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The development of functional drinks made from telang flower (*Clitoria ternatea*) with lime juice addition

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ABSTRACT

Telang (Clitoria ternatea) is a medicinal flower used in food and beverages. The beverages made from this flower are commonly available in Indonesia, but their properties have not been extensively investigated. Thus, this study aimed to determine the physicochemical properties of telang flower beverages, such as pH, vitamin C, anthocyanin, and hedonic level. The study employed a complete randomized factorial design. The weight of dried flowers and volume of lime juice were used as independent variables. The De Garmo effectiveness index selected the four best physicochemical characteristics of beverages. The addition of dried flower and lime juice resulted in a significant difference in anthocyanin, vitamin C, pH, and free radical inhibition activity. Four treatment beverages with good chemical qualities were chosen to be examined hedonically. The treatment with lime juice addition resulted in a significant difference in the hedonic level of color, sweetness, and acidity ($p<0.05$). There was, however, no significant difference in the aroma produced in the beverages ($p>0.05$). The best formulation of the telang flower beverage was based on hedonic analysis with a combination of 4.5 g of telang flower and 5 ml of lime juice, resulting in a beverage with less sugar and fewer calories.

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INTRODUCTION

The Covid 19 pandemic may enhance public awareness of the importance of safe and healthy food. Functional food and drink are becoming more popular due to its numerous health benefits, including lowering the risk of certain diseases and improving biological function (Tolun and Altintas 2019). Several new beverages on the market are designed to treat specific health conditions, such as dairy, probiotics, energy, fruit, and herb beverages.

For thousands of years, plants and herbs have been significant contributors to the quality of human life. Several are well-known as medicinal plants. They have recently become trendy due to several phytochemicals with potential or established biological activity, such as alkaloids, glycosides, polyphenols, and terpenes (Ahn 2017). The development of medicinal plants into functional beverages enhances general and physical health or reduces the risk of disease evolution (Bulman et al. 2021). Functional beverages made from medicinal plants such as *Hibiscus sabdarifa* flower (Omoarukhe 2017, Djaeni et al. 2018), ginger (Sugimoto et al. 2018), and curcumin (Astari 2020) have been published.

Telang flower (*Clitoria ternatea*) has long been recognized as one of the medicinal flowers that contain health-promoting compounds (Muhammad Ezzudin and Rabeta 2018). Because of its appealing colors, distinct aromas, and high antioxidant content, the flower is currently a well-known raw ingredient for food and beverages. In Southeast Asia, dried telang flowers are used to make a popular herbal tea (Adisakwattana et al. 2020).

Telang flower ethanol extract contains terpenoids, flavonoids, tannin, and steroids, which may act as antioxidants (Jeyaraj et al. 2021). The pentacyclic triterpenoids taraxerol and taraxerone are the main phytoconstituents discovered in this flower (Lijon et al. 2017). Studies on the antioxidant activity of telang flower extract have produced beneficial findings. In many model systems, the crude extracts demonstrated antioxidant activity (Jeyaraj et al. 2021). Natural antioxidants have been widely used in various dietary products to delay or prevent the lipid peroxidation process. They may operate in various ways during lipid peroxidation, including binding metal ions, scavenging free radicals, and degrading peroxides. Antioxidants also protect our immune system from reactive oxygen species (ROS), which are particularly dangerous throughout the aging process (Liu et al. 2018).

The development of medicinal flowers into functional beverages involves understanding the active component content. The methods are required to ensure that the taste and health benefits are acceptable. Functional beverages prepared from flowers and other plant components are mainly consumed fresh or in the form of dry powder. Ready-to-drink beverages are becoming much more popular in recent years. In addition to their health benefits, consumers prefer ready-to-drink beverages because of their nutrient profiles and ease of consumption due to their liquid structure.

The beverages made from telang flowers are commonly available in the community, but their sensory and chemical properties have not been well investigated. Lime juice will be added to telang beverages in this study, which is supposed to increase sensory and chemical properties. Lime has a pleasant acidic taste that can enhance the overall flavor while improving the nutritional content. Lime also includes citric acid, which might affect the color of telang drinks due to the flower's antioxidant properties. Because lime juice contains more vitamin C than lemon juice, it can be used instead of lemon juice in a long-term intervention focusing on weight loss. Lime is more economically feasible than lemon (Manuha et al. 2019). The previous study showed that soaking lime in infused water had a vitamin C concentration of 5-18 percent (Ivakdalam and Rehena 2020).

