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Formulation and Antioxidant Activity of Purple Rosela Flower (*Hibiscus sabdariffa* L.) Combination as Nutraceutical

(Formulasi dan Uji Aktivitas Antioksidan Kombinasi Bunga Rosela Ungu (*Hibiscus sabdariffa* L.) sebagai Nutrasetikal)

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Abstract: A nutraceutical is a functional food containing natural components that have the potential for health, especially from medicinal plants. The purple rosela (*Hibiscus sabdariffa* L.), one of many therapeutic plants that have been effectively transformed into herbal products, is one such example. High quantities of anthocyanin are found in bioactive substances found in rosela flowers. This study aimed to formulate and determine the antioxidant activity of purple rosela flower in combination with red ginger and cinnamon bark. Following the liking test, which includes the organoleptic test, and the calculation of shelf life with the Accelerated Shelf-Life Testing (ASLT) method, the brewed tea formulation was kept to determine the total content of phenolate. Antioxidant activity was measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. This study obtained an average result of 0.38±0.05% for the total phenol content of the three formulas. Formula I, II, and III showed antioxidant activity with IC_{50} values of 21.59 g/mL, 27.13 g/mL, and 20.05 g/mL, respectively. Organoleptic observations that have been observed for 20 days at three different temperatures (4 °C, 25 °C, and 37 °C) in the three formulas indicate no change in color, aroma, or taste, indicating the product remains stable. Based on the score results, the most preferred by panelists was Formula I, with its composition of purple, rose and red ginger.

Keywords: Antioxidant, formulation, nutraceutical, rosela

Abstrak: Nutrasetikal adalah jenis pangan fungsional yang mengandung komponen- komponen alamiah potensial yang memiliki manfaat terhadap kesehatan terutama dari tumbuhan yang berkhasiat sebagai obat. Banyak tumbuhan obat yang berhasil diolah menjadi produk herbal salah satunya bunga rosela (*Hibiscus sabdariffa* L.) ungu. Bunga rosela mengandung senyawa bioaktif dengan kadar antosianin yang tinggi. Berdasarkan hal tersebut, maka pada penelitian ini pada penelitian ini dibuat formulasi nutrasetikal kombinasi bunga rosela ungu dengan jahe merah dan kulit kayu manis sebagai minuman kesehatan. Seduhan dari formulasi tersebut dilanjutkan uji kadar fenolat total kemudian uji antioksidan dengan metode DPPH (2,2-difenil-1-pikrilhidrazil), lalu uji kesukaan yang meliputi uji organoleptik serta perhitungan umur simpan dengan metode *Accelerated Shelf-Life Testing* (ASLT). Dari penelitian ini didapatkan hasil rata-rata kadar fenolik total ketiga formula sebesar 0,38±0,05%. Formula I, II, dan III menunjukkan adanya aktivitas antioksidan dengan nilai IC_{50} berturut-turut sebesar 21,59 bpj; 27,13 bpj; dan 20,05 bpj. Pengamatan organoleptik yang telah diamati selama 20 hari pada tiga suhu yang berbeda (4 °C, 25 °C, 37 °C) pada ketiga formula tidak terdapat perubahan pada warna, aroma, dan rasa menunjukkan produk tetap stabil. Berdasarkan hasil uji skor yang paling disukai panelis adalah Formula I dengan komposisi rosela ungu dan jahe merah.

Kata kunci: Antioksidan, formulasi, nutrasetikal, rosela

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INTRODUCTION

BACK-TO-NATURE trend has made people tend to use natural products as preventive and curative, which has led to the growth of nutraceutical research. Nutraceuticals also contain nutrients and chemical content that is potentially effective for the body. Nutraceuticals come from the words "nutrition" and "pharmacy", which are defined as foods that provide medical benefits[1]. Rosela flowers, which are consumed as brewed tea, are frequently consumed.

