

Potential *Clitoria Ternatea* as Colourant for Gambir Leaves Tea: the Antioxidant Activity, Polyphenols, Anthocyanins, Catechin, and Epigallocatechin gallate

Tuty Anggraini^{a,*}, Winda Wahyuni Roza^a, Kesuma Sayuti^a, Neswati Alfi Asben^a

^a Faculty of Agricultural Technology, Andalas University, Padang, 25163, Indonesia
Corresponding author: *tuty@ae.unand.ac.id

Abstract— *Clitoria ternatea* and *Gambir* are potential sources of bioactive compounds, but the use of *Gambir* leaves has not been widely used as a beverage. This study investigated tea brewed properties from *Gambir* leaves, enriched with butterfly pea extract as a coloring agent and antioxidant enhancer to determine the optimal ratio of butterfly pea flower extract and *Gambir* tea. This study used a completely randomized design, consist of 5 treatments with three replications. The ratio of *Gambir* leaves tea extract (GLT) with butterfly pea flower extract (TF) used were 100%: 0% (as control); 90%: 10%; 80%: 20%; 70%: 30%; and 60%: 40%. The analysis was the color analysis of TF anthocyanin at various pH, measured antioxidant activity (IC₅₀ value), total polyphenols, anthocyanins, catechin and epigallocatechin gallate, and organoleptic. The result showed that the best ratio was 70% brewed *Gambir* leaf tea: 30% of butterfly pea flower extract. It had an average antioxidant value of IC₅₀ 415.27 mg / L, polyphenol 322.67 mg GAE / L, anthocyanin 9.47 mg/g, color 4.16, aroma 3.24, taste 3.68, catechins 58.84 µg / mg and epigallocatechin gallate 63.74 µg / mg. This beverage can contribute to human health due to the active compounds present in *Gambir* leaves and butterfly pea flowers. Epigallocatechin gallate is the beverage as the strongest antioxidant, which enriches the functional compound in the beverage. In addition to having many functional components, this drink was also very popular with high organoleptic values.

Keywords— Antioxidants; butterfly pea flower; *Gambir* leaves; herbal tea.

Manuscript received 12 Mar. 2020; revised 24 Nov. 2020; accepted 26 Jan. 2021. Date of publication 28 Feb. 2022.
IJASEIT is licensed under a Creative Commons Attribution-Share Alike 4.0 International License.



I. INTRODUCTION

Gambir is a commercially grown plant that is well known throughout Asia and one of the leading commodities in Indonesia. Indonesia is the third-ranked raw export of *Gambir* extract and the seventh-ranked for processed *Gambir* [1]. In West Sumatra, *Gambir* leaves have been extracted to make the *Gambir* dried extract. There are four varieties of *Gambir* grown in the West Sumatra, namely *Cubadak Gambir*, *Shrimp Gambir*, *Riau Gadang Gambir*, and *Riau Ketek Gambir*. All these *Gambir* types have the same antioxidant properties. The extract is processed from the Mangampo process leaves, including steaming, extraction, and clumping of extracts and shaping into pellets. It contains catechin, epigallocatechin, and epicatechin. The price of the dried extract *Gambir* has fluctuated that detrimental to *Gambir*'s farmers. However, making *Gambir* leaves into tea is expected to be a useful product and increase farmers' income to be more satisfied [2], [3].

Tea is mostly made from tea leaves (*Camellia sinensis*). Still, recently herbal teas developed from various parts of different plants, such as leaves, flowers, seeds, and roots, have increased in popularity due to their purported health benefits. Tea from *Gambir* leaves is one herbal tea that could well have health-enhancing properties. *Gambir* leaves contain quite a high concentration of the polyphenol compounds that are also found in traditional tea leaves (*Camellia sinensis*). However, *Gambir* leaf tea has an unappetizing color, which discourages consumer interest. Synthetic dyes, which may have adverse health effects if consumed to excess, are sometimes used to make food more attractive. These adverse health effects can be severe if manufacturers use coloring that has not been approved for human consumption to save money. A natural source of dye like butterfly pea flower extract could avoid these adverse effects while enhancing antioxidant properties.

The butterfly pea flower (*Clitoria ternatea* L) is a typical pea flower shape with a single blue petal. The butterfly pea flowers contain tannins, carbohydrates, saponins, triterpenoids, flavanol glycosides, proteins, alkaloids,

anthraquinone, volatile oils, and steroids [4]. These *anthocyanins*, which are natural pigments commonly found in plants, can be used as food dyes in butterfly pea flowers. Butterfly pea flower anthocyanins have already been found to have potential as beverage and yogurt coloring agents [5].