A study about the development of functional beverages based on telang flowers needs to be conducted. The objective of this research was to investigate the physicochemical characteristic of telang beverage, including pH, vitamin C content, anthocyanin content, and hedonic level.

METHODS

Materials

Clitoria ternatea flowers were collected from Kulonprogo Regency, Yogyakarta Province, Indonesia. It was harvested from the five-month-old plant in the morning and promptly moved to the following study phase. The flowers were then dried using a sun dryer with a black cloth cover for three days (moisture content less than 15% db).

2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, ethanol, buffer solution, chloric acid, and acetic acid were utilized as chemical reagents. Except otherwise mentioned, all reagents were analytical grade.

Experimental design

The beverage consisted of sugar, lemongrass, lime, and dried telang flower. The study used a complete randomized design of factorial. The independent variables were the weight of the dried flower (1.5, 2.5, 3.5, and 4.5 g) and the volume of lime squeezed (0, 5, 10, and 15 ml). The study provided 16 treatments with three repeats each, resulting in a total of 48 research trials.

The telang beverages were prepared as follows: 1 L of water, 30 g of lemongrass, and 120 g of sugar were cooked to a boil. Once after that, the flower was added, and the stove was turned off. While soaking the flower for 30 minutes, the lime juice was added. After that, the solution was filtered and prepared for packaging. All samples were then evaluated for pH, anthocyanin content, free radical inhibition activity, and vitamin C. The data was then examined using the De Garmo effectiveness index method to select the most effective treatment. Four samples with the best physicochemical characteristics were then chosen for sensory evaluation. The hedonic features of the four samples with the highest efficacy index were next studied.

Anthocyanin analysis

Anthocyanin content analysis was carried out using the pH differential method (Zu et al. 2012). A specified volume of telang flower extract is dissolved in two distinct buffer solutions. The first solution was dissolved in 0.025 M potassium chloride buffer pH 1.0, while the other solutions were dissolved in 0.4 M sodium acetate buffer pH 4.5. The number of samples utilized was determined, which results in the absorbance value at vis-max, which is within the linear range of the spectrophotometer. Additionally, scanning wavelengths between 200 nm and 750 nm were used to determine the anthocyanin content and viscosity of sample solutions in both buffers (KCl and Sodium Acetate). The absorbance of each solution was determined, and the result was calculated using the following equation.

$$A = (A_{540} - A_{700})_{pH1} - (A_{540} - A_{700})_{pH4.5}$$

Total monomeric anthocyanin from extract dried telang flower was estimated as cyaniding-3-glucoside based on the following equation.

$$MAP\ \left(\frac{mg}{L}\right) = \left[\frac{(AxMWxDFx10)}{\varepsilon} x\ 1\right]$$

description:

A: Absorbance of the solution

MW: Molecular weight

DF: Dilution factor

ε: Molar absorptivity of cyaniding-3-glucoside

b: the thickness of cuvette = 1

MAP: Monomeric Anthocyanin Pigment

Free radical inhibition activity analysis

Free radical inhibition activity was analyzed by DPPH methods (Tristantini et al. 2016). 1 ml of sample solution was added to the test tube, then adding 4 ml DPPH solution 50 μM and observed the change in its color. The mixture was homogenized and left for 30 minutes in a dark place, then measured by UV – Vis spectrophotometer at wavelengths 517 nm.

Sensory evaluation

The analysis involved 30 untrained panelists. The organoleptic test for the panelists used the hedonic LSD (Least Significance Difference) test by giving four samples of selected treatment drinks with the tested criteria, including color, sweetness level, acidity level, and aroma. The hedonic assessment scores include strongly like (5), like (4), moderate (3), dislike (2), and strongly dislike (1).

Vitamin C analysis

Vitamin C content was analyzed using spectrophotometer methods (Desai 2019). The standard ascorbic acid solution was prepared by dissolving 50 mg of ascorbic acids in 100 ml of distilled water (500 μg/ml).

pH analysis

Briefly, 5 g of the dried flower was homogenized in 50 ml of distilled water, and the filtrate was filtered using filter paper. The pH was measured using a pH meter.