Rosela flower tea is frequently used in the community to reduce fever, relieve colds and coughs, lower cholesterol levels, and stimulate appetite. Rosela flower has many pharmacological activities including antibacterial, diuretic, antihypertensive, antiinflammatory, anticholesterol, hepatoprotective, anthelmintic, and antioxidant[2–4]. High quantities of anthocyanin flavonoids found in rosela flowers work as antioxidants by blocking free radicals, which can stop cell aging and other ailments[5].

Rosela diversification continues to be developed, currently, rosela is consumed in tea, syrup, jam, packaged drinks, and candied rosela[6]. According to the Indonesian Ministry of Health No SPP 1065/35.15/0.5, every 100 g of rosella petals has the following nutritional content 1.145 g, fat 2.61 g, fiber 12 g, calcium 1.263 g, phosphorus 2.73 mg, malic acid 3.31%, fructose 0.82 g, sucrose 0.24%, carotene 0.029%, thiamine 0.117%, niacin 3.765 mg, iron 8.98 mg and vitamin C 244.4 mg. Compared to other fruits, rosela flowers have a higher vitamin C content[7,8].

Even though rosela flower tea is frequently consumed, its characteristic sour flavor prompts customers to combine it with other components, like sweet flowers, spices, or dried fruits are just a few examples of the ingredients. Based on this, a combination formulation of rosela flower with spices, namely red ginger and cinnamon bark, was conducted. Spices are a high source of vitamins and minerals and are also the most commonly used flavor additives in tea[9]. In Indonesia, these spices also grow well and are widely accessible. Purple rosela flowers were chosen because they are less acidic and have a higher anthocyanin content compared to red flowers[6]. This study aimed to formulate and determine the antioxidant activity of purple rosela flower in combination with red ginger and cinnamon bark.

MATERIALS AND METHODS

MATERIALS. The dried purple rosela flowers were obtained from a rosela plantation in Kediri, East Java, Indonesia. Cinnamon bark and red ginger purchased online at a market. These three samples were identified (No.B-418/IV/DI.01/3/2021) at Herbarium Bogor, Puslitbang Biologi LIPI, Cibinong, Bogor, West Java. Gallic acid standard (Merck, Germany), ethanol pro analysis (Merck, Germany), Na_2CO_3 (Merck, Germany). Vitamin C standard, DPPH (2,2-Diphenyl-1-picrylhydrazyl), and Foline-Ciocalteu reagent (Sigma Aldrich, Singapore).

Tools. Analytical balance, micropipette, and Spectrophotometer UV-Vis 1900 (Shimadzu, Kyoto, Japan).

METHODS. Sample Preparation. Using a blender, the dried purple rosela flowers, red ginger, and cinnamon bark were mashed and then sieved over a number of 4/18 sieves.

Formulation and Nutraceutical Evaluation of Rosela Flower Combination. Formulation and nutraceutical evaluation of rosela flower combination were made in 3 variations shown in Table 1. The evaluation was carried out based on organoleptic observation of changes in taste, color, and smell.

Table 1. Formulation of rosela flower combinaton.

Materials	Percentage (%)		
Purple rosela	50	50	50
Red ginger	50	-	25
Cinnamon bark	-	50	25

Total Phenolics Content (TPC) Determination. Approximately 100 mg of the steeping solution was diluted with 10 mL of 96% ethanol. Then 1 mL of the solution was pipetted for three replicates. Each solution was added with 0.4 mL of Folin-Ciocalteau reagent shaken for 4-8 minutes, followed by 4.0 mL of 7.5% sodium carbonate solution, shaken until homogenous, 10 mL of sterile distilled water and allowed to stand for 2 hours at room temperature. A blue complex will result from measured absorption at a maximum absorption wavelength of 764 nm. Three replicates were done so the content of total phenolics as mg equivalent gallic acid/100 mg fresh sample. In terms of gallic acid equivalents (GAE) per 100 g of dry weight, total phenolic values were defined[10,11].

