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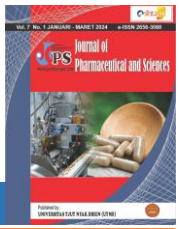

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Physicochemical and Antioxidant Activity Evaluation of Yellow Rope (*Anamirta cocculus* (L.) Wight & Arn.) Stem Extract Isotonic Drink

Evaluasi Mutu Fisikokimia dan Aktivitas Antioksidan Minuman Isotonik Ekstrak Batang Tali Kuning (*Anamirta cocculus* (L.) Wight & Arn.)

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Abstract

Background: *Anamirta cocculus* (L.) Wight & Arn., commonly known as yellow rope, is an endemic flora of Papua identified as having potential as a natural antioxidant agent. One potential way to utilize this plant is by innovating it into an isotonic drink product. **Objective:** This study aimed to evaluate the physicochemical properties and antioxidant activity of an isotonic drink formulated with yellow rope stem extract. **Methods:** he extraction process was conducted using the maceration method with 70% ethanol as the solvent. The isotonic drink was developed into three formulas, which were then tested for their physicochemical properties and antioxidant activity using the DPPH method. **Results:** The results indicated that the best formula was FII, with an overall hedonic score of 4,6 (neutral). All three formulas showed identical pH values (4,0) and Total Soluble Solids (TSS) of 7%, while the total acidity decreased progressively: FI (1,024%), FII (0,896%), and FIII (0,704%). The isotonic drinks from yellow rope stem extract for formulas FI, FII, and FIII produced IC_{50} values of 35,61, 46,56, and 57,69 µg/mL, respectively. Based on these data, FI and FII were identified as having very strong antioxidant intensity, while the FIII showed activity classified in the strong category. **Conclusion:**.The test results demonstrate that the isotonic drink made from yellow rope stem extract exhibits antioxidant potential with intensity levels ranging from strong to very strong.

Keywords*: *Free radicals, Yellow rope, Antioxidant, Isotonic drinks, DPPH.

Abstrak

Latar Belakang: *Anamirta cocculus* (L.) Wight & Arn., atau yang lebih dikenal sebagai tali kuning, merupakan flora endemik Papua yang teridentifikasi memiliki kapabilitas sebagai agen antioksidan alami. Salah satu cara potensial untuk memanfaatkan tanaman ini yaitu dengan menginovasikannya menjadi produk minuman isotonik. **Tujuan:** Penelitian ini diarahkan untuk mengevaluasi sifat fisikokimia dan aktivitas antioksidan minuman isotonik ekstrak batang tali kuning. **Metode:** Metode maserasi diterapkan dalam proses ekstraksi sampel dengan memanfaatkan etanol 70% sebagai cairan penyari. Minuman isotonik dibuat menjadi 3 formula, kemudian diuji sifat fisikokimianya dan aktivitas antioksidannya menggunakan metode DPPH. **Hasil:** Hasil menunjukkan formula terbaik diperoleh pada FII dengan skor hedonik keseluruhan 4,6 (netral). Ketiga formula menunjukkan nilai pH (4,0) dan TPT (7%) yang identik, namun kadar total asam menurun berturut-turut: FI (1,024%), FII (0,896%), dan FIII (0,704%). Minuman isotonik dari ekstrak batang tali kuning pada formulasi FI, FII, dan FIII menghasilkan nilai IC_{50} berturut-turut sebesar 35,61, 46,56, dan 57,69 µg/mL. Berdasarkan data tersebut, sediaan FI dan FII teridentifikasi memiliki efektivitas antioksidan dengan intensitas sangat kuat, sementara FIII menunjukkan aktivitas yang tergolong dalam kategori kuat. **Kesimpulan:** Hasil pengujian menunjukkan bahwa minuman isotonik ekstrak batang tali kuning menunjukkan potensi antioksidan dengan tingkat kekuatan antara kategori kuat hingga sangat kuat

Kata Kunci: Radikal bebas, Tali kuning, Antioksidan, Minuman Isotonik, DPPH.

