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Original Article

THE EFFECT OF A COMBINATION OF BLACK TEA WITH PEGAGAN LEAF TEA (*CENTELLA ASIATICA*) ON PH, TOTAL ACID, AND FLAVONOIDS

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ABSTRACT

Objective: There is no further research related to the development of Pegagan leaves; this innovation is expected to be able to process a product that is useful for health, one of which can be used as a functional drink. Therefore from that, he purpose is to research the effects of the combination of black tea with Pegagan leaves (Centella asiatica) on pH, total acids, and flavonoids.

Methods: The type of this study was experimental. This study was conducted by combining black tea with different Pegagan leaves. The research design used a Completely Randomized Design with 3 combinations of Pegagan leaves in black tea, which were P1 (0%:100, P2(25%:75), and P3(50%:50).

Results: The results showed the highest flavonoid levels in the combination of black tea with P3 Pegagan leaf tea (50%:50%) of 0.020%. There was no significant effect on the pH value and total acid content. However, on the flavonoid content, there was a significant effect on the combination of black tea and Pegagan leaf tea.

Conclusion: Based on the results of the study, it can be concluded that the combination of black tea and Pegagan leaf tea had no effect on pH value (p=0.911) and total acids (p=0.749). In the flavonoid level, the results of the study showed that there was an effect on the combination of black tea and Pegagan leaf tea (p=0.007).

Keywords: Black tea, Gotu kola leaf, pH value, Total acid content, Flavonoid content

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INTRODUCTION

Tea is the second most consumed beverage in the world after mineral water. In Indonesia, tea drinking is a cultural tradition that dates back to the colonial era. Made from the *Camellia sinensis* plant, tea is prepared by brewing dried leaves, shoots, or petioles. It is not only favored for its unique taste and fragrant aroma but also for its health benefits, such as the presence of anticholesterol activity in tea [1].

There are 3 types of tea differentiated from the differences in processing tea leaves, namely green tea, black tea, red tea (with or without fermentation), and oolong tea (semi-fermented) [2]. Green tea leaves are plants that are popular throughout the world [3]. Black tea is a type of tea made through several processes, starting with withering, milling, fermentation, and drying [4].

Parameters for determining the assessment of the quality of a tea product that is usually used are water content and ash content. The quality assessment is used because the parameters for determining have been determined and recognized by various tea quality standards throughout the world [5]. The water content value of black tea is 8%, while the ash content value of black tea is about 4-8%.

Recent developments have increased public consumption of herbal products such as tea, along with increasing public awareness about health [6]. Based on this reason, there is a study conducted by [7] regarding the innovation of herbal tea using Moringa leaves [8]. Besides Moringa leaves as an herbal tea mixture. Pegagan leaves can also be used as an innovative tea with health benefits.

Pegagan is one of the plants that is easily found and mostly used in traditional medicine in Indonesia [9]. Flavonoids, tannins, and saponins are bioactive components of Pegagan leaves with antibacterial properties [10]. One of the compounds, asiaticoside, is commonly used for wound healing and inhibits the proliferative activity of keloids and hypertrophic scars [11]. The results of the study by [12] showed that Pegagan leaf tea is able to lower blood pressure. Consuming Pegagan leaf tea can provide a diuretic effect that works by excreting more sodium in the blood through urine so that systolic and diastolic blood pressure can decrease significantly [13].

One of the measurements that can be carried out to find out the characteristics of food quality is by measuring the pH and total acidity of a food product. Total acid is closely related to pH value, where an increase in total acid indicates a decrease in pH so that it can find out the acidic properties shown in a food ingredient [14]. In black tea, there are phenolic components in the form of catechin, which interacts with amino acids so that they can generate acidic derivative compounds [15]. Meanwhile, Pegagan leaves have active ingredients of asiaticoside, thankuniside, isothankuniside, and madecassoside, which are aldehydes [16].