Butterfly pea flowers contain several good antioxidants, including anthocyanin. These compounds can absorb or neutralize free radicals and help prevent degenerative diseases such as cardiovascular diseases and cancers [6], [7]. This study was motivated by these possible health benefits and was designed to find the best concentration of butterfly pea flower extract (TL) to use in an herbal tea made from *Gambir* leaves (GLT). The proportions of TL to GLT were 100%: 0%; 90%: 10%; 80%: 20%; 70%: 30%; and 60%: 40%.

II. MATERIAL AND METHOD

A. Materials and Equipment

Young *Gambir* leaves harvested in Siguntur, South Pesisir, West Sumatra, Indonesia. Young leaves were chosen because they have a high content of catechins that function as antioxidants. Butterfly pea flowers were bought from the central Padang market. *Ethanol, HCl, potassium chloride, sodium acetate*, distilled water, DPPH solution, *methanol, Folin-ciocalteu* reagent, Na₂CO₃, shrimp larvae (*Artemia salina* Leach), *dimethylsulfoxide, catechin* standards, *epigallocatechin gallate* standards, THF (*tetrahydrofuran*/(CH₂)₄O), were used in the analysis.

B. Research Design

The research design was a completely randomized design with five treatments and three replications. Data from observations were analyzed using the F test of equality of variances, and if two values were found to be significantly different, then further testing using Duncan's New Multiple Test (DNMRT) was conducted at the 5% level. The treatments were labeled 100% GLT, TF10, TF20, TF30, and TF40 according to the percentage of TL used.

C. Brewing Gambir Leaf Tea

Young *Gambir* leaves were withered for 20 minutes at 70°C after picking, then blended and dried in an oven at 80°C for 30 minutes. The dried *Gambir* leaf fragments were sieved through a 20-mesh sieve. The GLT particles were then ready to be analyzed. *Gambir* tea was brewed using 10 g of dried *Gambir* leaves with 100ml of 90°C water for 5 minutes, then filter through a tea filter.

D. Making Butterfly Pea Flower Extract

A 250 ml of water was brought to the boil and combined with 20 g of butterfly pea flowers in a conical flask. After about 3 minutes, the water turned a bright blue color. This infusion was then filtered to produce TL for analysis.

E. Comparison of Different GLT/TL Mixtures

TL was added to the brewed GLT according to the ratio to be trialed, and 9 g of sugar was stirred in until the mixture was homogeneous. Sugar was used to make the tea palatable to local tastes.

F. Moisture Content Determination [8]

A 2 g of material was placed in a container with a known empty weight. The sample was dried in a 105°C oven for 4 hours, then put in a desiccator for 15 minutes to cool then weighed. This treatment was repeated until a constant weight was obtained. The difference between the initial and final weight was the water evaporated from the material. Moisture content was calculated using the formula:

$$\text{Moisture content (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100\% \quad (1)$$

G. Ash Content [8]

The ash content of tea can be up to 8% of dry weight and is determined as follows: 3-5 g samples of tea powder (Y) and a weighed heatproof container (X) were put into an incinerator and incinerated at 600°C. Until reduced to light grey ashes. The ash-filled container was cooled in a desiccator, then weighed (Z), and ash content calculated by the following formula:

$$\text{Ash Content (\%)} = \frac{(Z+X)}{Y} \times 100\% \quad (2)$$

Key:

X: container weight (g)

Y: Sample initial weight (g)

Z: total weight of container and ash after incineration (g)

H. Total Anthocyanin [9]

A 1-gram sample was mixed with 9 ml ethanol and 1 ml concentrated HCl. The solution is then mixed with a buffer solution of potassium chloride (4 ml, pH 1.0) and a sodium acetate buffer solution (4 ml, pH 4.5). The mixture's absorbance was measured at wavelengths of 510 nm and 700 nm using a UV-Vis spectrophotometer. Absorbance was calculated as $A = [(A_{510} - A_{700}) \text{ pH } 1,0] - [(A_{510} - A_{700}) \text{ pH } 4,5]$ using the molar extinction coefficient for anthocyanin of 26.900. Total anthocyanin is calculated as cyanidin-3-glucoside using the following equation:

$$\text{Anthocyanin} = \frac{A \times MW \times DF \times V \times 10^3}{\epsilon \times L \times m} \times 100\% \quad (3)$$

Key:

A: Absorbance

MW: Molecular weight of cyanidin-3-glucoside (449.2 Da)

DF: Dilution factor

V: Final volume (ml)

10³: Conversion factor (g to mg)

ε: Molar absorbance of cyanidin-3-glucoside (26.900)

L: Width of the cuvette (1 cm)

m: Sample weight (g)

I. Qualitative Analysis of Saponin Compounds [10]

100 mg of the sample was mixed with 10 ml water and filtered. The filtrate was placed in a test tube and shaken. The formation of a stable foam indicated the presence of saponin.