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Introduction

Indonesia is home to tens of thousands of plant species, with a total of 40,000 species. Of these, thousands possess medicinal properties, whether found in their natural habitats or through cultivation [1]. Despite being a primary hub for Indonesian medicinal flora, Papua's botanical wealth is poorly documented. Recent data collection efforts highlight the region's importance, but extensive research remains essential to bridge the existing knowledge gap [2].

Various plants serve as sources of medicinal compounds through the production of secondary metabolites for environmental adaptation. A key benefit of these compounds is their role as antioxidants, which protect the body from free radicals by inhibiting oxidative reactions [3]. Reactive oxygen species (ROS) are primarily produced within the body [4]. Antioxidants are molecules that can counteract the effects of free radicals. Free radicals are neutralized by antioxidants, thereby inhibiting or preventing the damage they cause [5].

Yellow rope (*A. cocculus* (L.) Wight & Arn.) is a plant known for its antioxidant activity. Yellow rope is a plant endemic to Papua, characterized primarily by its yellow stem [6]. Yellow rope stem contains terpenoid, alkaloid, flavonoid, and tannin compounds. [7]. Flavonoids and alkaloids exhibit antioxidant properties through distinct mechanisms. The hydroxyl groups in flavonoids act as reducing agents by donating hydrogen atoms to neutralize free radicals. Conversely, alkaloids function as antioxidants due to the unpaired electrons on their nitrogen atoms, which suppress free radical activity and prevent oxidative cell damage [8].

Based on the research conducted, the ethanol extract of yellow rope exhibits strong antioxidant activity with IC_{50} values ranging from 53,93-62,50 µg/mL [3]. Meanwhile, another study on antioxidant activity testing found that the ethanol extract of the yellow rope stem exhibited very strong to strong antioxidant activity, ranging from 16,16-129,03 µg/mL [7]. Currently, the use of yellow rope extract remains limited to basic activity testing and has not yet been developed into a functional consumer product. This offers a significant opportunity to innovate plant-based health products, such as antioxidant-rich isotonic drinks. Such a formulation would provide a practical solution for rapid energy replenishment while strengthening the body's defense against free radicals, which often increase due to high activity levels or poor physical conditions [9].

Based on the above discussion, this study aims to develop an isotonic drink formulation using yellow rope stem extract (*A. cocculus* (L.) Wight & Arn.) that possesses satisfactory physical and sensory qualities, with the hope of introducing new innovations in this field. This study is expected to provide a deeper understanding of health.

Experimental Section

Plant Samples Collection

Research samples were collected from Folley Village, East Misool, Raja Ampat (-1.780154, 130.307822). The specimen used was the yellow rope stem. Physically, the plant features a sturdy stem with gray bark and yellowish-white inner wood. For this study, relatively young stems with a diameter of ± 5 cm were harvested during the morning hours.

Materials and Apparatus

The equipment used in this study included a blender (Phillips), burette (Pyrex), refractometer (ATC), universal pH paper (Nesco), micropipette (Envilife), oven (Kenton), rotary evaporator (Buchi R-100),

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spectrophotometer UV-Vis (Thermo Scientific Genesys 150), test tubes (Pyrex), analytical balance (Ohaus), maceration container, vials, glass bottles, and a water bath (Memmert WNB 22).

The materials used in this study were samples of yellow rope (*A. cocculus* (L.) Wight & Arn.), sucrose, KCl, Ca. Lactate, Na. Citrate, Na. Bicarbonate, quercetin, DPPH (2,2-diphenyl-1-picrylhydrazyl), citric acid, distilled water, 70% ethanol, NaOH, phenolphthalein, and anhydrous ethanol.

Preparation of Yellow Rope Stem Samples

Yellow rope stems underwent wet sorting and washing under running water, followed by size reduction. The samples were then oven-dried at 50°C for 8 hours. Once sufficiently dry, dry sorting was conducted before the samples were finally ground into a powder using a blender [10].