Flavonoids are compounds have been known for anti-platelet and anti-coagulant properties, both *in vitro* and *in vivo* [17]. Flavonoid is a phenolic component that is usually found in stems, leaves, flowers, and fruits [18]; it is one of the natural antioxidants and is an active compound contained in black tea that can have a role in protecting the body from free radicals [19, 20]. The most important flavonoid in black tea is catechin, which is responsible for the properties of black tea [21]. Pegagan is also known to contain flavonoid compounds that have activities as antioxidants [22]. The most important bioactive contents in Pegagan leaves are triterpenoids and saponins, which are useful in improving the immune system of the human body [7]. Pegagan also contains *asiaticoside*, it can accelerate the healing of burns [23].

Because there is no further research related to the development of Pegagan leaves, this innovation is expected to be able to process a product that is useful for health, one of which can be used as a functional drink. Therefore, according to the background above, the researcher was interested in making Pegegan leaf tea and studied the effects of the combination of black tea with Pegagan leaves (Centella asiatica) on pH, total acids, and flavonoids.

MATERIALS AND METHODS

Tools and material

Tools used in making Pegagan leaf tea were a knife, spoon, cutting board, baking dish, oven, digital scale, bowl, paper sack, plastic gloves, and bottle. Tools used in testing pH value were beaker glass, measuring cup, dropper pipette, analytical scale, and pH meter. Tools used in testing total acids were burette and stative, Erlenmeyer tube, beaker glass, dropper pipette, and measuring cup.

Tools used in testing flavonoid contents were a spectrophotometer, ultrasonicator, Erlenmeyer, analytical scale, dropper pipette, volumetric flask, beaker glass, vortex mixer, and filter paper.

Materials used in making Pegagan leaf tea were Pegagan leaves and Kemuning black tea. Materials used in testing pH value were distilled water and Pegagan leaf tea. Materials used in testing total acids were distilled water, phenolphthalein indicator, 0.1 N NaOH solution, and Pegagan leaf tea. Materials used in testing flavonoid contents were AlCl3 10%, ethanol 60%, distilled water, NaNO3 0.5%, and Pegagan leaf tea.

Methods

The type of this study was experimental. This study was conducted by combining black tea with different Pegagan leaves. The research design used a Completely Randomized Design with 3 combinations of Pegagan leaves in black tea, which were P1(0%:100, P2(25%:75), and P3(50%:50). Making Pegagan leaf tea was conducted in the Laboratory of Food Ingredient Science UMS. Testing of pH value and total acids was conducted in the Laboratory of Food Quality Analysis UMS. Moreover, testing flavonoid content was conducted in the Chem-Mix Pratama Bantul Laboratory. This study was conducted in July 2023.

Preparation of making pegagan leaf tea and the combination of pegagan leaf in black tea

Method of making Pegagan leaf tea refers to the study by [24]. The experiment took place in biomaterial laboratory of Universitas Muhammadiyah of Surakarta and took in week started from June 26th until 8th Juli 2023. The first step was preparing Pegagan leaf and washing them until clean, cutting Pegagan leaf into small pieces, and putting the cut leaves in the oven at 50 °C for 120 min. Moreover, the combination of Pegagan leaf and black tea as started by preparing paper sacks, putting black tea into the paper sacks with the combinations of 100%, 75%, and 50%, adding Pegagan leaf tea in the paper sacks with the combinations of 0%, 25%, and 50% of the total weight of black tea with 2 g respectively.

Procedures for testing pH value on pegagan leaf tea

Testing pH value refers to the study by [25], which was conducted by measuring 200 ml Pegagan leaf tea, then taking 70 ml of them and homogenizing using a beaker glass for 3 min, then the solution was measured its pH value using pH meter for 5 min. The pH value is the reading of the pH value after a constant position.

Procedures for testing total acids on pegagan leaf tea

Testing total acids refers to the study by [25] using titration methods, which was by measuring 10 ml of Pegagan leaf tea then dissolving it with distilled water until 100 ml, then homogenizing for approximately 3 min and filtering, 10 ml of filtrate was taken and added with 3 drops of phenolphthalein indicator, then the solution was titrated using NaOH 0.1 N solution until red and stable according to the standard solution. Then, analyzing the titration acidity using formula is as follows [25]:

$$Total\ Acid\ (\%) = \frac{V \times N \times BM}{BS \times 1000} \times 100\% \quad \text{..... eq. 1}$$

Notes:

V: Volume of NaOH (ml)

N: Normality of NaOH (N)

BM: Molecular weight of lactic acid

BS: Weight of Pegagan leaf tea (gram)

Procedures for testing flavonoid content on pegagan leaf tea

Testing flavonoid content refers to the study by [21] using the spectrophotometric method. There were 2 stages carried out: making extraction and testing flavonoid.