J. Antioxidant Analysis [11]

To make a control solution, 4 mg DPPH was mixed with 30 ml distilled water and 70 ml methanol, allowed to stand for 15 minutes then mixed again. The liquid was placed in a cuvette, and absorbance at 517 nm measured using a UV-Vis spectrophotometer. To determine the sample's absorbance at 517 nm, it was diluted with methanol to produce a regularly

spaced series of dilutions before being placed in the UV-Vis spectrophotometer in a cuvette. The % inhibition was obtained using the formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\% \quad (4)$$

From this, the value of IC₅₀ was calculated statistically using linear regression:

$$Y = a + bX \quad (5)$$

Where,

Y: % Inhibition (dependent variable)

X: concentration of sample solution (independent variable)

a: intercept

b: regression coefficient

K. Total Polyphenols [12]

A 1 ml of sample was diluted with distilled water with a 1:10 dilution. 1 ml of this sample mixture was put into a test tube along with 1 ml of ethanol, 2 ml of distilled water, 1 ml of *Folin-ciocalteu* reagent (50%), and mixed in a vortex mixer. After 5 minutes, 1 ml of Na₂CO₃ (10%) was incorporated into the liquid until homogeneous. The reaction mixture can stand in a dark place wrapped in *aluminum foil* for 60 minutes, and then the absorbance value is measured at a wavelength of 725 nm. Standard curves were made in the same way, with gallic acid of various concentrations replacing the samples. The total content of polyphenols is expressed in mg / L of solution.

L. Brine Shrimp Toxicity Test [13]

A 40 mg of sample was dissolved in 4 ml methanol to make a 10 mg/ml solution. 750 µl of this concentrate was pipetted into methanol to obtain a final concentration of 1000 µg/ml, and 50 µl for a concentration of 100 µg/ml. The 10, 100, or 1000 µg/ml solutions were placed in 3 test vials each. A control solution was prepared in 3 vials without the addition of the sample solution. The mixtures in the vials were dried by evaporation, and 50 µl *dimethylsulfoxide* (DMSO) and 2 ml of seawater were added. *Artemia salina* Leach shrimp larvae were obtained by incubating shrimp larvae in a culture container and filled with seawater. The container was divided into a light section and a dark section. The shrimp to be hatched were placed in the dark section. After hatching, the larvae went to the light section. Six test vials and two control vials were used for each sample. Tests were repeated three times. Ten shrimp larvae were then placed in each vial and seawater added to make 5 ml. The median lethal concentration LC₅₀ (the concentration before half the larvae is dead) was calculated by using Probit analysis and regression equations, as below:

$$Y = A + BX \quad (6)$$

Where,

Y: Probit value which is set at 5 for LC₅₀

A and B: intercept and slope of a regression line

X: log (Concentration)

M. Catechins and Epigallocatechin Gallate HPLC Method [3]

1) *Standard Preparation*: A 10 mg of the standard was dissolved in acetonitrile solvent (with 1% formic acid) until homogenized in a 10 ml measuring flask. 0.1 ml of this was

pipetted into a 5 ml flask with the addition of solvent to make 5ml.

2) *Sample Preparation*: One ml of this solution was pipetted and put in a centrifuge tube. To this was added 30 ml of chilled methanol, which had been sonicated for 20 minutes. The sample was mixed in a vortex mixer and ultrasonicated for 15 minutes at room temperature, then filtered through Whatman paper. Mobile Phase A consisted of 250 ml of distilled water (for injection) homogenized with THF 1% and H₃PO₄ 0.2%. Mobile Phase B consisted of 250 ml of acetonitrile homogenized with THF 1%. The flow rate was 0.8 ml/min. For the injection process, 95% mobile phase A and 5% mobile phase B were used. System was run 27 minutes after injection of the sample).

III. RESULTS AND DISCUSSION

A. Raw Material Analysis

The moisture content, ash content, anthocyanin, and IC₅₀ value of GLT and TL can be seen in Table 1. The moisture content of *Gambir* leaf tea powder was 5.71%. This value is relatively low compared to traditional teas in Indonesia. High moisture content can cause the tea to become moist and easily damaged [14]. Water content is the most critical factor related to the shelf life of tea. Tea is hygroscopic, which can absorb the water content in the air. So, one factor that must be considered during tea storage is good packaging. If the packaging is not right, the water content will increase so that it is easy to overgrow microorganisms. So, the appropriate moisture content for tea is around 4-8%.

TABLE I
RESULTS OF RAW MATERIAL ANALYSIS

Analysis	GLT	TL
	Mean ± SD	Mean ± SD
Moisture content (%)	5.71 ± 0.76	-
Ash content (%)	1.99 ± 0.49	-
Anthocyanin (mg/g)	-	36.95 ± 0.28
IC ₅₀ value (mg/L)	8.41 ± 8.79	288.56 ± 26.57

(-) No testing was done

The *Ash* content of *Gambir* leaf powder was 1.99%. This value is an indication of mineral content or purity and cleanliness of a material produced. Ash content indicates the mineral content contained in food, and minerals are one of the micronutrients needed by the human body. So, processing raw materials that contain high minerals into drinks will increase the product's functional value.