Treatment selection

Determination of the best combination of telang flower beverages using the De Garmo effectiveness index method (Fauzi and Palupi 2020, Wulandari et al. 2020) based on the content

of vitamin C, anthocyanins, and pH of the products. The results of the De Garmo calculation are based on the effectiveness values (EV) and weight of each parameter. The parameters in this study were weighted in vitamin C, anthocyanins, and pH with the values of 3, 2, and 1, respectively. The total multiplication of weight and EV is the effectiveness index for the basic selection of the four best treatments.

Statistical analysis

The data were analyzed using one-way ANOVA and Duncan Multiple Range Test (DMRT) using SPSS IBM 25. The hedonic scores were converted to interval data by Methods of Successive Interval (MSI) before one-way ANOVA. The significant difference was a 95% confidence range. Selection of the best combination treatment using the De Garmo effectiveness index method.

RESULTS AND DISCUSSION

The chemical characteristics of telang flower beverage in this study included anthocyanin content, free radical inhibition, pH, and vitamin C. Chemical characteristic tests were carried out for all treatments. The *Clitoria ternatea* flower beverages from all treatments contained anthocyanin levels ranging from 111.33 to 4630.16 µg/ml. Table 1 shows that the addition of dried flower and lime juice resulted in a significant difference in the anthocyanin content of the beverages ($p<0.05$). According to a statistical study, there was an interaction between the addition of telang flower concentration and lime juice on the anthocyanin content of beverage products. The data indicated that when the concentration of telang flower increased, the anthocyanin content increased as well. The highest anthocyanin level with the value of 4630.16 µg/ml was reported in the treatment with 4.5 g of dried flower and 15 ml of lime. The previous study showed that dried telang flower exhibits antioxidant activity with an IC_{50} of 36-49.67 ppm (data not shown). From this value, dried flower is classified as a strong antioxidant (Adisakwattana et al. 2020).

Table 1 The chemical characteristic of telang flower beverages

Treatment		Antocyanin content (µg/l)	Free radical inhibition activity (%)	Vitamin C (%)	pH
Dried telang (g)	Lime juice (ml)				
1.5	0	0.11 ± 0.04^{a}	62.74 ± 3.69^{abc}	424.85 ± 10.67^{b}	7.17 ± 0.21^{h}
2.5	0	0.24 ± 0.04^{a}	64.49 ± 1.09^{abcd}	437.13 ± 2.73^{b}	6.73 ± 0.06^{g}
3.5	0	2.58 ± 0.44^{cd}	61.77 ± 0.01^{b}	585.55 ± 3.80^{d}	6.67 ± 0.29^{g}
4.5	0	4.07 ± 0.23^{f}	65.36 ± 2.18^{bcd}	797.54 ± 3.91^{h}	6.50 ± 0.26^{g}
1.5	5	0.58 ± 0.08^{a}	61.77 ± 0.00^{a}	424.49 ± 6.53^{b}	3.03 ± 0.06^{de}
2.5	5	1.31 ± 0.47^{b}	65.89 ± 3.10^{cd}	534.63 ± 9.99^{d}	3.10 ± 0.17^{de}
3.5	5	2.25 ± 0.14^{c}	66.23 ± 2.86^{d}	670.06 ± 6.79^{f}	3.47 ± 0.21^{f}
4.5	5	4.14 ± 0.18^{f}	63.08 ± 1.68^{abcd}	841.60 ± 22.53^{i}	3.37 ± 0.12^{ef}
1.5	10	1.20 ± 0.24^{b}	64.68 ± 0.00^{abcd}	453.38 ± 2.14^{bc}	2.83 ± 0.06^{bcd}
2.5	10	2.96 ± 0.17^{d}	62.50 ± 1.16^{ab}	595.31 ± 3.48^{e}	2.87 ± 0.12^{bcd}
3.5	10	$3.05 \pm 0.54d^{e}$	64.05 ± 1.34^{abcd}	672.59 ± 3.57^{f}	2.90 ± 0.10^{bcd}
4.5	10	4.23 ± 0.2^{fg}	64.92 ± 0.67^{abcd}	795.38 ± 9.69^{h}	2.93 ± 0.06^{cd}
1.5	15	1.27 ± 0.18^{b}	64.53 ± 0.00^{abcd}	419.07 ± 7.84^{a}	2.83 ± 0.45^{bcd}
2.5	15	2.76 ± 0.14^{d}	62.21 ± 1.16^{ab}	473.96 ± 5.37^{c}	2.43 ± 0.32^{a}
3.5	15	3.45 ± 0.37^{e}	63.03 ± 0.75^{abcd}	727.12 ± 12.21^{g}	2.53 ± 0.25^{ab}
4.5	15	4.63 ± 0.20^{g}	62.26 ± 0.42^{ab}	858.58 ± 4.10^{i}	2.63 ± 0.06^{abc}