Preparation of Yellow Rope Stem Extract

A maceration process was employed to extract 500 g of yellow rope stem powder using 70% ethanol at a 1:4 (w/v) ratio. The extraction was maintained for 72 hours at ambient temperature, with stirring performed every 24 hours, and subsequently filtered to obtain the liquid extract [11]. The percentage yield was derived by weighing the final concentrated extract and applying the following calculation [12]:

$$\%Yield = \frac{Wextract}{Wpowder} \times 100\%$$

Formulation of Isotonic Drinks

The isotonic drink formulation was adapted from previous studies, with the addition of yellow rope stem extract as the active ingredient. All ingredients were weighed, mixed, and heated at 50°C for 10 minutes. The resulting beverage was immediately transferred into sterile glass bottles and stored at cold temperatures (<4°C) to maintain the stability and quality of the preparation [13]. The preparation process involves weighing the extract and other excipients according to the proportions specified in the formulation details in **Table 1**.

Table 1. Formulation of an Isotonic Drink Containing Yellow Rope (*A. cocculus* (L.) Wight & Arn.) Stem Extract

Composition (%)	FI	FII	FIII
Yellow rope stem extract	0,125	0,25	0,5
Sucrose	7	7	7
KCl	0,06	0,06	0,06
Ca. Lactate	0,02	0,02	0,02
Na. Citrate	0,16	0,16	0,16
Na. Bicarbonate	0,14	0,14	0,14
Citric Acid	0,18	0,18	0,18
Aquadest	ad 200 mL	ad 200 mL	ad 200 mL

Organoleptic Test of Isotonic Drinks

This sensory evaluation was conducted as a preliminary sensory study. The hedonic test involved 10 untrained panelists who met the inclusion criteria, which included being non-smokers, in good health, and regular consumers of isotonic drinks. Assessments were performed using a 7-point hedonic scale (1=strongly dislike, 7=strongly like). The collected data were assumed to be interval data, thereby enabling statistical analysis. The overall acceptance was then calculated using proportional weighting: aroma (40%), color (30%), and taste (30%) [14].

pH Test of Isotonic Drinks

The pH value was measured using universal pH paper by immersing it in the isotonic drink sample at room temperature. The resulting color change on the paper was then compared against a standard pH indicator scale to determine the acidity level [15].

Determination of Total Soluble Solids (TSS) in Isotonic Drinks

The sugar content of the samples was estimated by determining the Total Soluble Solids (TSS) through refractometric analysis. A sample was carefully applied to the refractometer lens using a dropper. The measurement was recorded by directing the instrument toward a light source to ensure accurate readings [16].

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Determination of Total Acid Content in Isotonic Drinks

Total acidity was determined via titration. The procedure involved adding 2-3 drops of phenolphthalein indicator to a 10 mL isotonic drink sample. The solution was then titrated with 0,1 N NaOH until the endpoint was reached, characterized by the appearance of a persistent pink color [16]. Total acid concentration was quantified based on the equation below [16].

$$Total\ Acidity\ (\%) = \frac{(mL\ NaOH\ \times N\ NaOH\ \times MW\ dominant\ acid\ \times DF\ \times 100\%)}{sample\ weight\ (g) \times 1000 \times acid\ valence}$$

Definition:
DF : Dilution Factor
MW : Molecular Weight
Dominant Acid : Citric Acid (192)
Acid Valence : Citric Acid (3)
N NaOH : 0,1 N

Antioxidant Activity Testing using Ultraviolet-Visible Spectrophotometry

Preparation of DPPH Solution

A stock solution of 0,4 mM DPPH was obtained by dissolving 0,0157 g of DPPH crystals in a 100 mL volumetric flask. Analytical-grade (p.a.) ethanol was used as the solvent, and the mixture was diluted until the volume reached the graduation mark [17].

Determination of DPPH Maximum Wavelength (λmax)

To optimize the maximum wavelength, a mixture consisting of 1 mL of 0,4 mM DPPH in 5 mL of analytical-grade ethanol was prepared. The absorbance of this solution was then measured across a spectrum of 400 to 800 nm using a UV-Vis spectrophotometer to pinpoint the λmax [17].

Determination of Operating Time

To determine the operating time, the absorbance of a 0,4 mM DPPH solution (diluted to 5 mL) was monitored at 1-minute intervals. This process was carried out at the maximum wavelength for a duration of 0 to 90 minutes using analytical-grade ethanol until no further changes in absorbance were detected [18].

