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Formulation and Antioxidant Activity of Old Coconut Water Syrup (*Cocos nucifera* L) and Red Fruit Extract (*Pandanus conoideus* L) Using the DPPH Method

Formulasi Dan Aktivitas Antioksidan Sirup Air Kelapa Tua (*Cocos nucifera* L) Dan Ekstrak Buah Merah (*Pandanus conoideus* L) Dengan Metode DPPH

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Abstrak

Background: Mature coconut water (*Cocos nucifera* L.) has the potential as a health drink but is often discarded as waste. Red fruit (*Pandanus conoideus* L.) is an endemic plant of Papua that is rich in antioxidants. The combination of both is expected to produce a beneficial herbal syrup preparation. **Objective:** To formulate a combination syrup of mature coconut water and red fruit extract and to determine its antioxidant activity using the DPPH method. **Methods:** Red fruit extract was obtained by maceration using 96% ethanol. Phytochemical screening was performed on both ingredients. The syrup was prepared with the addition of sugar, citric acid, vanilla, and CMC. Physical evaluation included organoleptic, homogeneity, pH, viscosity, and clarity tests. Antioxidant activity was tested using the DPPH method at a wavelength of 517 nm using a UV-Vis spectrophotometer. **Results:** Phytochemical screening showed that mature coconut water contains alkaloids, while red fruit extract contains flavonoids, tannins, saponins, and alkaloids. The syrup evaluation met SNI requirements: organoleptic (reddish-orange color, characteristic odor, sweet taste), homogeneous, pH 5–6, viscosity 250 mPa·s, and clear. The IC50 values of the syrup in three consecutive replicates were 550.34 µg/mL, 2282.3 µg/mL, and 2863.4 µg/mL, which are classified as weak antioxidant activity (IC50 > 200 ppm). **Conclusion:** The combination syrup of mature coconut water and red fruit extract exhibits weak antioxidant activity but meets the physical quality standards of the preparation. This preparation has the potential to be developed as a herbal health drink.

Kata Kunci: Sirup, Air kelapa tua, Buah merah, Antioksidan.

Abstrak

Latar Belakang: Air kelapa tua (Cocos nucifera L.) berpotensi sebagai minuman kesehatan namun sering menjadi limbah. Buah merah (Pandanus conoideus L.) adalah tanaman endemik Papua yang kaya antioksidan. Kombinasi keduanya diharapkan dapat menghasilkan sediaan sirup herbal yang bermanfaat. **Tujuan:** Memformulasikan sirup kombinasi air kelapa tua dan ekstrak buah merah serta mengetahui aktivitas antioksidannya dengan metode DPPH. **Metode:** Ekstrak buah merah diperoleh dengan maserasi etanol 96%. Dilakukan skrining fitokimia pada kedua bahan. Sirup dibuat dengan tambahan gula, asam sitrat, vanila, dan CMC. Evaluasi fisik meliputi uji organoleptik, homogenitas, pH, viskositas, dan kejernihan. Aktivitas antioksidan diuji dengan metode DPPH pada λ 517 nm menggunakan spektrofotometer UV-Vis. **Hasil:** Skrining fitokimia menunjukkan air kelapa tua mengandung alkaloid; ekstrak buah merah mengandung flavonoid, tanin, saponin, dan alkaloid. Evaluasi sirup memenuhi syarat SNI: organoleptik (warna oranye kemerahan, bau khas, rasa manis), homogen, pH 5–6, viskositas 250 mPa·s, dan jernih. Nilai IC50 sirup pada tiga replikasi berturut-turut adalah 550,34 µg/mL; 2282,3 µg/mL; dan 2863,4 µg/mL, yang tergolong aktivitas antioksidan **lemah** (IC50 > 200 ppm). **Kesimpulan:** Sirup kombinasi air kelapa tua dan ekstrak buah merah memiliki aktivitas antioksidan lemah namun memenuhi standar mutu fisik sediaan. Sediaan ini berpotensi dikembangkan sebagai minuman kesehatan herbal.

Keywords: Syrup, Old Coconut Water, Red Fruit, Antioxidants

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Introduction

Indonesia is a tropical country with a rich biodiversity that has been used for generations as traditional medicine. It is this natural wealth that communities utilize to meet their healthcare needs, both through traditional methods and as a complement to modern medicine. One of the regions with the largest forest areas in Indonesia is Papua. Papua's forests serve as habitats for a diverse array of endemic flora and fauna found exclusively in that region, one of which is the Province of Southwest Papua [1]. Papua is renowned for its vast forests and fertile soil, home to various types of food crops, plantation crops, and medicinal plants. Key commodities for the Papuan people include betel nut, coconut, sago, matoa, and red fruit. The use of plants as ingredients for traditional medicine has become an integral part of the community's basic needs. Nearly all Indonesians, from Sabang to Merauke, utilize plant species growing near settlements or in forested areas as sources of traditional medicine. In Papua, some well-known herbal plants include red fruit, ant nests, bungkus leaves, and betel nut.