Data are mean ± standard deviation (n = 3). Mean values with different alphabet superscripts are significantly different ($p < 0.05$)

Table 2 Effectiveness index of telang flower beverages formulation

Treatment		Effectiveness value			Effectiveness value x weight			Effectiveness index
Dried telang (g)	Lime juice (ml)	Vitamin C	Anthocyanin	pH	Vitamin C	Anthocyanin	pH	
1.5	0	66.84	0	0	133.68	0	0	133.68
2.5	0	93.26	29.56	91.54	186.51	88.67	91.54	366.73
3.5	0	412.6	546.8	105.63	825.2	1640.39	105.63	2571.22
4.5	0	868.69	876.85	140.84	1737.38	2630.54	140.84	4508.76*
1.5	5	66.06	103.45	873.18	132.12	310.34	873.18	1315.65
2.5	5	303.04	266.01	859.09	606.09	798.03	859.09	2263.21
3.5	5	594.41	472.91	781.64	1188.83	1418.72	781.64	3389.18
4.5	5	963.48	891.63	802.76	1926.96	2674.88	802.76	5404.6*
1.5	10	128.22	241.38	915.43	256.44	724.14	915.43	1896.01
2.5	10	433.58	630.54	908.39	867.15	1891.63	908.39	3667.17
3.5	10	650.25	901.34	1199.7	1950.74	901.34	4051.79	4051.79
4.5	10	911.33	894.3	1728.05	2733.99	894.3	5356.35	5356.35*
1.5	15	256.16	915.43	108.81	768.47	915.43	1792.72	1792.72
2.5	15	586.21	999.93	345.02	1758.62	999.93	3103.57	3103.57
3.5	15	738.92	978.8	1434.35	2216.75	978.8	4629.91	4629.91
4.5	15	1000	957.68	2000	3000	957.68	5957.68	5957.68*

*) The selected treatment for the hedonic test

Telang flower has antioxidant characteristics, one of which inhibits free radicals. The total antioxidant that may block free radicals using 1,1-diphenyl-2picrihidazil was assessed in this study. Since DPPH is a stable free radical molecule, it can be dissolved and read at 515-520 nm (Çoklar and Akbulut 2017). An increase in telang flower and lime juice concentration significantly affected free radical inhibitory activity ($p<0.05$). The interaction between telang flower and lime juice was not significantly different ($p>0.05$). Telang flower beverages have a free radical inhibition activity of 61.77-66.23 % (Table 1). The findings revealed that increasing the anthocyanin content of telang flowers did not always result in increased free radical inhibitory activity. Because free radical inhibition is a measure of a product's total antioxidants, it's possible that other antioxidant substances in telang flower played a role in this condition. The flowers contain taraxerol and taraxerone, among other chemicals. The flowers were analyzed spectrophotometrically, and 14 flavonol glycosides were discovered (Muhammad Ezzudin and Rabeta 2018).

pH and vitamin C content

Telang flower and lime treatments significantly changed the vitamin C content of the beverages ($p<0.05$). Table 1 shows that the amount of vitamin C and the concentration of lime increased. The most efficient treatment was found to be 4.5 g of dried flower and 15 ml of lime with a vitamin C level of 858.58 ppm.

The previous study showed that soaking lime in infused water had a vitamin C concentration of 5-18 percent (Ivakdalam and Rehena 2020). This study found that the vitamin C content of some drink formulations was higher than that of orange juice drinks mentioned in the previous study (493 ppm) (Devianti and Wardhani 2018). Lime, as a source of vitamin C, has numerous health benefits. Vitamin C is an antioxidant that can neutralize free radicals produced by fat oxidation, preventing diseases such as cancer, heart disease, and premature aging. The rate of deterioration of vitamin C is strongly dependent on storage conditions. The rate of vitamin breakdown in orange juice is highly controlled by storage temperature; at 7^{o}C, the degradation rate is lower than at 28^{o}C. Citrus fruits stored for 15 days lower vitamin C levels from 18.90 mg/110 g to 17.18 mg/100 g (Herlina et al. 2020).