Preparation of Isotonic Drinks Stock Solution

This study utilized three isotonic drink formulations (FI, FII, and FIII) with initial concentrations of 1250 ppm, 2500 ppm, and 5000 ppm, respectively. Intermediate stock solutions of 1000 ppm were prepared, followed by serial dilutions to obtain a 100 ppm working stock. This stock was further diluted to prepare a series of working concentrations at 10, 20, 30, 40, and 50 ppm [19].

Antioxidant Activity Assay of Isotonic Drinks

To achieve working concentrations of 10, 20, 30, 40, and 50 ppm, specific volumes (0,5, 1,0, 1,5, 2,0, and 2,5 mL) of samples FI, FII, and FIII were pipetted into light-resistant 5 mL volumetric flasks. Each sample was reacted with 1,0 mL of 0,4 mM DPPH solution and diluted to volume with analytical grade ethanol. Absorbance was determined at the maximum wavelength via a UV-Vis spectrophotometer once the predetermined operating time had elapsed [3,19].

Preparation of 1000 ppm Quercetin Stock Solution

A stock solution of quercetin (1000 ppm) was obtained by dissolving 25 mg of the substance in 25 mL of analytical ethanol. A subsequent 100 ppm working solution was prepared by accurately pipetting 1 mL of this stock into a 10 mL volumetric flask, followed by the addition of ethanol up to the graduation mark [17].

Preparation of Quercetin Working Standard Solutions

A series of quercetin standards (2, 4, and 6 ppm) was generated through the dilution of a 100 ppm stock solution. To each concentration, 1 mL of 0,4 mM DPPH was added within a 5 mL volumetric flask, which was then filled to the mark with solvent. After being incubated in a dark environment for the predetermined operating time, the absorbance was recorded at the peak wavelength using UV-Vis spectrophotometry [17].

Data Analysis of Isotonic Drinks Organoleptic Tests

Data analysis was performed using the One-Way ANOVA method via SPSS software. If significant differences were revealed between groups, the analysis was followed by Duncan's Multiple Range Test (DMRT) at a 95% confidence level (α=0,05) to identify specific differences among the treatment groups [20].

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Percentage Inhibition of DPPH Free Radical Compounds

The potency of antioxidant activity in scavenging free radicals was analyzed using the DPPH method. The radical scavenging effectiveness was obtained by calculation using the following formula [3].

$$\%Antioxidant\ Activity = \frac{(Absorbance\ of\ blank - Absorbance\ of\ sample)}{Absorbance\ of\ blank} \times 100\%$$

Definition:
Absorbance of blank : DPPH absorbance
Absorbance of sample : Antioxidant absorbance of isotonic drink containing yellow rope stem extract

Determination of 50% Inhibitory Concentration IC_{50}

The following linear regression equation was used to describe the linearity relationship between the x and y values in the resulting graph [3].

$$y = ax + b$$

Definition:
y = %inhibition
x = Sample concentration
a = Y-intercept of the curve
b = Slope of the curve

The IC_{50} calculation was performed by substituting the value of 50 for the y-variable in the regression equation to obtain the x-value. The a and b parameters in this linear model are constants derived from the empirical data obtained from the testing [3].

Results and Discussion

Yield of Yellow Rope Stem Extract

The extraction process of yellow rope stems yielded 33 g of thick extract with a yield value of 6,6%. This yield reflects the quantity of extract successfully recovered from the crude drug (simplisia), where a higher percentage indicates a greater recovery. However, the value obtained in this study is below the minimum threshold established by the Farmakope Herbal Indonesia, which specifies a yield of no less than 10% [21]. The yield percentage obtained in this study does not meet the aforementioned requirements. Several crucial parameters that may influence the extraction efficiency include the physicochemical properties of the raw material and the selection of the solvent concentration. Furthermore, operational factors such as the maceration duration, the particle size of the crude drug powder, and the extraction temperature also play a significant role in the final extract yield. The effectiveness of the maceration process is significantly influenced by the particle size of the raw material and the intensity of the agitation employed. The use of powder with a specific fineness (40–60 mesh) has been proven to expand the surface contact area, thereby facilitating more optimal solvent penetration into the plant cell matrix [22]. Generally, smaller particle sizes enhance extraction efficiency through the optimization of solvent penetration and solute diffusion; however, excessively fine particles must be approached with caution as they can trigger over-adsorption and complicate the subsequent filtration process [23]. Beyond the particle size factor, periodic stirring or the use of a shaker during the maceration process also plays a crucial role in improving homogeneity and extraction efficiency. Nevertheless, most studies have not described the intensity or frequency of stirring in detail, making it difficult to quantitatively determine the extent to which this factor influences the resulting yield. This becomes particularly relevant considering that in this yellow rope stem extraction process, stirring was only performed every 24 hours, which is suspected to have caused the formation of a saturated layer on the material surface and hindered the diffusion rate of the active compounds [22]. Yield data is listed in **Table 2**.