Among these various biological resources, coconuts and red fruit are commodities with significant potential for development, both as food sources and for health purposes. The coconut (*Cocos nucifera* L) is a smallholder crop found in nearly all districts and cities in the Sorong region and its surroundings (BPS, 2024). One part of the coconut with significant potential for utilization is coconut water. In the market, there are two types of coconut water: young coconut water and mature coconut water. Young coconut water is generally used as a refreshing beverage, whereas mature coconut water is often simply discarded or goes unused, becoming waste and potentially causing unpleasant odors, as it is a byproduct of coconut grating for coconut milk production [2] . Coconut water is known to contain bioactive compounds such as vitamin B1, vitamin C, amino acids, minerals, and antioxidants [3].

Red fruit (*Pandanus conoideus* Lam) is one of the endemic medicinal plants of Papua. The red fruit plant itself is widely distributed throughout the Papua region, ranging from lowlands to elevations of 2,300 meters above sea level (masl), including in the city/regency of Sorong and its surroundings [4] . Red fruit (*Pandanus conoideus* Lam) has potential as a traditional medicine, food ingredient, and dye. Red fruit is known to contain secondary metabolites such as flavonoids, alkaloids, tannins, and saponins [5]. Flavonoids are one of the secondary metabolites in red fruit that exhibit antioxidant activity [6].

Antioxidants play a crucial role in protecting cells from damage caused by free radicals formed during oxidative stress. These compounds work to neutralize free radicals, converting them into non-toxic metabolic byproducts that can then be effectively eliminated by the body. Regular consumption of fruits and vegetables rich in antioxidants is known to help reduce the risk of various diseases triggered by free radical activity [7]. Free radicals are unstable molecules with unpaired electrons, making them highly reactive, which are formed during normal cellular metabolism. These molecules undergo chain reactions and can cause continuous damage within the body [8]. One trigger for free radicals is an unhealthy diet and drinking habits. Therefore, the development of natural antioxidant sources based on local commodities, such as mature coconut water and red fruit, is crucial in supporting efforts to improve public health.

Given this potential, the utilization of mature coconut water and red fruit extract as natural antioxidant sources in functional food products is highly promising for development. However, the use of mature coconut water as a raw material for functional beverages remains very limited, which has the potential to generate waste. On the other hand, the development of functional syrup products based on a combination of mature coconut water and red fruit extract with antioxidant activity has rarely been reported. The combination of these two natural ingredients is expected to produce a beverage with high antioxidant activity while utilizing mature coconut water waste, which has been underutilized until now. One of the most popular and easily applicable food formulations is syrup.

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Syrup is a type of light beverage consisting of a thick sugar solution with a variety of flavors and a high sugar content; it has a relatively shorter shelf life due to its high water content. Syrup without added preservatives generally lasts for about three weeks under specific storage conditions [9].

With the passage of time and increasing public awareness of healthy lifestyles, the use of local food commodities as raw materials for food products has been on the rise. One of the most common forms of food processing is the production of syrup beverages [10] . Several studies have developed syrup products based on local ingredients as part of efforts to advance food development while enhancing the value of these commodities.

Based on the above explanation, this study aims to formulate a syrup preparation combining mature coconut water and red fruit extract as a functional beverage with antioxidant activity. The antioxidant activity of the syrup was evaluated using the DPPH method to assess the free radical scavenging capacity of the resulting preparation. It is hoped that the results of this study on the combination syrup formulation of mature coconut water and red fruit extract can serve as a healthy, beverage based on local food resources from Southwest Papua, possessing potential as a natural antioxidant and supporting the sustainable utilization of local commodities.

Experimental Section

Materials and Apparatus

The equipment required for this study included aluminum foil, glass bottles, porcelain *dishes*, *Pyrex®* funnels, beakers *Pyrex®*, measuring cylinders *Pyrex®*, hot plate *Faithful*, filter paper, watch glasses, measuring flasks *Iwaki®*, micropipettes *Dlab*, plastic wrap, spectrophotometer *Thermo Genesys 150* UV-Vis, analytical balance *Dhaus*, tissue, vials, and sample containers.

The materials required for the study were coconut water samples *(Cocos nucifera* L), citric acid, red fruit extract (*Pandanus conoideus* L), CMC, DPPH (*2,2-diphenyl-1-pycrylhydrazyl*), 96% ethanol, p.a. ethanol, granulated sugar, vitamin C, and vanilla.