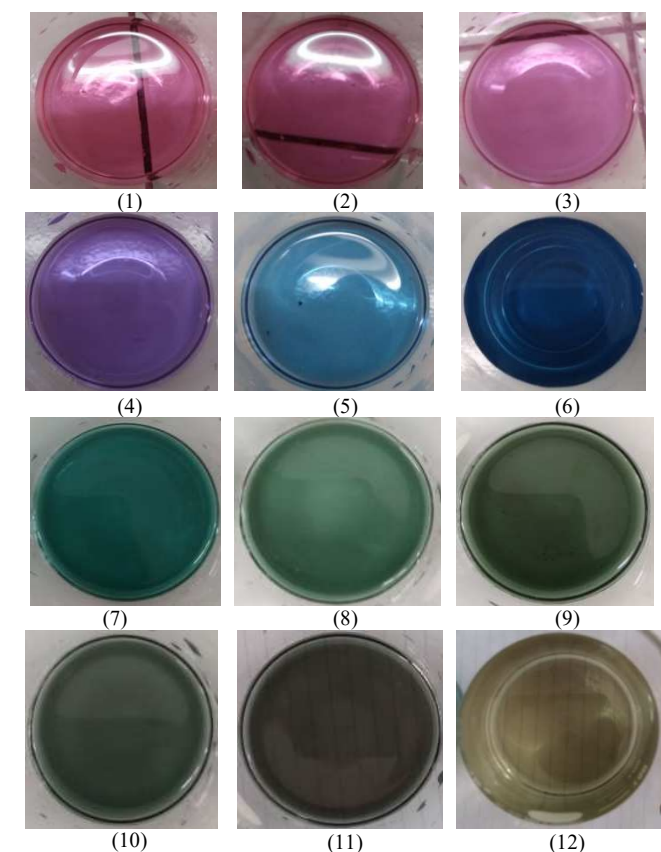
GLT had an antioxidant activity (IC₅₀) of 8.41 mg / L, and in butterfly pea flower extract has an antioxidant activity of 288.56 mg / L. IC₅₀ value in testing antioxidant activity is the amount of sample concentration that can counteract 50% of the free radicals. The lower the IC₅₀ value, the higher the free radical scavenging activity [15]. The data showed that the antioxidant content in GLT is very high compared to the antioxidant content found in TL. However, somehow, the antioxidant content found in TL also contributes to the product produced.

No saponin was found in the TF as no foam was formed. Saponins are susceptible to heat being lost after a long heating process [16]. Manjula, Mohan, Sreekanth, Keerthi, and Devi (2013) found an average saponin content in butterfly pea

flowers of 2.00 mg/g [17]. A high saponin concentration can trigger allergic reactions and digestive disorders, so its elimination of heating is advantageous for human consumption.

B. Color Analysis of Butterfly pea Flower Anthocyanin at Various pH

The colors found from butterfly pea flower anthocyanin at various pH can be seen in Figure 1. Anthocyanin has hydrophilic properties, which make it easy to dissolve in water. Besides being hydrophilic, anthocyanin can also dissolve in polar organic solvents such as ethanol, methanol, acetone, and chloroform.



(1) pH 2; (2) pH 3; (3) pH 4; (4) pH 5; (5) pH 6; (6) pH 7; (7) pH 8; (8) pH 9; (9) pH 10; (10) pH 11; (11) pH 12; and (12) pH 13

Fig. 1 Anthocyanin color various pH

Antioxidants are compounds that function to increase the body's immunity. Antioxidants enhance immunity through their role in protecting cells and human organs. A robust immune system is needed in the face of viruses and bacteria, especially in the present condition, where there is a coronavirus pandemic (covid-19). So, in addition to maintaining cleanliness, food and drinks become essential factors that must be considered. A healthy diet will make the immune system strong. In contrast, if the consumption pattern is not right, the immune system will weaken.

TF *anthocyanin* reacted with acids to produce a soft red color at pH 3; purplish-red at pH 4; purple at pH 5-6; and blue at pH 7. With bases, the color produced is light green. According to Marco et al. (2011), at pH 1-2, the dominant anthocyanin is the cation flavylium with a red color. While at pH <6, it turns into *carbinol*, and part of it becomes a blue

quinoidal with purple is observed. At pH 5 to 9, *quinoidal* is dominant, blue, and at pH > 9 *chalcone* is dominant, which is yellow [18]. The blue color of butterfly pea flowers can be extracted using water solvents because quinoidal is polar. Absorption values of a water extract of butterfly pea flowers between 510 nm and 700 nm showed that the total anthocyanin content was 36.95 mg/g.