Total phenolics (C) = Cu x Va x Fp/Bu (mg/g GAE)

Cu : sample concentration (mg/mL); Va: final volume (mL); Fp : dilution factor; Bu: weight (g)

Determine The Antioxidant Activity. The vitamin C, test, and blank solutions were all incubated for 30 minutes at 37 °C in an incubator. Then measured spectrometrically at a maximum absorption wavelength of 517 nm[9,10]. We applied the following equation to determine the inhibition:

Inhibition (%) = (Abs DPPH - Abs Sample)/(Abs DPPH) x 100%

A number of 0% indicates that there is no free radical activity, whereas a value of 100% indicates that all free radical activity has been suppressed, and further dilution of the test substance is required to determine the concentration limit of its activity Furthermore, a correlation the concentration of the test solution and the percentage of DPPH inhibition was created, and the IC_{50}, the concentration of the test solution that results in 50% DPPH inhibition, was calculated. The IC_{50} value is determined by intersecting the line between the inhibition power and the concentration axis with the regression equation y= a+bx, where y= 50 and the x value shows IC_{50}.

Hedonic Test. The degree of preference test on the best nutraceutical formula was done on 30 untrained panelists who were separated into two groups based on gender, namely men and women with an age range of 20-60 years, each with 15 persons using a questionnaire. The color, taste, and smell tests of the brewed purple rosela tea in combination were included in the like test. Hedonic test methodology and non-parametric statistical analysis, Kruskal-Wallis, were used to perform favorability test data analysis[12].

Shelf Life Analysis. The Accelerated Shelf Life Testing (ASLT) technique is used to calculate shelf life. This approach involves examining physical qualities with the use of sensory senses. The observation focuses on organoleptics such as color, smell, and taste. This method is carried out by adjusting the temperature according to storage conditions at cold (2-8 °C), room (25-30 °C), and warm (37 °C) temperatures for 20 days[12,13].

RESULTS AND DISCUSSION

Plant Determination. The results of the determination (No.B-418/IV/DI.01/3/2021) carried out at the Laboratory of the Biology Research Center of LIPI, Bogor showed that the samples were purple rosela (*Hibiscus sabdariffa* L.) flowers, red ginger (*Zingiber officinale* Roscoe var. Sunti Val.) and cinnamon bark (*Cinnamomi burmanni* Cortex).

Organoleptic Evaluation. The three formulations had the same organoleptic results shown in Table 2. This showed that the product was remained stable over the length of the 20-day storage term. The smell and taste of purple rosela flowers were improved by the distinctive smell and taste of red ginger and cinnamon bark. Cinnamon in the beverages helped to keep the anthocyanins stable throughout storage[14]. Recent researches has shown that these compounds and their reaction products can stabilize anthocyanins through co-pigmentation processes. Cinnamaldehyde slowly oxidizes to form cinnamic acid, and studies have shown that when anthocyanins are acylated with cinnamic acid, it proves more stable[14,15].

Total Phenolics Content (TPC) Determination. From the results of the gallic acid BP series solution (Figure 1), the value of a= -0.0256, and b= 0.1298 so that the regression line equation y= -0.0256 + 0.1298 x was obtained. with a value of r= 0.981 close to 1 which indicated that the regression equation is linear. In determining the level with three measurements on each sample, the results were obtained described in

Table 2. Evaluation of rosela flower combination as nutraceutical (organoleptic).