Extraction Preparation

The extraction process was performed using the maceration method with 96% ethanol. A 100-g sample of red fruit was macerated with a 96% ethanol solution at a sample-to-solvent ratio of 1:10 (w/v), i.e., 100 g of powder in 1000 mL of solvent. The maceration process was carried out for three 24-hour periods at room temperature in the dark, with occasional stirring. The mixture was then concentrated using a water bath at 50°C until a thick extract formed. The resulting thick extract was weighed using an analytical balance.

Ethanol-Free Test

The ethanol-free test was performed by adding 1 mL of glacial acetic acid and 1 mL of concentrated sulfuric acid to a sample, then heating it on a hot plate at 40°C for 10 minutes. A sample was considered ethanol-free if, after heating, no ester (fruity) odor was detected [11].

Phytochemical Screening

The extract is then dissolved in an appropriate solvent (96% ethanol), filtered, and 1 mL is taken using a pipette and placed into separate test tubes to be treated with specific reagents to identify the compounds. Flavonoids (Pb(II) acetate), alkaloids (Dragendorff, Mayer, Bouchardat), tannins (1%$FeCl_3$), and saponins (distilled water) [12]. The extract changed color after being mixed with each reagent. Flavonoids were detected by the appearance of a reddish-orange color, alkaloids by an orange color, tannins by a blackish-green color, and saponins by the formation of foam that lasted for ten seconds [13].

Making Syrup

Bring the filtered mature coconut water to a boil while stirring constantly to prevent it from thickening. Add granulated sugar, citric acid, vanilla, food coloring (red fruit extract), and CMC. Once cooled, pour into prepared bottles [14].

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Formulation

The syrup formulation used in this study was developed based on a previous formulation by the Indonesian Institute of Sciences (2017)), which has been patented under patent number P00201709576, and was subsequently modified by the researchers as shown in Table 1.

Table 1. Formulation of Syrup Preparation from Mature Coconut Water and Red Fruit Extract

Ingredients	Function	Amount
Mature coconut water	Active ingredient	500 mL
Granulated sugar	Sweetener	500 grams
Citric acid	Preservative	1.25 grams
Vanilla	Flavor and aroma enhancer	0.25 grams
Red fruit extract	Colorant	1.25 grams
CMC	Thickener	1 gram

Physical Stability Test for Preparation Evaluation

a) Organoleptic Test

After the syrup preparation is complete, an organoleptic test is conducted to assess the color, odor, and taste of the syrup preparation [15] .

b) Homogeneity Test

A homogeneity test is performed to check for the presence of lumps or sediment in the syrup solution [15] .

c) pH Test

A pH indicator was used to determine the syrup's pH. A sample of the syrup was placed in a beaker for this test and then observed [15] .

d) Viscosity Test

A sufficient amount of syrup sample is placed in a beaker, then the spindle is lowered until it is submerged in the syrup preparation, and the device is turned on by pressing the button. The device speed is set to 50 rpm. Differences in rpm are observed for each measurement [15] .

e) Clarity Test

The clarity test is performed visually by observing the syrup preparation. A good result is when the preparation is clear and contains no impurities [16]

Antioxidant Activity Testing Using UV-Vis Spectrophotometry

a) Preparation of the DPPH Solution

A 0.4 mM DPPH solution was prepared by weighing 0.0157 grams of DPPH and dissolving it in a 100 mL volumetric flask with 96% p.a. ethanol up to the mark [17] .

b) Preparation of Blank Solution

The blank was prepared by mixing 1 mL of 0.4 mM DPPH solution with p.a. ethanol to a volume of 5 mL, without adding a sample. The absorbance of the blank was measured at a wavelength of 517 nm.

c) Determination of the DPPH Wavelength (λmax)

A 5 mL volumetric flask covered with aluminum foil is filled with 1 mL of 0.4 mM DPPH solution, and the volume is adjusted with p.a. ethanol. A wavelength range of 400–600 nm is used to measure absorbance. Absorbance is measured at wavelengths of 400–600 nm [18] .

d) Preparation of Stock Solutions and Syrup Formulations

Weigh 10 mL of syrup and add p.a. ethanol to a beaker until homogeneous. Stock solutions were prepared in five concentrations, namely 50 ppm, 60 ppm, 70 ppm, 80 ppm, and 90 ppm, by pipetting 0.5, 0.6, 0.7, 0.8, and 0.9 mL of the stock solution into a 5 mL volumetric flask and adding p.a. ethanol up to the mark [19].

e) Determination of Operating Time

After adding 1 mL of the syrup test solution to 1 mL of DPPH solution, measurements were taken at a wavelength of 517 nm for 1 hour. The time-absorbance curve was used to determine the operating time after the absorbance value stabilized [20].

