BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 1, April 2025 | Pages: 113-122 | DOI: 10.14421/biomedich.2025.141.113-122 ISSN 2540-9328 (online)

Optimization of Total Polyphenol Content In Herbal Tea Formula Bay Leaves, Cinnamon, and Black Tea

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Manuscript received: 16 December, 2024. Revision accepted: 08 May, 2025. Published: 14 May, 2025.

Abstract

Polyphenols are one of the secondary metabolites found in bay leaves, cinnamon and black tea. This mixture is formulated for therapy, one of which is chronic degenerative diseases, such as hypertension. This research aims to optimize the formulation of bay leaves, cinnamon and black tea on total polyphenol levels. This research method includes: determining the polyphenol content of the three simplices, designing the formulation design, determining the optimization of the total polyphenol content of the 14 formulations and the conformation of the selected formula using Design Expert 13. The results of the research obtained the total polyphenol content of bay leaves (49.36 mg GAE/g), cinnamon (61.36 mg GAE/g), and black tea (61.73 mg GAE/g). Optimization of the highest polyphenol content was 58.419 mg GAE/g. The optimal formula conformation consisted of 1 gram of bay leaves, 8 grams of cinnamon and 1 gram of black tea, resulting in a polyphenol content of 59.63 mg GAE/g. The conclusion of this research is the optimization of the highest polyphenol content of 58.419 mg GAE/g with a formulation composition of Bay Leaves 10%, of Cinnamon 80%, and of Black Tea 10%.

Keywords: optimization; total polyphenols; bay leaves; cinnamon; black tea.

INTRODUCTION

Hypertension is a condition where blood pressure is above normal for an extended period. Hypertension is commonly referred to as high blood pressure or chronic degenerative cardiovascular disease which is most common in Indonesia (Rusli et al., 2018).One of the people's efforts to overcome hypertension is by consuming herbal medicines in the form of tea, because they have relatively fewer side effects compared to synthetic medicines.

Tea is a drink obtained from brewing the leaves or shoots of plants using hot water. Based on the processing process, tea is divided into several types, one of which is black tea. Black tea is a type of tea that is made through processing processes such as withering, milling, oxidating, and drying. Black tea has a higher caffeine content than green tea (Wilantari, 2018). According to (Supriyo & Pudjihastuti, 2021), black tea contains the chemical compound polyphenols, these compounds are the highest concentration of substances in black tea. In general, black tea contains polyphenolic compounds (5-27%) consisting of catechins (flavonols) and gallic acid (Evitasari & Susanti, 2021). Polyphenolic compounds are the largest group in black tea which can be helpful in preventing cardiovascular disease by reducing the rate of fat oxidation (Sudaryat et al., 2016). Polyphenolic compounds are also found in natural ingredients such as bay leaves (*Eugenia polyantha* Wight) (Dawley, 2014) and cinnamon (*Cinnamomi Burmannii* Cortex) (Handayani & Paneo, 2021).

Bay leaves (*Eugenia polyantha* Wight) are one of the spices that many people use as a complementary ingredient or flavoring for cooking, apart from that, bay leaves also have pharmacological activity for health (Haryanto et al., 2023). Bay leaves contain polyphenol chemical compounds, essential oils (citral, eugenol), and carbohydrates (Dawley, 2014).Bay leaves contain polyphenolic compounds of (163.27 µg/ml CGAE). The chemical compounds in bay leaves that can act as antihypertensives are polyphenols which function as antioxidants.

The polyphenol compounds found in bay leaves are flavonoids, which have the benefit of preventing oxidation of body cells so that they can prevent hypertension by preventing blockages in blood vessels (Haryanto et al., 2023). According to (Sadik, 2021), polyphenol compounds have the advantage of providing activity to the vascular system, namely by blocking platelets from sticking to each other to form blockages (platelet aggregation), improving endothelial function, so that blood pressure can be reduced. Apart from bay leaves, there are natural ingredients that have similar pharmacological effects on health, such as cinnamon.

Cinnamon (*Cinnamomi Burmannii* Cortex) contains polyphenolic compounds, phytosterols and essential oils (Handayani & Paneo, 2021). Cinnamon has antioxidant activity in the form of polyphenols which can fight free radicals (Munthe, 2021). Cinnamon has pharmacological benefits such as antiseptic, analgesic, anti-inflammatory, antioxidant, and lowering of blood pressure and cholesterol. The polyphenol compounds in cinnamon can play a role in lowering blood pressure with three working mechanisms, namely, destroying blood clots, absorbing blood circulation, and inhibiting cholesterol absorption, so the chemical compounds contained in cinnamon have the properties of preventing and destroying blood clots on the walls of blood vessels. and reduces blood viscosity so that hypertension can be prevented (Sari et al., 2021). According to (Handayani & Paneo, 2021), cinnamon water extract has polyphenolic compounds that have the potential to act as antihypertensives by inhibiting the activity of Angiotensin Converting Enzyme (ACE). Polyphenolic compounds can contribute to human biological activity because these secondary metabolites are included in bioactive compounds. In previous research, cinnamon extract contained polyphenolic compounds reaching 682 mg GAE/g (Ajie et al., 2023).