C. Antioxidant Activity of the Gambir Tea Leaves with Butterfly pea Extract

The *Gambir* leaf tea beverage was dissolved with methanol with three concentrations, 50 mg / L, 100 mg / L, and 150 mg / L, to determine the antioxidant activity using an IC₅₀ (inhibitory concentration) curve. IC₅₀ values are generally used to express a test material's antioxidant activity using the DPPH free radical immersion method. IC₅₀ is the concentration needed to reduce or counteract 50% of free radicals. IC₅₀ does not represent the number of antioxidants contained in an ingredient directly but the antioxidant strength. The IC₅₀ value is inversely proportional to the antioxidant ability. The IC₅₀ of the *Gambir* leaf tea drinks indicating antioxidant activity can be seen in Table 2.

TABLE II
IC₅₀ VALUE AND TOTAL POLYPHENOL CONTENT OF GLT WITH TF

Treat-ment	IC ₅₀ (mg/L)	Total Polyphenol (mg GAE/g) ± SD	Anthocyanin (mg/L) ± SD
GLT	320.33 ± 8.32 ^a	176.00 ± 9.60 ^a	0.00 ± 0.00 ^a
TF 10	727.77 ± 1.60 ^b	237.67 ± 3.51 ^b	5.69 ± 1.05 ^b
TF 20	553.24 ± 4.57 ^c	280.00 ± 1.00 ^c	8.04 ± 0.32 ^c
TF 30	415.27 ± 3.82 ^d	322.67 ± 0.57 ^d	9.47 ± 0.52 ^c
TF 40	384.71 ± 6.34 ^e	348.33 ± 1.00 ^e	10.84 ± 0.75 ^d

These values range from 320 mg/L to 727 mg/L, and there was a significant difference among the treatments. According to Fitriana *et al.* [19], a compound has a powerful antioxidant activity intensity when the IC₅₀ value is less than 50 mg / L, is said to be strong if the IC₅₀ is 50 to 100 mg / L, moderate if the IC₅₀ is 100 to 250 mg / L, and weak if the IC₅₀ is 250 to 500 mg / L [19]. The *Gambir* leaf tea beverages' antioxidant activity was weak, having IC₅₀ > 250 mg / L. The lowest IC₅₀ and strongest antioxidant activity were found in the TF10 treatment, and the highest/weakest was in the pure GTL tea. In addition to anthocyanins, butterfly pea flowers contain *taraxerol* and β -sitosterol ranging from 0.358 ± 0.006 to 1.04 ± 0.024 mg / g and 0.183 ± 0.004 to 0.334 ± 0.009 mg / g [20].

Compared to the IC₅₀ value of the raw materials, *Gambir* leaf tea powder, and butterfly pea flower extract, *Gambir* leaf tea beverages have decreased antioxidant activity. This result may be because antioxidant compounds tend to be unstable and susceptible to high temperatures. Antioxidant activity in GLT is catechins and their derivatives, *flavonoids*, *phenolics*, and Vitamin C [21]. Other active components found in butterfly pea flowers are *gallic acid*, *catechin*, *ferulic acid*, and *rutin*. The butterfly pea flower also contains the *flavonols kaempferol*, *kaempferol 3-glucoside*, *kaempferol 3-robinobioside-7-rhamnoside*, *quercetin*, and *quercetin 3-glucoside*, which have antioxidant properties [22].

D. Polyphenol Content

GLT *polyphenol* contents can be seen in Table 2. The average total polyphenols in the *Gambir* leaf tea beverages

ranged from 176 mg GAE / L - 348.33 mg GAE / L, and there was a significant difference among the treatments. The lowest being in the TL40 treatment and the highest total polyphenols in the tea made from pure GLT. The standard curve using gallic acid had the parameters $y = 0.023x + 0.122$ with $R^2 = 0.998$ and was based on four concentrations; 10, 30, 50, and 70 ppm. The higher the volume of TF added to the tea, the lower the total polyphenols. TF has a low total polyphenol content of 1.9 mgGAE / L [23]. *Quercetin glycosides* and *ternatin anthocyanins* are two phenol components found in butterfly pea flowers [24].

E. Anthocyanin

Table 2 shows the results of the anthocyanin analysis of GLT drinks. This research found a significant difference among the treatments, except TF20 and TF30. The test used wavelengths of 510 nm and 700 nm and was conducted at pH 1.0 and pH 4.5. The highest anthocyanin value (10.84 mg / L) was found in the TF40 treatment, which has the highest TF concentration, which was the anthocyanin source. This anthocyanin gave a blue tinge to the drink, improving the GLT beverage's attractiveness, usually golden yellow or reddish-brown. TF does not change the taste or aroma of food products because anthocyanin dyes are the only compounds that are detectable to the senses and have no aroma or flavor of their own, so they are used in making drinks and creams in cakes [5]. The most common type of anthocyanin found in butterfly pea flowers is delphinidin [25].