f) Measurement of Antioxidant Activity of the Syrup Preparation

A total of 2 mL of test solution from each concentration was taken and 1 mL of 0.4 mM DPPH was added; the mixture was then incubated for 5 minutes and 30 seconds in the dark based on the determined

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operating time. Absorbance was measured at a wavelength of 517 nm, and the results were calculated to obtain the percentage inhibition value.

g) Preparation and Measurement of Antioxidant Activity of Vitamin C Reference Solution

A 100 ppm vitamin C solution was prepared. 10 mg of vitamin C was weighed and mixed with p.a. ethanol, then stirred until homogeneous. The volume of the solution was adjusted to 10 mL of p.a. ethanol. The 1000 ppm vitamin C solution was diluted to 100 ppm. To obtain 5 concentration series, namely 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm to measure the antioxidant activity of the vitamin C solution, 0.05 mL, 0.1 mL, 0.15 mL, 0.2 mL, and 0.25 mL of the 100 ppm stock solution were pipetted into 5 mL volumetric flasks, and 1 mL of 0.4 mM DPPH and ethanol were added up to the mark. The solutions were then tightly sealed and allowed to stand for 30 minutes. Afterward, the absorbance of the solutions was measured using a UV-Vis spectrophotometer [18].

Data Analysis Technique

Antioxidant activity can be calculated using the equation:

$$\%\ \text{aktivitas inhibisi} = \frac{(absorbansi\ blanko - absorbansi\ sampel)}{absorbansi\ blanko} \times 100\%$$

Using the syrup formulation concentration (ppm) as the x-axis and the percentage inhibition activity value as the y-axis, the calculation results are entered into a linear regression equation. The formula y = bµ + an is used to determine the IC50 value [17] .

Results and Discussion

Table 2. Ethanol-free test of red fruit extract (*Pandanus conoideus* Lam)

Identification	Reference	Observation results
Ethanol-free test	When heated, there is no ester (fruity) odor [11]	No ester odor is detected.

Based on **Table 2.** the results of the ethanol-free test indicate that the red fruit extract (*Pandanus conoideus* Lam) is free of ethanol solvent because no ester (fruity) odor is detected. The ethanol-free test was conducted to ensure that the red fruit extract (*Pandanus conoideus* Lam) is free of ethanol solvent, thereby yielding a pure extract without contamination. Thus, this red fruit extract can be used in the next stage.

Table 3. Phytochemical screening test of red fruit extract (*Pandanus conoideus* L) and mature coconut water (*Cocos nucifera* L)

Sample	Compound Group	Reaction	Reference	Observation Results	Notes
Red fruit extract	Alkaloids	Dragendroff	Orange-colored precipitate [21]	An orange-colored precipitate forms	+
		Bouchardat	Brown precipitate [21]	Brown sediment formed	+
		Mayer	Yellowish-white precipitate [22]	A yellowish-white precipitate has formed	-
	Flavonoids	PbII acetate	Orange to red color[23]	A reddish-orange color forms	+
	Tannin	FeCl3	Greenish-black color [23]	A color change to greenish black occurs	+
	Saponin	Distilled water	Stable foam forms [24]	Stable foam forms	+
Old coconut water	Alkaloids	Dragendroff	Orange-colored precipitate [21]	An orange-colored precipitate forms	+
		Bouchardat	Brown precipitate [21]	Brown sediment formed	+
		Mayer	Yellowish-white precipitate [22]	A yellowish-white precipitate forms	+
	Flavonoids	PbII acetate	Orange to red color [23]	A white precipitate forms	-
	Tannin	FeCl3	Greenish-black color [23]	A yellowish-white color change occurs	-
	Saponin	Distilled water	Stable foam forms [24]	No stable foam formed	-

Notes:
(+) : Compound formation
(-) : No compound formation

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Based on the data in **Table 3**, the phytochemical screening test of red fruit extract (*Pandanus conoideus* Lam) revealed the presence of secondary metabolites in the form of flavonoids, alkaloids, tannins, and saponins, whereas old coconut water (*Cocos nucifera* L) contained only alkaloids as secondary metabolites. The results of the flavonoid test using Pb (II) acetate reagent showed that the red fruit extract tested positive for flavonoids, whereas the mature coconut water tested negative, indicating that no flavonoids were detected. The mechanism of the reaction between flavonoids and the Pb (II) acetate reagent, resulting in the formation of a precipitate, is due to the presence of a hydroxyl group (-OH) on the benzene ring in the flavonoid structure, which reacts with the reagent to produce a yellow precipitate[25] .