Table 3 Telang beverage's hedonic scores

Treatment		Color	Sweetness level	Acidity level	Aroma	Overall
Dried telang (g)	Lime juice (ml)					
4.5	0	3.27±1.12[a]	3.92±0.89[b]	3.47±0.85[ab]	3.92±0.89[a]	3.64±0.92[a]
4.5	5	3.89±0.76[b]	3.94±0.81[b]	3.77±0.75[b]	3.72±0.91[a]	3.83±0.91[b]
4.5	10	3.97±0.75[b]	3.80±0.89[b]	3.53±1.01[ab]	3.64±0.89[a]	3.74±0.90[b]
4.5	15	3.88±0.88[b]	3.25±1.02[a]	3.19±1.08[a]	3.62±0.98[a]	3.48±0.92[a]

Data are mean ± standard deviation (n = 30). Mean values with different alphabet superscripts are significantly different ($p < 0.05$)

Table 4 Nutrition content of the best telang beverages

Nutrition content	Value
Fe (mg/100g)	1.34
Vitamin C (mg/100g)	35.34
Total sugar (%)	14.60
Ca (mg/100g)	0.03
K (mg/100g)	7.37
Fat (%)	0.21
Protein (%)	0.17
Calori (cal)	360.35
Vitamin A (mg/100g)	Negative
Cholesterol (mg/100g)	Negative

The best treatment of beverages formulation

Telang flower drink formulation was chosen based on the De Garmo effectiveness index. The chemical properties of the drink are used to weigh the effectiveness value. The weights for anthocyanin, vitamin C, and pH content were calculated using the priority scale, with the value of 3, 2, and 1, respectively.

The highest effectiveness index in each treatment with 15 ml of lime addition, with values of 4508.76, 5404.60, 5356.35, and 5957.68, respectively. The four treatments were regarded to be beverages with favorable chemical properties. The chosen formulation was subsequently subjected to sensory evaluation.

Beverages hedonic level

The four highest effectiveness index products were the selected telang flower drink formulations to be tested for consumer acceptance based on hedonic level analysis. The lime juice treatment showed a significant difference in the hedonic level of color, sweetness level, and acidity level ($p<0.05$). However, there was no significant difference in the aroma of telang flower drink ($p>0.05$).

The average hedonic score is in the range of 3.27-383 (Table 3). This indicates that all flower drink formulations are in the "like" category. The beverages without lime juice addition had a lower preference level than the other three formulations on the color criteria. The product resulted in a dark blue color. The panelist prefers the product with a purple color that can be found in lime addiction treatment. The anthocyanin in telang flower produced a color change from blue to purple at high acidity levels, making the resulting beverage product more appealing.

The level of acidity in some formulations of telang flower drink varies due to differences in the concentration of added lime juice. Lime contains citric acid, which has a high acidity level. J4T4 had the lowest pH and the highest acidity level, which reduced the panelists' preference level. Based on the average organoleptic score, it can be seen that the formula with a concentration of 5 ml of lime juice and 4.5 g of dried flower received the highest score of the panelists' preference level.

The beverage nutrition content

The best telang flower drink from the research was then analyzed for its nutritional content, including Fe, vitamin C, total sugar, Ca, K, fat, protein, calories, and cholesterol content. The results of the analysis of the beverage content are shown in Table 4. The beverage in this study contains less sugar than commercial apple beverages. The total sugar content of a product affects its caloric value. The beverage in this study contained significantly fewer calories than the apple beverage. Even though low-calorie beverages are currently popular, the flower beverage has the potential to be developed. The flower drink contains less fat, more protein, and cholesterol-free. The previous research mentioned that consumption of *Clitoria ternatea* beverage rise plasma antioxidant activities without hypoglycemia in healthy subjects (Chusak et al. 2018).

CONCLUSIONS

The formulation of telang flower drink with different treatments of dried flower and lime juice showed significant differences in the anthocyanin content, free radical inhibitory activity, pH, and vitamin C content in the resulting beverage. The best formulation of the beverage was chosen based on the four best physicochemical characteristics using the De Garmo method followed by hedonic analysis, which is with a combination of 4.5 g of dried flower and 5 ml of lime juice, with the criteria of color, sweetness level, acidity level, and aroma in the "like" category (3.83) with a score range of 1-5.

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