Table 2. Total Yield Value of Ethanol Extract from Yellow Rope (*A. cocculus* (L.) Wight & Arn) Stem

Massa (g)		Extraction Yield (%)
Sample	Extract Weight	
500	33	6,6

Results of Organoleptic Test of Isotonic Drinks

The color element is a crucial parameter that determines consumer decision-making when selecting food products. This is attributed to the role of color as the primary sensory stimulant, which has a significant

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impact on preference assessments and the acceptability level among panelists [14]. The organoleptic testing results indicated that the panelists assigned the highest scores to isotonic drinks FII (6,1) and FIII (6,0), both of which were categorized within the 'like' scale. In contrast, FI received a score of 4,1, falling into the 'neutral' scale. There was an observable trend of increasing visual preference for FII and FIII. This was attributed to the higher concentrations of yellow rope stem extract, which produced a more appealing color intensity compared to FI. The organoleptic color test results for the isotonic drinks are presented in **Figure 1**.

Aroma functions to balance the overall flavor profil [14]. The data in **Figure 1.** indicate a higher panelist preference for the aroma of isotonic drink FIII (5,1), which was categorized as somewhat like. Conversely, FI and FII only obtained scores of 4,1 and 4,3, respectively, falling into the neutral category. The increase in the concentration of yellow rope stem extract proved to have a significant effect on the resulting aroma profile of the product. There was a positive correlation where a higher extract concentration led to a more distinct herbal aroma, which ultimately enhanced the panelists' preference levels.

Taste serves as a fundamental parameter in the organoleptic evaluation of food products. The panelist assessment results presented in **Figure 1** indicate that FII obtained the highest score of 3,2 (categorized as 'somewhat dislike'), followed by FI and FIII with scores of 2,3 and 1,7, respectively (categorized as 'dislike'). Although FII possessed a higher extract concentration and a higher mean preference score compared to FI, statistical analysis indicated that this difference was not statistically significant ($p>0,05$).

This suggests that increasing the extract concentration to a certain level has not yet exerted a significant impact on changes in panelist taste preferences. Based on the studies conducted by [24] regarding the hedonic taste test of yellow sweet potato syrup with the addition of bilimbi juice (mL), results indicated the panelists' level of preference for the taste. Specifically, 48,3% of panelists gave a score of 5 (strongly like) for F2 (3:1), while 40% gave a score of 4 (somewhat like) for F1 (3:2), and 42.6% gave a score of 5 (strongly like) for F3 (3:3). The assessment of the panelists' preference level showed that the most preferred syrup was F2, with a score of 5 (strongly like).

The taste of the syrup was dominated by the characteristics of yellow sweet potato with a slightly acidic note from the bilimbi. Evaluating taste is not an easy task, as it is heavily influenced by individual preferences. Consequently, it can be concluded in this study that F1 received a lower score than F2 due to the influence of each panelist's individual preferences. In a study conducted by [25] regarding the hedonic test of coconut isotonic drinks influenced by the addition of sucrose, the analysis of hedonic quality for the taste parameter showed that increasing sucrose levels from 6% to 8% was followed by an increase in the preference score from 3,6 to 3,76 (the highest score). This indicates that an 8% sucrose concentration is the optimal point for panelist preference.