Alkaloid testing was performed using three main reagents: Dragendroff, Bouchardat, and Mayer. The Dragendroff reagent produces an orange precipitate, the Bouchardat reagent produces a dark brown precipitate, and the Mayer reagent produces a white or yellowish precipitate. Furthermore, the results of the phytochemical screening test for alkaloids in red fruit extract and old coconut water indicate the presence of alkaloid secondary metabolites; however, in the red fruit extract, the Mayer alkaloid did not form a yellowish-white precipitate. The mechanism of the alkaloid reaction indicates that the precipitate is formed due to the formation of a potassium-alkaloid complex. Alkaloids possess a lone pair of electrons on the nitrogen atom that binds with the K+ ion in the alkaloid reagent [21]. Furthermore, based on the results of phytochemical screening for tannins using the $FeCl_3$ reagent on red fruit extract and old coconut water, the red fruit extract tested positive for tannins, while the old coconut water tested negative, indicating the absence of tannin compounds. The reaction mechanism for tannins involves the formation of a complex between tannins and Fe^{3+} ions, which produces a dark green color [21].

Furthermore, based on the results of the saponin test using distilled water on red fruit extract and old coconut water, it was found that the red fruit extract tested positive for saponins, while the old coconut water tested negative, indicating the absence of saponin compounds. The saponin reaction mechanism involves the formation of foam, as glycoside compounds can create foam in water and break down into glucose and other compounds. This reaction demonstrates the foaming process associated with saponin compounds [21].

Phytochemical test results indicate that mature coconut water (*Cocos nucifera* L) contains the secondary metabolite alkaloid, which differs from the studies by Afunwa (2025) [26] , Anyiam and Opara (2023) [27] , as well as Akpomie (2020) [28] . Thus, it can be concluded that high or low coconut water content influences the phytochemical results, leading to the detection of different secondary metabolite compounds. Consequently, researchers can conclude that when conducting phytochemical tests on mature coconut water, the water content level must be carefully considered to ensure the detected results demonstrate appropriate accuracy.

Table 4. Evaluation test of syrup preparation mature coconut water (*Cocos nucifera* L) and red fruit extract (*Pandanus conoideus* L)

Tests/Parameters	Replication	Observation results	SNI	Information
Organoleptic (color)	-	Reddish orange color	Normal	Qualify
Organoleptic (odor)	-	Distinctive odor of old coconut water with hints of red fruit	Normal	Qualify
Organoleptic (taste)	-	Sweet taste	Normal	Qualify
Homogeneity	-	Homogeneous	Homogeneous	Qualify
pH	1	5	4-7	Qualify
	2	6	4-7	Qualify
	3	6	4-7	Qualify
Viscosity	-	250 mPa.s.	30-397 cP	Qualify
Clarity	-	Clear	Hygienic	Qualify

Based on **Table 4,** the results of the organoleptic evaluation of the mature coconut water syrup and red fruit extract preparations showed a reddish-orange color, the characteristic odor of mature coconut water with a slight aroma of red fruit, and a sweet taste. Organoleptic testing was conducted to assess any changes in color, odor, and taste parameters that could affect the stability of the preparations. The results of the observations indicate that, overall, the color, odor, and taste of the syrup formulation remain consistent with the characteristic features of mature coconut water, accompanied by a slight aroma of red fruit. As for the taste parameter, the taste of the syrup preparation itself is predominantly sweet. This is due to the addition of sweeteners in the formulation, which is consistent with the characteristics of syrup preparations that generally contain sucrose in a concentration of no less than 64% [14].

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The results of this study are consistent with the research on (Solang *et al.*, 2017), which found that the addition of sweeteners or sucrose produced a distinctive color and aroma, as well as a sweet syrup. However, due to limitations in the research equipment used, the sugar content indicating a sweet taste in accordance with SNI standards could not be presented as a percentage in the table. However, the results of the organoleptic taste test indicated a sweet flavor. Thus, it can be concluded that the preparations of old coconut water syrup and red fruit extract possess distinctive color and aroma, and the sweet taste of the syrup meets SNI requirements, i.e., it is normal and compliant.

Based on the homogeneity test results, the preparations of old coconut water syrup and red fruit extract were found to be stable, homogeneous, and clear. The homogeneity test for the syrup preparations was conducted by placing them in a transparent container and observing any parts that were not well-mixed. The homogeneity test results for the old coconut water syrup and red fruit extract preparations met the requirements because the syrups were homogeneous.

