	1	0,682	18,18	18,2	9103,88	45,52
	2	0,684	18,23			

F: Formulation, R: Repeat, [A]: Absorbance

Based on Table 11, analysis of vitamin C levels in the three formulations showed that the highest value was found in FIII at 9103.88 µg/mL compared to FI (5245.43 µg/mL) and FII (5479.45 µg/mL). This is in line with research by Wahyani and Fera (2022), the results of the analysis of the highest average vitamin C content found in red ginger powder at 18.11 mg/100 g compared to large ginger and emprit ginger[50]. Ginger has a relatively high vitamin C content compared to with other rhizome plants[51]. The vitamin C content in the ginger plant is not a high dose of vitamin C. Therefore, it is more used as an alternative for prevention and immune booster[52]. The vitamin C content in 100 g of fresh ginger is 7.7 mg[53.]

Vitamin C plays a crucial role in immune function, particularly in neutrophils. It accumulates in phagocytic cells, enhancing chemotaxis, phagocytosis, and reactive oxygen species generation, leading to improved microbial killing[54]. Vitamin C is also essential for neutrophil apoptosis and clearance by macrophages, reducing tissue damage Activated neutrophils can rapidly accumulate vitamin C to concentrations as high as 14 mM through a recycling mechanism involving oxidation, translocation, and intracellular reduction[55.] Vitamin C deficiency impairs immunity and increases susceptibility to infections, while infections deplete vitamin C levels due to increased inflammation and metabolic demands[56]. Vitamin C deficiency has the potential to result in impaired immunity and higher susceptibility to infection. In contrast, infection significantly impacts vitamin levels C due to increased inflammation and metabolic needs[57].

The research regarding the effect of adding lime juice to red ginger powder on organoleptic acceptability and vitamin C levels, where the antioxidant content is relatively high from red ginger plus vitamin C and flavonoids in lime, was carried out on 25 panellists, showing the influence in terms of colour, aroma. There is no real impact. Then, of the three treatments, the red ginger powder product had the highest levels of vitamin C, namely F3 at 1.06 mg[39].

Several researchers have explored various ginger and orange preparations' vitamin C content and antioxidant properties. Red ginger was found to have higher vitamin C levels (6.372%) compared to emprit ginger (4.338%) in garlic-based preparations[58]. Another study reported higher vitamin C content in red ginger powder (18.11 mg/100g) compared to emprit ginger powder (10.21 mg/100g)[59]. Adding emprit ginger powder to sweet orange peel herbal tea significantly increased the vitamin C content, with a 20% addition resulting in 25.56 mg/g tea powder[60]. Analysis of vitamin C using iodimetry in various food samples showed levels ranging from 0.044 to 2.286 mg/g, highlighting the variability in vitamin C content in various sources[61]. These studies demonstrate the potential of ginger and citrus preparations as valuable sources of vitamin C and antioxidants.

This study results show that FIII, with the highest concentration of lime juice, has the highest vitamin C content, namely 9103.88 µg/mL, so when using the preparation, there is 45.52 mg/5 mL.

CONCLUSION

This study shows the potential of nutraceutical beverages containing red ginger and lime juice as functional beverages with antioxidant and vitamin C properties. Among the three formulas evaluated, FIII showed the highest concentration of lime juice, the most potent antioxidant activity, and the highest vitamin C content. Increasing the lime juice content can significantly increase this beverage's antioxidant and vitamin C benefits.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

All authors conceptualized, designed, analyzed the data experiment, and edited the manuscript. All authors have read and approved the final manuscript.

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