However, at a sucrose concentration of 10%, the taste score dropped drastically to 2,48. This decline indicates that excessive addition of sweetener beyond a certain threshold negatively impacts the acceptance level of the isotonic drink's taste. Thus, it can be concluded in this study that the lower score obtained by F1 is also suspected to be caused by a low extract concentration that was masked by sucrose, consequently reducing the level of taste acceptance by the panelists. Conversely, the significant decrease in the score for FIII indicates a negative effect resulting from the excessive increase in extract concentration. This is presumed to be caused by the high content of secondary metabolites, such as berberine alkaloids, in the yellow rope stem. These compounds trigger an excessive bitter sensation, which subsequently dominates the flavor profile of the formulation [26].

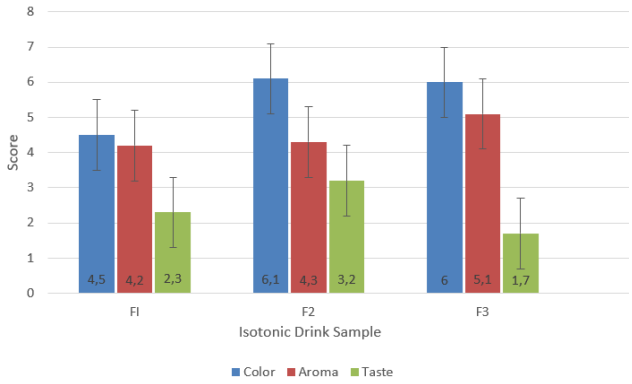

Figure 1. Graph of Organoleptic Test Results

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Results of pH Analysis, Total Soluble Solids (TSS), and Total Titratable Acidity (TTA) Measurements of Isotonic Drinks

pH (Potential of Hydrogen) is an indicator of the acidity or alkalinity of a solution and serves as a crucial parameter in the food industry [16]. This study utilized universal pH indicator strips as the measurement instrument. The primary advantage of using universal indicator paper lies in its operational simplicity, as it requires no specific maintenance or calibration procedures. Unlike litmus paper, which only identifies the acidic or basic nature of a substance, universal indicators can estimate pH values within a 0–14 range through a simple immersion process and colorimetric reaction, without involving the use of electrodes. Furthermore, this method is significantly more cost-effective compared to digital pH meters. Nevertheless, universal indicators have notable drawbacks regarding accuracy and precision. The accuracy tends to be lower due to the subjective nature of visual interpretation by the observer. Additionally, this method has sensitivity limitations, as it can only provide whole-number pH values without the decimal precision offered by calibrated digital pH meters [27]. Based on the pH measurement results, all three formulations of the yellow rope stem extract isotonic drink showed a constant value of 4,0. These data indicate that variations in extract concentration did not exert a significant influence on the product's pH. The obtained pH values demonstrate compliance with applicable quality standards, referring to SNI regulations, this product meets the quality requirements as its pH value remains within the permissible limit (maximum 4,0) [28]. The pH measurement results of isotonic drinks can be seen in **Table 3**.

Total Soluble Solids (TSS) is an indicator representing the concentration of dissolved substances within a solution system, which is commonly interpreted as the sugar content in °Brix units. Specifically, the Brix degree refers to the mass of dissolved sucrose, 1°Brix is quantified as equivalent to 1 g of sucrose in 100 mL of solution, or a sugar concentration of 1% [29]. The research results indicate that all three formulations of the yellow rope stem extract isotonic drink have a consistent Total Soluble Solids content of 7%. Referring to the SNI standards, which establish a minimum Total Soluble Solids threshold of 5%, all samples in this study are declared to have met the applicable quality requirements The results of the Total Soluble Solids test of isotonic drinks are shown in **Table 3**.

Total acid content analysis was carried out using a titration method by reacting the sample with a standardized base solution until the endpoint or equivalence point of the reaction was reached. The obtained value represents the total organic acid content present in the product [29]. The results demonstrated that the highest total acid content was observed in FI (1,024%), whereas the lowest value was recorded in FIII (0,704%). The decrease in acid concentration, which was inversely proportional to the increasing extract concentration, is presumed to be associated with the phytochemical characteristics of the yellow rope stem extract. The presence of basic secondary metabolites in the extract is thought to exert a neutralizing effect on the acidic components of the formulation. Yellow rope stem is known to contain alkaloid compounds [3,7]. Most alkaloids are basic in nature due to the presence of a lone pair of electrons on the nitrogen atom. The basicity of these compounds is influenced by the stability and availability of the lone electron pair, which plays an important role in determining the chemical interactions of the compounds in solution [30]. The total acid values of the isotonic drinks are shown in **Table 3**.