Table 5. Results of the antioxidant activity test of the syrup of mature coconut water (*Cocos nucifera* L) and red fruit extract (*Pandanus conoideus* L), replication 1

Concentration (ppm)	% Inhibition	IC50 μg/mL
50	92.92	550.34 μg/mL
60	92.61	
70	91.84	
80	89.53	
90	90.15	

The pH test is used to assess the acidity of a solution. Additionally, the purpose of the pH test is to ensure the product's pH in relation to the safety of the active ingredient's solubility. pH testing of the syrup formulation is important because the syrup consumed enters the stomach, which can cause problems if the syrup's pH is inappropriate. The SNI pH standard for syrup formulations is 4–7 [29] . pH measurements of the old coconut water and red fruit extract syrup were conducted to determine whether the syrup formulation meets the pH specified in the reference, ensuring the resulting syrup is safe for use. Based on the research results, it can be concluded that the old coconut water and red fruit extract syrup preparations are deemed safe for consumer consumption because their pH falls within the 5–6 range, thus meeting safety standards and fulfilling the quality requirements set forth in the Indonesian National Standard (SNI).

Additionally, a viscosity test was conducted to assess the thickness of the syrup formulation under study. This viscosity test was performed using a Brookfield viscometer with a spindle 3 and a speed of 50 rpm. According to SNI regulations, the viscosity value of the syrup formulation should be within the range of 30–397 Cp; thus, the viscosity test results in this study comply with SNI regulations and meet the requirements. The concentration of CMC-Na affects the syrup's viscosity, indicating an increase in thickness. The higher the CMC-Na concentration, the higher the viscosity. The viscosity test results for the old coconut water syrup and red fruit extract were 250 mPa·s, indicating that the viscosity is acceptable. Cp (centipoise) and mPa·s (millipascal-seconds) are equivalent units, where 1 cP = 1 mPas. Furthermore, in the clarity test conducted visually to examine the syrup preparation, it must be clear and free of impurities. The results of the clarity test for the old coconut water and red fruit extract preparations showed that the resulting coconut water syrup had good clarity. Thus, the clarity test results obtained by the researchers were in accordance with SNI regulations and met the requirements.

Based on the evaluation results, the syrup formulation combining mature coconut water and red fruit extract meets the quality parameters for syrup as specified by SNI and is deemed acceptable. The results of the analysis (observation) indicate that the syrup formulation combining mature coconut water and red fruit extract has a reddish-orange color, the characteristic odor of mature coconut water with a hint of red fruit, and a sweet taste; it meets the requirements because the syrup is homogeneous, of good quality, and safe for consumer consumption as the pH is within the 5–6 range and meets the requirements. The viscosity of 250 mPa·s indicates that it is suitable and meets the requirements, and it also has good clarity and meets the requirements. Based on the results of the operating time determination, the test sample solution showed that the absorbance began to stabilize within a time range of 4–5 minutes with an absorbance value of 0.071. The operating time (OT) determination aims to identify the time required for the test sample solution to fully react. The operating time (OT) is determined based on the time when the absorbance value begins to stabilize, as indicated by the difference in absorbance values at each time interval [30].

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Table 6. Results of the antioxidant activity test of the syrup of mature coconut water (*Cocos nucifera* L) and red fruit extract (*Pandanus conoideus* L), replication 2

Concentration (ppm)	% inhibition	IC50 µg/mL
50	93.07	2282.3 µg/mL
60	95.07	
70	94.15	
80	96.46	
90	91.38	

Table 7. Results of the antioxidant activity test of the syrup of mature coconut water (*Cocos nucifera* L) and red fruit extract (*Pandanus conoideus* L), 3 replicates

Concentration (ppm)	% inhibition	IC50 µg/mL
50	92.92	2863.4 µg/mL
60	95.07	
70	94.15	
80	96.46	
90	91.38	

Table 8. Results of the comparative antioxidant activity test of vitamin C solutions

Concentration (ppm)	% inhibition	IC50 µg/mL
1	47.38	0.31 µg/mL
2	76	
3	89.38	
4	94.46	
5	94.61	