F. Organoleptic Test

An organoleptic test determines the characteristics of food using the subjective senses. In this case, the color, taste, and aroma of the beverages were examined. Twenty-five untrained panelists rated each property on a scale of 1 to 5. The average value of the color scores can be seen in Table 3.

TABLE III
RESULT OF ORGANOLEPTIC ANALYSIS

Treatment	Color ± SD	Aroma ± SD	Taste ± SD
GLT	3.32 ± 0.90	3.08 ± 0.27	3.08 ± 0.27
TF 10	3.60 ± 0.86	3.24 ± 0.52	3.24 ± 0.52
TF 20	3.76 ± 0.52	3.36 ± 0.56	3.36 ± 0.56
TF 30	3.88 ± 0.72	3.40 ± 0.57	3.40 ± 0.57
TF 40	4.16 ± 0.68	3.60 ± 0.64	3.60 ± 0.64

The addition of butterfly pea flower extract to *Gambir* leaf tea drinks had a statistically significant effect ($\alpha < 0.05$) on organoleptic color. The highest value was obtained in the TF40 treatment at 4.16 (likes), while the lowest value was in the GLT treatment at 3.32. The bright blue color due to TF's most enormous volume was attractive to the participants. The amount of TF used has a significantly perceptible effect ($P < 5\%$) on the *Gambir* tea beverage's brightness. This color comes from the TF's anthocyanin dye, particularly the delphinidin derivatives 3,3', 5'-triglucoside, *ternatins* A3, B3 – B4, C1 – C5, D3, and the *preternatins* A3 and C4 [22]. Color is one of the critical factors in making beverage products. Because attractive colors will increase the desire to consume them, this drink uses natural color, which functions as a coloring agent and functions as an antioxidant.

The aroma results showed that the addition of butterfly pea flower extracts significantly ($P < 5\%$) affected *Gambir* leaf tea

beverages' aroma to the panelists. The most favored aroma was from the plain GLT tea (3.60), while the lowest value was the TF10 treatment (3.08). The addition of TF reduced the aroma, and it appears that the panelists found the moderately strong aroma of the TF10 brew more attractive than the stronger GLT or the weaker TF40. All tea beverages had the distinctive *Gambir* aroma due to *1,2-benzenediol*, *catechol*, *1,3,5-benzenetriol*, *dimethyl terephthalate*, and *terephthalic acid* in the GLT [26]. The aroma is also an essential factor that must be considered in making beverages. Usually, beverages with an active component tend to have a preferred aroma, but these drinks have an aroma that panelists can receive.

Analysis of variance indicated no significant differences ($P > 5\%$) in *Gambir* leaf tea drinks' flavor. Panelists found the teas moderately palatable, with average scores ranging from 3.68 for TF40 to 3.60 for TF30.

In general, the taste of the beverages was slightly astringent, like ordinary tea. This flavor comes from the catechin found in *Gambir* leaves and butterfly pea flowers [27-28]. So, this beverage can be accepted by panelists. With an attractive color and functional components in this drink, this drink is highly recommended for daily consumption.

G. Toxicity

The LC_{50} of *Gambir* leaf tea drink products with butterfly pea flower juice's addition were 1.7×10^8 ppm, indicating they were nontoxic and safe for consumption. The classification of toxicity of plant-based materials is divided into three categories, $LC_{50} < 30$ ppm is very toxic, $31 \text{ ppm} < LC_{50} < 1000$ ppm is toxic, and while $LC_{50} > 1000$ ppm is nontoxic [29]. Some natural ingredients that contain active components have specific doses that are safe for consumption. Like cinnamon and nutmeg, although it has a high antioxidant content, there are safe limits for consumption. This drink is one drink that is formulated to have no toxic effects on the body, so it is safe for consumption.

H. Analysis of Catechins and Epigallocatechin Gallate

Data from the catechin and epigallocatechin gallate analysis can be seen in Table 4. The levels of catechin compounds in *Gambir* leaf tea beverage products ranged from 87.98 $\mu\text{g} / \text{mg}$ for pure GLT drink to 58.84 $\mu\text{g} / \text{mg}$ for TF40. of catechin compounds. We can see that there is a decrease in catechin compounds with an increasing volume of TF. The catechin presence is seen by comparing sample absorption at a wavelength of 280 nm with the standard catechin solution.