Storage Temperature	Formula	F1			F II			F III		
	Day									
Cold (4-10°C)	0	UMG	J	A	UM	KM	AA	UMG	KM	A
	5	UMG	J	A	UM	KM	AA	UMG	KM	A
	10	UMG	J	A	UM	KM	AA	UMG	KM	A
	15	UMG	J	A	UM	KM	AA	UMG	KM	A
	20	UMG	J	A	UM	KM	AA	UMG	KM	A
Room (25-30°C)	0	UMG	J	A	UM	KM	AA	UMG	KM	A
	5	UMG	J	A	UM	KM	AA	UMG	KM	A
	10	UMG	J	A	UM	KM	AA	UMG	KM	A
	15	UMG	J	A	UM	KM	AA	UMG	KM	A
	20	UMG	J	A	UM	KM	AA	UMG	KM	A
Warm (37°C)	0	UMG	J	A	UM	KM	AA	UMG	KM	A
	5	UMG	J	A	UM	KM	AA	UMG	KM	A
	10	UMG	J	A	UM	KM	AA	UMG	KM	A
	15	UMG	J	A	UM	KM	AA	UMG	KM	A
	20	UMG	J	A	UM	KM	AA	UMG	KM	A

Note: UMG = Dark Red Purple, UM = Red Purple, J = Ginger, K = Cinnamon, A = Sour, AA = Mildly Sour.

Table 3. In the previous study, the TPC in rosella flower red petals amounted to 1.20 g GAE/100 g extract. These results are different from this study is 0.38%.

Environmental factors influence the formation of secondary metabolites. Temperature and carbon dioxide levels are two elements that influence secondary metabolite formation. The greater the temperature and carbon dioxide levels, the more secondary metabolites are created. Many elements impact rosella growth, including temperature, humidity, rainfall, and altitude[16,17].

The Antioxidant Activity. The antioxidant activity of brewed tea in formulations I, II, and III was shown by IC_{50} values of 21.59±0.61ppm, 27.13±0.11ppm, and 20.05±0.20ppm, respectively, indicating that the concentration may eliminate 50% of free radicals. The antioxidant activity results in Table 4. The lower the IC_{50} value, the higher the antioxidant activity as a free radical scavenger[17]. The antioxidant activity is defined to be very strong if its IC_{50} value is less than 50 ppm, strong if it is between 50 and 100 ppm, medium if it is between 100 and 250 ppm, and weak if it is greater than 250 ppm.

According to the test results, the nutraceutical formula has very strong antioxidant activity, with the highest level of antioxidant activity (20.05 ppm) observed in the third formula, which contains purple rosela flowers, red ginger, and cinnamon bark. As indicated by the results, adding ginger and cinnamon increases antioxidant activity. They can be used in cuisine as a natural antioxidant. Antioxidant activity is enhanced by phenolic chemicals and volatile components. Cinnamon includes numerous antioxidants that are essential in decreasing oxidative stress, which is related to its different flavonoid components[18].

Cinnamon oil showed strong potential DPPH radical scavenging action, similar to commercial antioxidants. Some phenolic compounds in ginger oil, such as shogaols, zingerone, gingerol, and gengerdiols, were identified as small components (2% of oil). Ginger oil is a very complex blend of a number of chemical compounds which can have either synergistic or antagonistic effects on the process of lipid oxidation or radical scavenging[19,20].

Hedonic Test. The results of the comparison table 6 of score values and hedonic quality performed on 30 panelists reveal that there are differences in color, smell, and taste in each sample. According to the taste parameter, panelists preferred the sample when sugar was added, indicating that the taste of the sample improves with the addition of sugar when compared to those that didn't use sugar. From three formulas, panelists preferred the color, smell, and taste of Formula I* (purple rosela + red ginger + sugar) because Formula I* concluded that the formula was purple (3), smelled good (4), and tasted slightly sour (4).

Shelf Life Analysis. There are no changes in color, smell or taste detected after 20 days during storage at three different temperatures in the three formulas (Table 5)[12,13]. This indicates that the product can be stored for more than 20 days with the same quality as before, so the estimation of shelf life using the Arrhenius method with the ASLT approach cannot be processed because there is no data on the decrease in the quality of color, smell, and taste in the three formulas stored at various storage temperature conditions.