Making extraction was started by weighing 1 g of Pegagan leaf tea, then adding 25 ml of ethanol 60%; extraction was carried out at 40 °C for 15 min using an ultrasonicator, then the solution was filtered and the filtrate was collected, while the rest was re-extracted with 25 ml of ethanol 60% and repeated 3 times, then the filtrate was collected into one 100 ml of volumetric flask, ethanol 60% solvent was added in the filtrate until the limit line was indicated, the concentration of the extract solution obtained was 10 mg/ml.

Flavonoid testing was started by adding 4 ml of distilled water and 1 ml of extracted Pegagan leaf tea, then homogenizing using vortex mixer, then adding 0.3 ml of NaNO3 0.5% and homogenized, then leaving for 5 min, then adding 0.3 ml of AgCl3 10% and homogenized, then leaving for 5 min, then adding distilled water until the solution became 0 ml and homogenized, then leaving at room temperature for 15 min. Flavonoid absorbance measurement was conducted at a wavelength of 510 nm, and the quercetin calibration curve to obtain the total flavonoid value was conducted by converting the absorbance value. The calibration curve was made according to the quercetin concentration series of 20, 40, 60, 80, and 100 µg/ml. Flavonoid content can be calculated using the formula from [7]:

$$F = \frac{c \times V \times f \times 10^{-6}}{m} x\ 100\% \quad \text{..... eq. 2}$$

Notes:

F: The amount of flavonoid AlCl3 method

c: Quercetin equivalence (µg/ml)

V: Volume of total extract

f: Dilution factor

m: Weight of Pegagan leaf ta (g)

Data analysis

Data analysis used the SPSS 26 application. The research data were tested for normality test using Shapiro-Wilk and the homogeneity test. Data of analysis results were tested using the One Way ANOVA test, where the data were normally distributed and homogeneous ($p \geq 0.05$). However, if the data were not normally distributed and not homogeneous ($p<0.05$), the Kruskal Wallis test was conducted. If the results of the Kruskal Wallis test indicate the effect, with p-value<0.05, on pH, total acids, and flavonoids, then it is followed by the Dunnet test to find out the differences between each combination.

RESULTS AND DISCUSSION

pH value in the combination of black tea and pegagan leaf tea

The normality test used shapiro wilk because of the number of data<50 (12 data). The results of the Wilk test showed a p-value>0.05, whereas the results of the homogeneity test showed a p-value>0.05. According to these results, the data were normally distributed and homogeneous, so the One Way ANOVA test was conducted to find out the effect of the combination of black tea and Pegagan leaf tea on pH value.

The results of pH value in the combination of black tea and Pegagan leaf ta can be seen in table 1 below.

Table 1: The analysis results of pH test in the combination of black tea and pegagan leaf tea

Combination	Ph test				Average±SD
	Test I		Test II		
	Analysis 1	Analysis 2	Analysis 1	Analysis 2	
0%	7.5	7.0	6.9	6.8	7.02±0.34
25%	7.2	6.9	7.1	6.9	7.00±0.18
50%	7.2	7.1	7,2	6.9	7.07±0.18
Sig value					0.911

Based on table 1, the combination of 0% Pegagan leaf tea showed an average pH value of 7.02. Meanwhile, the combination of 25% Pegagan leaf tea showed an average pH value of 7.00, and the combination of 50% Pegagan leaf tea showed an average pH value of 7.07. The results of the One Way ANOVA test showed that the pH test had a p-value=0.911 (p>0.05), which means that there was no significant effect on the pH value in the combination of black tea and Pegagan leaf tea.

According to the research conducted by [7], Pegagan leaves exhibited a higher pH value (pH>7) compared to black tea. The table shows that as the combination of Pegagan leaves increased from 25% to 50%, there was a slight rise in pH value. However, this change was not significant, as the pH remained within the neutral range.