TABLE IV
RESULT OF CATECHIN AND EPIGALLOCATECHIN GALLATE ANALYSIS

	Treatment	
	GLT	TF 40
Catechin (μg)	87.98	58.84
Epigallocatechin gallate (μg)	96.40	63.74

The catechin and epigallocatechin gallate found in *Gambir* leaf tea beverage products can be seen in Table 4. There is a decrease in levels of epigallocatechin gallate as the volume of TF was increased. *Gambir* leaves are rich in catechin compound epigallocatechin gallate, but TF is not [30]. The addition of TF, therefore, dilutes the epigallocatechin in the beverage. The GLT presence of 96.40 $\mu\text{g} / \text{mg}$

epigallocatechin gallate and 63.74 µg / mg in TF40. Epigallocatechin gallate is the catechin responsible for the astringent taste of teas and why the GLT products had the typical tea taste. However, epigallocatechin gallate's dilution with TF's addition to the tea was not sufficient to perceptively diminish this flavor.

IV. CONCLUSION

All teas were nontoxic, and none had detectable saponin. *Gambir* leaf tea was found to be moderately palatable to the panelists. Butterfly pea flower extract gave the *Gambir* leaf tea drink an attractive color liked by panelists, who also appreciated the reduction in aroma when added at a ratio of TF10. Butterfly pea flower extract increased antioxidant properties, but these were much reduced from the dry ingredients' levels. This result may have been due to sugar in the beverages, but further research is necessary to determine whether alternative sweeteners may better preserve antioxidant properties.

ACKNOWLEDGMENT

Andalas University funds this research through the Acceleration for Professorship Research program with contract number: T/45/UN.16.17/PP.IS-KRP2GB/LPPM/2019.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are included in the article