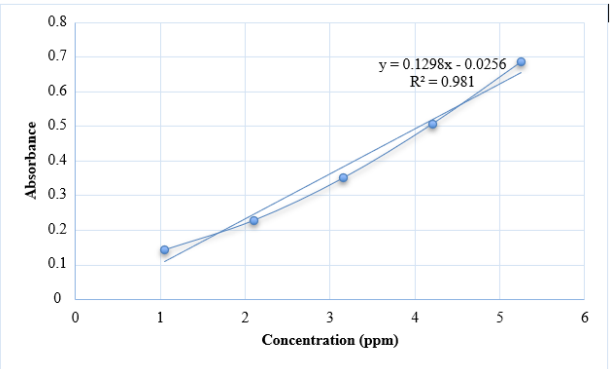

Figure 1. Standard curve of gallic acid.

Table 3. Result of total phenolic assay.

Formula	Replication	Concentration (ppm)	Content (%)	Average
I	1	0.4284	0.42	
	2	0.4284	0.42	0.42
	3	0.4284	0.42	
II	1	0.4438	0.39	
	2	0.4438	0.42	0.41±0.02
	3	0.4361	0.41	
III	1	0.3513	0.32	
	2	0.3513	0.32	0.32
	3	0.3513	0.32	

Table 4. Result of antioxidant activity.

	IC_{50} values (ppm)						
Replication	Vit C	Brewed water of rosella flower	Brewed water of red ginger	Brewed water of *Cinnamomum burmanii* Cortex	F I	F II	F III
1	5.88	21.80	33.68	34.01	22.11	27.18	19.83
2	5.01	20.31	33.59	31.18	21.75	27.20	20.23
3	4.81	18.36	33.65	31.53	20.92	27.00	20.08
Average±SD	5.23±0.57	20.16±0.91	33.64±0.05	32.24±1.54	21.59±0.61	27.13±0.11	20.05±0.20

Table 5. Result of shelf life.

Storage Temperature	Formula	F1			F II			F III		
	Day	W	A	R	W	A	R	W	A	R
Cold (4-10◦C)	0	9	9	9	9	9	9	9	9	9
	5	9	9	9	9	9	9	9	9	9
	10	9	9	9	9	9	9	9	9	9
	15	9	9	9	9	9	9	9	9	9
	20	9	9	9	9	9	9	9	9	9
Room (25-30◦C)	0	9	9	9	9	9	9	9	9	9
	5	9	9	9	9	9	9	9	9	9
	10	9	9	9	9	9	9	9	9	9
	15	9	9	9	9	9	9	9	9	9
	20	9	9	9	9	9	9	9	9	9
Warm (37◦C)	0	9	9	9	9	9	9	9	9	9
	5	9	9	9	9	9	9	9	9	9
	10	9	9	9	9	9	9	9	9	9
	15	9	9	9	9	9	9	9	9	9
	20	9	9	9	9	9	9	9	9	9

Description: W = colour, A=smell, R = taste.

Table 6. Result of hedonic test.

Sample Code	Colour	Smell	Taste	Average
F1	3	4	3	3,33
F2	3	3	3	3,00
F3	2	3	3	2,67
F1*	3	4	4	3,67
F2*	3	3	4	3,33
F3*	2	3	4	3,00

Description:
F1: Purple rosela + red ginger; F2: Purple rosela+cinnamomum; F3: Purple rosela+red ginger+cinnamomum
F1*: Purple Rosela+red ginger+sugar; F2*: Purple rosela+cinnamomum+sugar; F3*: Purple rosela+red ginger+cinnamomum+sugar

CONCLUSION

Nutraceutical products that are useful as drinks can be made by combining purple rosela flowers with red ginger and cinnamon bark. The third formula has the strongest antioxidant activity, with an IC_{50} value of 20.05 ppm. Red ginger and cinnamon bark can reduce bitterness while enhancing antioxidant activity.

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