This study used the finished product "Gambyong" black tea from Kemuning, Karanganyar Regency, Central Java. Based on the study by [26], the pH content in black tea is lower than the pH content in Pegagan leaves, where the pH value in black tea ranges from 4.9 to 5.5, while according to the study conducted by [39], based on the results of pH test in Pegagan leaf, pH value ranges from 8.08 to 9.96 [27].

[28] Stated that in the oxidation process, polyphenol components in black tea can generate acidic derivative compounds due to high water content. This acid compound is generated from phenolic components that have oxidation. Based on [15], the increasing temperature is accompanied by a decrease in phenolic acids.

[29] Stated that some proteins will be damaged in the leaves during the heating process of drying tea leaves. This causes the amino acids in the protein to be more free. When heated at high temperatures, catechins that are polyphenol components interact with amino acids to form aldehyde compounds to give aroma to tea [30].

Pegagan leaves have triterpenoid active ingredients, namely asiaticoside, thankuniside, isothankuniside, and madecassoside, which are aldehydes [27]. Thus, if compounds in Pegagan leaves are mixed with compounds in black tea, they will generate acidic properties in the combination of the two ingredients.

According to SNI 3553-2015, the pH of mineral water ranges from 6.0-8.5, which means that Pegagan leaf tea brewed using mineral water allows it to generate a pH that tends to be neutral to alkaline so that acid compounds in Pegagan leaf tea are lost due to the influence of the neutral brewing water.

The total acid in the combination of black tea and pegagan leaf tea

The normality test used Shapiro Wilk because of the number of data<50 (12 data). The results of the Shapiro-Wilk test showed a p-value>0.05, and the results of the homogeneity test showed a p-value>0.05. According to the test, the data were normally distributed and homogeneous, so the One Way ANOVA test was followed to find out the effect of the combination of black tea and Pegagan leaf tea on total acids.

The results of the total acids on black tea with the combination of pegagan leaf tea can be seen in table 2 below.

Table 2: The analysis results of total acid test in the combination of black tea and Pegagan leaf tea

Combination	Total acid test %				Average±SD
	Test I		Test II		
	Analysis 1	Analysis 2	Analysis 1	Analysis 2	
0%	2.10	4.80	3.00	4.50	3.60±1.27
25%	3.90	3.00	4.80	1.50	3.30±1.40
50%	3.00	2.55	1.50	4.50	2.88±1.24
Sig value					0.749

Based on table 2, the combination of 0% Pegagan leaf tea showed an average total acid of 3.60%. Meanwhile, the combination of 25% Pegagan leaf tea showed an average total acid of 3.30%, and the combination of 50% Pegagan leaf tea showed an average total acid of 2.88%. The results of the One Way ANOVA test showed that the total acid test had a p-value = 0.749 (p>0.05), which means that there was no significant effect on the total acid in the combination of black tea and Pegagan leaf tea.

From the results of the study by [2], black tea has a high total acid, which is 3.22%, compared to total acids in Pegagan leaf tea [31]. Moreover, the results of the study conducted by [32] showed that ready-to-serve drinks with Pegagan leaf obtained total acid ranging from 0.0279% to 0.0495%. This means that Pegagan leaf only has very low total acid, so there is no effect or limitation to the total acidity in SNI 01-1902-1995 regarding black tea.

Adding other food ingredients to black tea can make the acidity level of black tea increase or decrease. From the results of the study conducted by [33] on yogurt drinks, after adding Pegagan leaf and rosella flower and testing the total acidity, there was no significant difference in total acids. This is the same as the study conducted in black tea, where after adding Pegagan leaf and testing the total acids, apparently, there was no effect of the combination of black tea and Pegagan leaf tea on total acids.

Tea polyphenols are sensitive to pH and stable in low pH conditions. However, it easily causes damage by strong acids, strong alkalis, strong light radiation, and high temperature [34]. Theaflavin and thearubigin, which are the results of polyphenol compound fermentation, are the most important components affecting the quality of brewing tea. Theaflavin in brewed tea provides slightly acidic properties [35].

Pegagan leaves have triterpenoid active ingredients, namely asiaticoside, thankuniside, isothankuniside, and madecassoside, which are aldehydes [36]. Thus, if compounds in Pegagan leaves are mixed with compounds in the tea, they will generate acidic properties in the combination of the two ingredients.