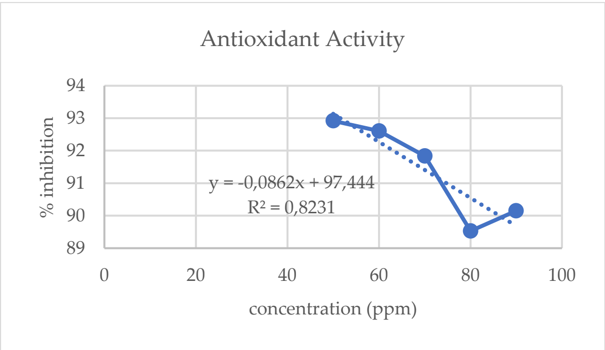

Figure 1. Standard curve of antioxidant activity, replicate 1

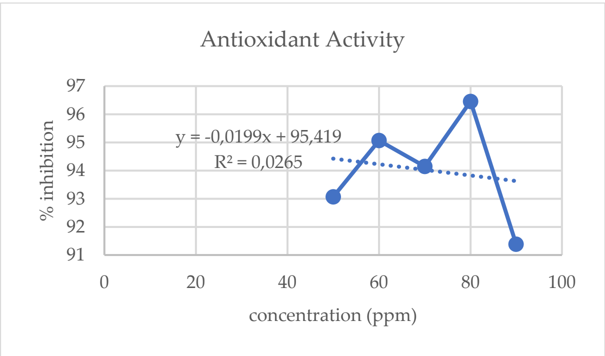

Figure 2. Standard curve of antioxidant activity, replicate 2

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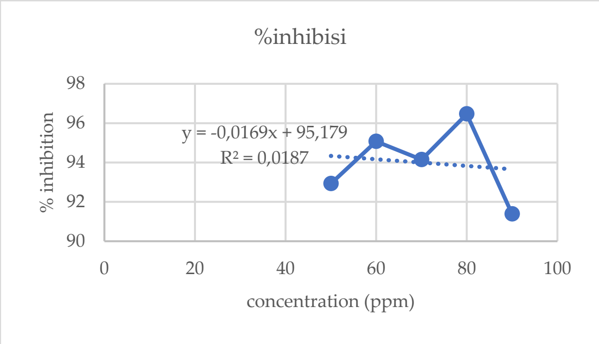

Figure 3. Standard curve of antioxidant activity, replicate 3

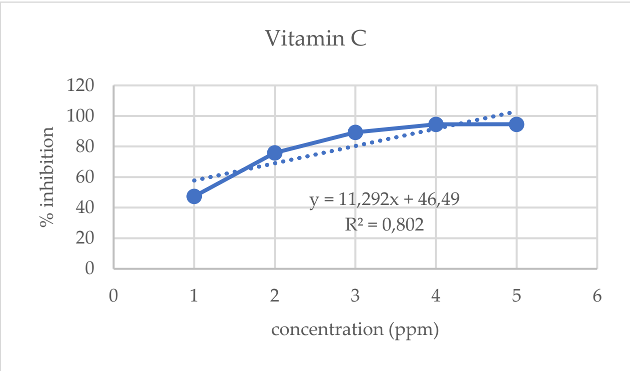

Figure 4. Vitamin C standard curve

Antioxidant activity testing of the syrup formulation of mature coconut water (*Cocos nucifera* L) and red fruit extract (*Pandanus conoideus* Lam) was conducted using the DPPH (*2,2-diphenyl-1-pycrylhydrazyl*) method. This method is a quantitative assay for determining antioxidant activity via UV-Vis spectrophotometry. As shown in **Table 5, 6, and 7** the absorbance of the old coconut water and red fruit extract syrup preparations was measured at a wavelength of 517 nm using a concentration series of 50 ppm, 60 ppm, 70 ppm, 80 ppm, and 90 ppm. The IC50 values from the three replicates were as follows: replicate 1 at 550.34 μg/mL, replicate 2 at 2282.3 μg/mL, and replicate 3 at 2863.4 μg/mL. Thus, the old coconut water syrup and red fruit extract preparations exhibit weak antioxidant activity since their IC50 values are above 200 ppm. Increasing sample concentrations showed a consistent rise in the percentage inhibition value, resulting in IC50 values indicative of high antioxidant activity [31] . The quality of antioxidant activity is inversely proportional to the IC50 value, where a lower value indicates a compound with greater potential to neutralize free radicals.

The DPPH radical is a stable purple free radical with a maximum wavelength of 517 nm. In this method, the mechanism of antioxidant action involves an oxidation-reduction reaction, in which the antioxidant compound donates a hydrogen atom or an electron to the DPPH radical. The release of these hydrogen atoms or electrons converts DPPH into the reduced form DPPH-H, which is non-radical and has a paler or yellow color, resulting in a decrease in the purple color intensity of the solution. This color change is measured as a decrease in absorbance at a wavelength of 517 nm using UV-Vis spectrophotometry. Antioxidant activity is calculated based on absorbance values, inhibition percentage, and determined by the IC50 value—the sample concentration capable of inhibiting 50% of DPPH free radical activity [32] . Thus, the lower the IC50 value, the lower the compound concentration required to inhibit 50% of DPPH free radicals, indicating stronger antioxidant activity. This aligns with the mechanism of action of antioxidant compounds, which involves the donation of a proton or hydrogen atom to the radical compound to replace the missing electron in the free radical and prevent the chain reaction resulting from free radical formation [33] .