REFERENCES

- [1] A. Rauf, Rahmawaty, and A. Z. Siregar, "The Condition of Uncaria Gambir Roxb. as One of Important Medicinal Plants in North Sumatra Indonesia," *Procedia Chem.*, vol. 14, pp. 3–10, 2015, doi: 10.1016/j.proche.2015.03.002.
- [2] Anggraini, Neswati, and A. Asben, "Book of Gambir Pengolahan, Komponen dan Manfaat." Universitas Andalas, 2018, [Online]. Available: <http://repo.unand.ac.id/15857/>.
- [3] T. Anggraini and A. A. Neswati, "Mangampo: A Traditional Method from West Sumatra to Extract Gambir from Uncaria gambir," *Pakistan J. Nutr.*, vol. 18, no. 2, pp. 146–152, 2018.
- [4] Ezzudin M and Rabeta MS, "A Potential of Butterfly pea Tree (*Clitoria ternatea*) in Human Health," *Food Research*, Vol. 2 No, 5 pp. 415 – 420, 2018
- [5] H. E. Khoo, A. Azlan, S. T. Tang, and S. M. Lim, "Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits," *Food Nutr. Res.*, vol. 61, no. 1, p. 1361779, Jan. 2017, doi: 10.1080/16546628.2017.1361779.
- [6] Amodio R, "Nutritional Antioxidants as Antidegenerative Agents. *International Journal of Developmental Neuroscience*", Vol. 18 No. 4-5, pp. 356 – 366, 2000.
- [7] Ramli EM and Shalleh RM, "A potential of Butterfly pea tree (*Clitoria ternatea*) in human health," *Food Research*, Vol. 2(5), pp. 415-420, 2018.
- [8] AOAC, "Official Methods of Analysis" Association of Official Analytical Chemist. Inc. Washington DC, pp. 185-189, 1995.
- [9] Coklar H and Akbulut M, "Anthocyanins and phenolic compounds of Mahonia aquifolium berries and their contributions to antioxidant activity," *Journal of Fuctional Foods*," Vol. 35, pp. 166-174, 2017.
- [10] Tiwari PB, Kumar M, Kaur G, Kaur H, Kaur, "Phytochemical Screening and Extraction: A Review," *International Pharmaceutica Scientia*, Vol. 1 No. 1, pp. 98-106, 2011.
- [11] Okoh SO, Asekun OT, Familoni OB and Afolayan AJ, "Composition and antioxidant activities of leaf and root volatile oils of *Morinda lucida*," *J. Nat. Prod. Commun* Vol. 6, pp. 1537–1541, 2011.
- [12] Anggraini T, Wilma S, Syukri D and Azima F, "Total Phenolic, Anthocyanin, Catechins, DPPH Radical Scavenging Activity, and Toxicity of *Lepisanthes alata* (Blume) Leenh," *International Journal of Food Science*, 2019.
- [13] Supraja N, Prasad TNVKV, Gandhi AD, Anbumani D, Kavitha P and Babujanarthanam R, "Synthesis, characterization and evaluation of antimicrobial efficacy and brine shrimp assay of *Alstonia scholaris* stem bark extract mediated ZnONPs," *Biochem Biophys*, Vol. 14, pp. 69-77, 2018.
- [14] Liang G, Dong C, Hu B, Zhu H, Yuan H, Jiang Y and Hao, G, "Prediction of Moisture Content for Congou Black Tea Withering Leaves Using Image Features and Nonlinear Method," *Scientific Reports Nature*. Publishing Group. Vol. 8 pp. 7854, 2018.
- [15] Mandic AI, Dilas SM, Cetkovic GS, Brunet JMC and Tumbas VT, "Polyphenolic Composition and Antioxidant Activities of Grape Seed Extract," *International Journal of Food Properties*, Vol. 11 (4), pp. 713 – 726, 2008.
- [16] Heng L, "Flavour Aspects of Pea and Its Protein Preparations in Relation to Novel Protein Foods," [Thesis] Wageningen Universiy, Netherland, 2005.
- [17] Manjula P, Mohan CH, Sreekanth D, Keerthi B and Devi BP, "Phytochemical Analysis of *Clitoria ternatea* L., a Valuable Medicinal Plant," *Journal of Indian Botanical Society*," Vol. 92 No.3-4, pp.173-178, 2013.
- [18] Marco PH, Poppi RJ, scarminio IS and Tauler R, "Investigation of the pH effect and UV radiation on kinetic degradation of anthocyanin mixtures extracted from *Hibiscus acetosella*" *Food Chemistry*, Vol. 125, pp. 1020-1027, 2011.
- [19] Fitriana WD, Ersam T, Shimizu K and Fatmawati S, "Antioxidant Activity of *Moringa oleifera* Extracts," *Indonesian Journal of Chemistry*, Vol. 16 No. 3, pp. 297 – 301, 2016.
- [20] Makasana J, Holakiyad BZ, Gajbhiye NA, Bishoyi AK and Raju S, "Assessment of chemical diversity in *Clitoria ternatea* accessions byan improved and validated HPTLC method," *Indian Journal of Agricultural Sciences*, Vol. 86 (9), pp. 1133–9, 2016.
- [21] Chen LH, Chen IC, Chen PY and Huang PH, "Application of Butterfly Pea Flower Extract in Mask Development," *Sci. Pharm*, Vol. 86, pp. 53, 2018.
- [22] Oguis GK, Gilding EK, Jackson MA and Craik DJ, "Butterfly Pea (*Clitoria ternatea*), a Cyclotide-Bearing Plant with Applications in Agriculture and Medicine," *Front Plant Sci*, Vol. 10, pp. 645. 2019.
- [23] Kamkaen and Wilkinson, "The antioxidant activity of *Clitoria ternatea* flower petal extracts and eye gel," *Phytother Res*, Vol. 23(11), pp. 1624-5, 2009.
- [24] V. Nair, W. Y. Bang, E. Schreckinger, N. Andarwulan, and L. Cisneros-Zevallos, "Protective Role of Ternatin Anthocyanins and Quercetin Glycosides from Butterfly Pea (*Clitoria ternatea* Leguminosae) Blue Flower Petals against Lipopolysaccharide (LPS)-Induced Inflammation in Macrophage Cells," *J. Agric. Food Chem.*, vol. 63, no. 28, pp. 6355–6365, Jul. 2015.
- [25] Terahara N, Oda M, Matsui T, Osajima Y, Saito N, Toki K, Honda T, "Five New Anthocyanins, Ternatins A3, B4, B3, B2, and D2, from *Clitoria ternatea* Flowers," *J. Nat. Prod*, Vol. 59, pp. 139–144, 1996.
- [26] Nandika D, Syamsu K, Arinana, Kusumawardhani DT and Fitriana Y, "Bioactivities of Catechin from Gambir (*Uncaria gambir*Roxb.) Against Wood-decaying Fungi," *BioResources*, Vol 14(3), pp. 5646-5656, 2019.
- [27] Anggraini T, Tai A, Yoshino T and Itani T, "Antioxidative activity and catechin content of four kinds of *Uncaria gambir* extracts from West Sumatra, Indonesia," *African Journal of Biochemistry Research*, Vol. 5(1), pp. 33-38, 2011.
- [28] Al-Snafi AE, "Pharmacological importance of *Clitoria ternatea* – A review," *IOSR Journal of Pharmacy*, Vol. 6, Issue, pp. 68-83, 2016.
- [29] M. Angelina, I. D. Dewijanti, S. Hartati, and L. Meilawati, "Acute Oral Toxicity and Brine Shrimp Lethality of *Pterocarpus indicus* Standardized Ethanol Extract," *Int. J. PharmTech Res.*, vol. 6, no. 2, 2014.
- [30] Musdja MY, Rahman HA and Hasan D, "Antioxidant Activity of Catechins Isolate of *Uncaria Gambier* Roxb in Male Rats," *International Journal of Health and Life Sciences*, Vol.4 No.2, pp.34-46, 2018