The acids in the tea needed by the body are lactic acid, which is important for the human digestive system; acetic acid as a preservative to inhibit bacteria so that it gives distinctive aroma and taste of the tea; and malic acid, which is important in the body detoxification process. Gluconic and butyric acids work together to fight yeast infections. Oxalic, nucleic, and amino acids are important in producing energy for the body, regeneration and cell division, improving damaged tissue, and able to form antibodies to fight bacteria and viruses [31].

The flavonoid level in the combination of black tea and pegagan leaf tea

The normality test used Shapiro Wilk because of the number of data<50 (12 data). The results of the Shapiro-Wilk test showed a p-value>0.05, and the results of the homogeneity test showed a p-value<0.05. According to the test, the data were normally distributed but not homogeneous, so the Kruskal Wallis test was conducted to find out the effect of the combination of black tea and Pegagan leaf tea on the flavonoid level.

The results of flavonoid level in the combination of black tea and Pegagan leaf tea can be seen in table 3 below.

Based on table 3, the combination of 0% Pegagan leaf tea showed an average flavonoid level of 0.013%. Meanwhile, the combination of 25% Pegagan leaf tea showed an average flavonoid level of 0.017%, and the combination of 50% Pegagan leaf tea showed an average flavonoid level of 0.020%. The results of the Kruskal Wallis test showed that the flavonoid test had a p-value = 0.007 (p<0.05), which means that there was a significant effect of flavonoid level on the

combination of black tea and Pegagan leaf tea. Furthermore, a Post Hoc test was conducted with Dunnet T3 to find out the significant difference of each combination. The test results showed that all combinations had significant differences.

Table 3: The analysis results of the flavonoid test in the combination of black tea and pegagan leaf tea

Combination	Flavonoid test %				Average±SD
	Test I		Test II		
	Analysis 1	Analysis 2	Analysis 1	Analysis 2	
0%	0.0144	0.0124	0.0145	0.0123	0.013±0.00[a]
25%	0.0167	0.0179	0.0166	0.0178	0.017±0.00[b]
50%	0.0203	0.0204	0.0204	0.0205	0.020±0.00[c]
Sig value					0.007

Note: Letter notation indicates there is a real difference in the results of the Dunnet T3 analysis.

Based on the results of the study by [15], the average flavonoid level in black tea was 0.00391%, while according to the study by [32], the average flavonoid level in Pegagan leaf Simplicia powder was 0.19674%. Therefore, the results of this study are in accordance with the standards for flavonoids of Pegagan leaf and black tea, as the study conducted by [32].

Flavonoid is a polyphenol compound with the highest content in tea. One of the most important flavonoid compounds is catechin, which is responsible for the tea's properties as an antioxidant to protect the body from free radicals [3]. Besides being an antioxidant that is healthy for the body, Catechin also has a role in determining the properties of tea products, such as taste, color, and aroma [30].

Pegagan leaf is also known to contain flavonoid compounds that work as antioxidants, so it is assumed that flavonoid compounds, which have potential as antidiabetics, have a role in lowering blood sugar levels. Furthermore, flavonoids, known as active ingredients in Pegagan leaf, are also believed to be able to neutralize free radicals in the body [22]. The most important bioactive contents in Pegagan leaves are triterpenoid and saponin antioxidants, which are useful in improving the immune system of the human body [37].

CONCLUSION

Based on the results of the study, it can be concluded that the combination of black tea and pegagan leaf tea had no effect on pH value (p=0.911) and total acids (p=0.749). In the flavonoid level, the results of the study showed that there was an effect on the combination of black tea and pegagan leaf tea (p=0.007). The greater the percentage of the combination of pegagan leaf tea, the higher the flavonoid level. At a combination of 50% pegagan leaf tea showed the highest flavonoid level.

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AUTHORS CONTRIBUTIONS

Eni Purwani was contributed to provide ideas for research titles, guided the completion of the manuscript and revising the manuscript; Aliya Maharani Putri has contributed to creating a manuscript, conducting research and revising the manuscript; Aan Sofyan has contribution for provided input during the research and gave input on manuscript writing.

CONFLICT OF INTERESTS

The authors declare that this research has no conflicts of interest

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