A compound is said to have very high antioxidant activity if the IC50 value is less than 50 ppm. Antioxidant activity is categorized as strong if the IC50 value is in the range of 50–100 ppm, moderate if it is in the range of 100–150 ppm, and weak if the IC50 value is in the range of 150–200 ppm[34] . Based on the data in Figure 1, a linear equation (y) = -0.0862x + 97.444 with an R^2 value of 0.8321 was obtained; in Figure 2, a linear equation (y) = -0.0199x + 95.419 with an R^2 value of 0.0265 was obtained; and in Figure 3, yields the linear equation (y) = -0.0169x + 95.179 with an R^2 value of 0.0187. The R^2 value is used to assess the degree of linearity of a curve; the better the linearity of the curve, the closer the R^2 value will be to 1. The R^2 value indicates how

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well the data on the independent variable can explain changes in the dependent variable. If the R^2 value approaches 1, then the independent variable is highly effective in predicting the dependent variable [35] .

When compared to a previous study reporting an IC50 value of 54.67 ppm for a single red fruit extract—classified as having strong antioxidant activity [36]. the IC50 value of the combined red fruit extract and mature coconut water syrup formulation in this study, at 550.34 μg/mL, indicates a reduction in antioxidant activity to the weak category. This condition indicates that the combination with mature coconut water in the form of a syrup preparation does not provide a synergistic effect; rather, it tends to reduce the antioxidant capacity of the red fruit extract.

However, organoleptically, the mixture of DPPH solution with the red fruit and mature coconut water syrup formulation showed a noticeable color change from purple to yellow, which qualitatively indicates antioxidant activity. However, UV-Vis spectrophotometric measurements at a wavelength of 517 nm showed a relatively low inhibition percentage, so the calculated IC50 value remained in the weak category. This discrepancy between visual observations and spectrophotometric data is suspected to be related to color interference and the turbidity of the syrup matrix, which increased the sample's baseline absorbance, as well as the use of a blank solution that did not fully compensate for the preparation's inherent color. In addition, technical factors such as the calibration conditions of the spectrophotometer, which could potentially affect the absorbance values obtained, as well as the cleanliness and uniformity of the cuvettes, may also play a role.

Table 8. shows that ascorbic acid**,** or vitamin C, had an IC50 value of 0.31 μg/mL. A comparison of the IC50 value from this study with that of a previous study also indicates that a value of 0.46 μg/mL for vitamin C demonstrates very strong antioxidant activity [36]. A low IC50 value indicates that vitamin C possesses very strong antioxidant activity, as a smaller IC50 value means a lower concentration of the compound is required to inhibit 50% of DPPH free radicals. This is because a smaller IC50 value implies that a lower concentration of the compound is needed to inhibit 50% of DPPH free radicals. Ascorbic acid was used as a reference and acts to neutralize free radicals through an electron-donating mechanism, thereby protecting cells from damage[18] . Ascorbic acid is known for its strong and consistent antioxidant properties; it was used as a reference (positive control) to assess antioxidant activity. The calculated % inhibition was used to determine the antioxidant activity of vitamin C.

Various classes of secondary metabolites are known to act as antioxidants through different mechanisms. Flavonoid compounds have antioxidant potential because the hydroxyl groups attached to the aromatic ring can donate hydrogen atoms, thereby neutralizing free radicals generated during lipid peroxidation reactions. Alkaloids exhibit antioxidant activity because they contain nitrogen atoms with lone pairs of electrons, which can neutralize free radicals in the body [37]. Tannins act as secondary antioxidants by inhibiting free radical formation through iron ion chelation[38]. Saponins possess antioxidant activity because they can scavenge superoxide free radicals by forming hydroperoxide intermediates, thereby preventing biomolecular damage caused by free radicals [37].

Conclusions

Based on the results of the analysis, it can be concluded that the findings of this study specifically, the antioxidant activity test of a syrup formulation combining mature coconut water (*Cocos nucifera* L) and red fruit extract (*Pandanus conoideus* L) using the DPPH method indicate that mature coconut water contains alkaloids, while red fruit extract contains flavonoids, alkaloids, tannins, and saponins. Evaluation of the syrup formulation indicates that the combination syrup of mature coconut water and red fruit extract complies with SNI standards and meets the requirements, characterized by a reddish-orange color, the distinctive odor of mature coconut water with a slight hint of red fruit, a sweet taste, homogeneity, a pH range of 5 to 6, a viscosity of 250 mPa·s, and good clarity. Antioxidant activity testing of the syrup formulation combining mature coconut water and red fruit extract revealed weak antioxidant activity, with IC50 values of 550.34 μg/mL in the first replicate, 2282.3 μg/mL in the second replicate, and 2863.4 μg/mL in the third replicate. Thus, the innovation of the syrup formulation combining mature coconut water and red fruit extract has the potential to be developed as a herbal health drink rich in antioxidants. The combination syrup formulation of mature coconut water and red fruit extract can be further improved to explore plant-based preservatives and antioxidants to prevent a decline in antioxidant activity during storage, and further testing can be conducted on the combination syrup formulation of mature coconut water and red fruit extract, such as stability testing of the syrup formulation in follow-up studies.

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