Table 3. Measurement Results of pH, Total Soluble Solids (TSS), and Total Acidity of Isotonic Drinks Containing Yellow Rope Stem Extract (*A. cocculus* (L.) Wight & Arn)

Formula	pH	Total Soluble Solids (TSS)	Total Titratable Acidity
FI	4,0	7	1,024%
FII	4,0	7	0,896%
FIII	4,0	7	0,704%

Results of Antioxidant Activity

The antioxidant activity of the samples was determined using the DPPH assay, the basic principle of which involves the transfer of hydrogen atoms from antioxidant substances to stabilize the free electrons in DPPH molecules. This neutralization process transforms DPPH radicals into a stable form, diphenylpicrylhydrazine, physically, this is marked by a color transition of the solution from purple to yellow and a reduction in absorbance values during UV-Vis spectrophotometric measurements [16]. Referring to the data presented in **Figure 2.** and **Table 4.**, the antioxidant activity of the three isotonic drink formulations containing yellow rope stem extract showed varying levels of effectiveness. FI obtained the lowest IC_{50} value at 35,61 µg/mL, followed by FII with an IC_{50} value of 46,56 µg/mL, both results indicate antioxidant activity

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classified as very potent. Meanwhile, FIII obtained an IC_{50} value of 57,69 µg/mL, categorizing the sample within the strong antioxidant activity intensity. All three formulations demonstrated 50% inhibition against DPPH radicals within the concentration range of 10-50 µg/mL. The lower the IC_{50} value, the stronger the antioxidant capacity [31]. In this study, the highest concentration did not yield very potent antioxidant activity. This finding is inconsistent with the theory that a higher concentration added to a treatment results in a higher inhibition percentage. Based on this phenomenon, it is suspected that pro-oxidant compounds are present in the yellow rope stem. According to previous research, the yellow rope plant is known to contain flavonoid compounds [3,7]. Flavonoids are polyphenolic compounds that possess various biological activities, such as antidiabetic, antibacterial, anticholesterol, antihyperlipidemic, antiviral, anticancer, anti-inflammatory, and antioxidant properties [32]. Aside from their antioxidant properties, several polyphenols can demonstrate pro-oxidant behavior. This mechanism stimulates the formation of Reactive Oxygen Species (ROS), such as hydroxyl radicals, superoxide anions, and hydrogen peroxide [33].

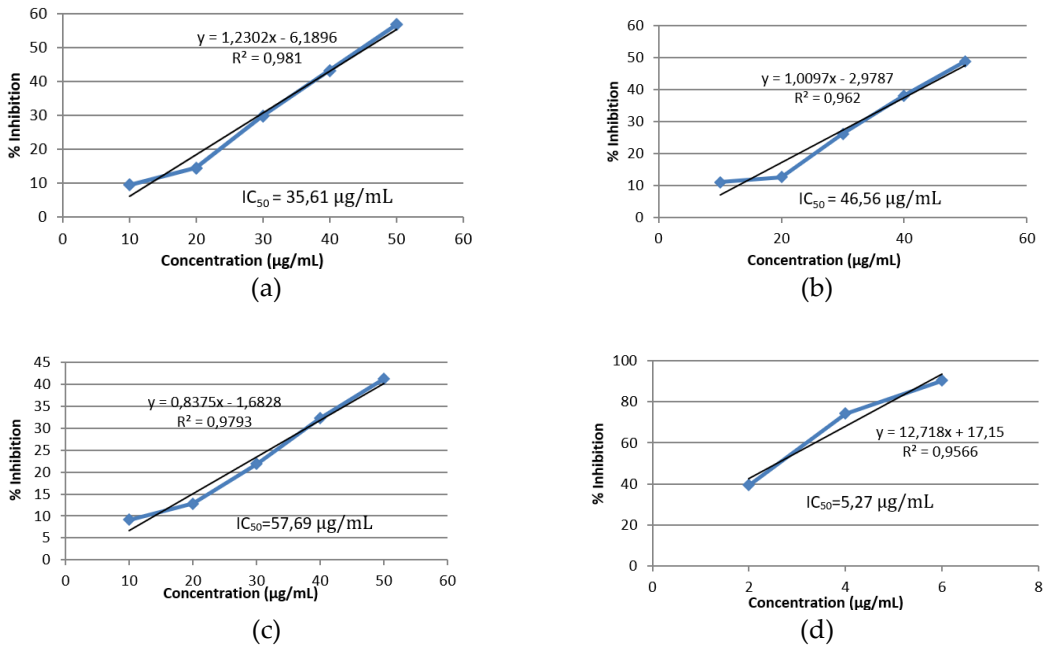

Figure 2. Results of Antioxidant Activity Test (a) Isotonic Drink FI, (b) Isotonic Drink FII, (c) Isotonic Drink FIII, (d) Quercetin

Table 4. Antioxidant Activity of Isotonic Drink from Yellow Rope Stem Extract (*A. cocculus* (L.) Wight & Arn)

Formula	Concentration (ppm)	Antioxidant Activity (%)	IC_{50} Value (µg/mL)
FI	10	0,468	35,61 µg/mL
	20	0,442	
	30	0,363	
	40	0,294	
	50	0,224	
FII	10	0,460	46,56 µg/mL
	20	0,452	
	30	0,382	
	40	0,320	
	50	0,265	
FIII	10	0,470	57,69 µg/mL
	20	0,451	
	30	0,404	
	40	0,350	
	50	0,304	

Quercetin was used as the comparative standard. It was selected as the standard solution in this assay because it is a flavonoid derivative with a broad pharmacological spectrum. In addition to its very potent antioxidant potential, quercetin is also known to exhibit efficacy as an anti-inflammatory, antiviral, anticancer,

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and antibacterial agent [34]. Based on the test results, quercetin yielded an IC_{50} value of 5,27 µg/mL, indicating very potent antioxidant activity. Quercetin demonstrated 50% inhibition against DPPH radicals within the concentration range of 2- 6 µg/mL. It is categorized as a secondary antioxidant, often classified as an exogenous antioxidant, which serves as a preventive defense system within the body. Its primary mechanism involves inhibiting the formation of reactive oxygen species through metal chelation or the degradation of their precursors [35]. The antioxidant activity profile of quercetin is demonstrated in **Figure 2.** and **Table 5**.

Table 5. Antioxidant Activity of Quercetin

No.	Concentration (ppm)	Antioxidant Activity (%)	IC_{50} Value (µg/mL)
1.	2	0,313	5,27 µg/mL
2.	4	0,133	
3.	6	0,05	

Conclusions

Based on the research results, the best antioxidant activity was obtained from FI with an IC_{50} value of 35,61 µg/mL, this was followed by FII with an IC_{50} value of 46,56 µg/mL, and FIII with an IC_{50} of 57,69 µg/mL. The optimal formulation for the yellow rope stem (*A. cocculus* (L.) Wight & Arn) extract isotonic drink was FII, which exhibited the most preferred organoleptic characteristics among panelists, receiving an overall hedonic score of 4,6 (neutral) for taste, aroma, and color. All three formulations showed identical pH (4.0) and TSS (7%) values; however, the total acid content decreased sequentially: FI (1,024%), FII (0,896%), and FIII (0,704%). For future research, it is highly recommended to conduct a shelf-life stability study over a period of 7–14 days at both 4°C and 25°C, focusing on the monitoring of pH stability, antioxidant activity retention, and total acid levels to ensure the product maintains its functional properties throughout its intended duration. To improve the accuracy of the chemical profile, pH measurements should be performed using a digital pH meter calibrated with standard buffer solutions, thereby ensuring precise data recorded to two decimal places. Furthermore, subsequent studies should expand the scope of chemical analysis to include the determination of sodium and potassium levels. Finally, formulation optimization is suggested to enhance palatability by exploring flavor-masking agents and natural sweeteners, supported by comprehensive sensory profiling to achieve a flavor profile that is more highly preferred by panelists.

Conflict of Interest

The authors confirm that there are no conflicts of interest, including financial or personal relationships, that could bias the findings presented in this study.

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