Due to this similarity, because it contains polyphenolic compounds, black tea can be combined with bay leaf simplicia and cinnamon to become a drink that contains benefits for the health of the body or is called herbal tea. Herbal tea is tea made from ingredients other than tea leaves, namely flowers, seeds, leaves, roots and parts of other plants (Haras et al., 2018). Apart from that, herbal tea can be used as an alternative medicinal drink to treat diseases, one of which is hypertension, by using natural ingredients such as bay leaves.

Based on the similarities in the properties and chemical content of bay leaves (*Eugenia polyantha* Wight), cinnamon (*Cinnamomi Burmannii* Cortex), and "X" black tea because they contain polyphenols, these three natural ingredients will be combined into herbal tea and optimization of the polyphenol content of several formulas to obtain optimal polyphenol content so that it can later be used as an antihypertensive therapy drink. this research aimed to optimize the formula for bay leaves, cinnamon and black tea on total polyphenol levels.

MATERIALS AND METHODS

Research Design

This research used the Completely Randomized Design (CRD) method of several herbal preparation formulas combining bay leaves, cinnamon and black tea, which were designed using expert design software version 13.0. The formulation of the herbal tea underwent quantitative analysis, namely determining the polyphenol content in herbal tea using the UV-Vis spectrophotometry method. The test result data was then processed using Expert Design software version 13.0 to determine the optimal polyphenol content of several selected formulas and conformations.

Materials

The tools used in this research were knives, ovens, baking sheets, analytical scales (Pioneer), 10 mL measuring cups (Pyrex), beakers (Pyrex), 10 mL, 50 mL, and 100 mL measuring flasks (Iwaki), stirring rod (pyrex), volume pipette (DURAN), measuring pipette (DURAN), test tube (pyrex), test tube rack, herb filter (Maksindo), filter paper (Whatman), cuvette, and UV-Vis spectrophotometer instrument (Genesys). The ingredients used in this research were black tea, bay leaves, cinnamon, standard gallic acid, 96% ethanol, sodium carbonate (Na_2CO_3), and distilled water.

Research Procedures

Plant Determination

Determination of bay plants (*Eugenia polyantha* Wight) and cinnamon (*Cinnamomi Burmannii* Cortex) was carried out by the Batu Herbal Materia Medica Laboratory UPT.

Preparation of Bay Leaf Simplicia (Eugenia polyantha Wight)

Take leaves from the bay plant (*Eugenia polyantha* Wight), then sort them to remove dirt stuck to the leaves. Wash the sorted leaves thoroughly using running water. Drain the leaves that have been washed clean, then place the leaves on a baking sheet. Dry the leaves using an oven with a heating temperature of 50°C until the leaves are dry. Dried leaves are characterized by the fact that they are easy to crush with your hands until they are crushed. After the leaves are dry, they are ground using a blender until the appropriate degree of simplicia powder is obtained. (Inorah., 2013).

Preparation of Cinnamon Simplicia (Cinnamomi Burmannii Cortex)

Prepare the cinnamon bark, then sort it to remove dirt stuck to the cinnamon bark. Wash the cinnamon bark using running water. Drain the cinnamon skin which has been washed clean, then place it on a baking sheet. Dry the leaves using an oven at a heating temperature of 50°C until the cinnamon bark is dry. Dried cinnamon bark is characterized by being easy to knead with your hands until it crumbles. After obtaining dry cinnamon bark, then grind the cinnamon bark using a blender until the appropriate degree of simplicia powder is obtained (Inorah., 2013).

Determination of Total Polyphenol Content

Preparation of Gallic Acid Standard Solution

Weigh out 50 mg of gallic acid. Dissolve gallic acid with 96% ethanol in a 100 mL volumetric flask until a solution concentration of 500 ppm is obtained (Fitria, 2020).

Determination of Maximum Wavelength (λ_{max}) of Gallic Acid

Pipette 3 mL of a standard solution of gallic acid with a concentration of 500 ppm, then dissolve it with 96% ethanol: distilled water (1:1) into a 25 mL volumetric flask until a solution concentration of 60 ppm is obtained. Pipette 0.5 mL of a standard gallic acid solution with a concentration of 60 ppm, then add 5 mL of Folin Ciocalteu reagent (diluted 1:10 distilled water). Add 4 mL of 7.5% Na_2CO_3 solution, then shake until homogeneous and leave at room temperature for 15 minutes. Measuring the solution at the maximum absorption wavelength with a UV-Vis Spectrophotometer (Fitria, 2020).

Determining Operating Time

Pipette 3 mL of a standard solution of gallic acid with a concentration of 500 ppm, then dissolve it with 96% ethanol: distilled water (1:1) into a 25 mL volumetric flask until a solution concentration of 60 ppm is obtained. Pipette 0.5 mL of a standard gallic acid solution with a concentration of 60 ppm, then add 5 mL of Folin Ciocalteu reagent (diluted 1:10 distilled water). Add 4 mL of 7.5% Na_2CO_3 solution, then shake until homogeneous and leave at room temperature for 30 minutes. Determine the maximum wavelength of gallic acid by scanning at a wavelength of 500 – 800 nm. The measurement time that shows the absorbance results remain stable is determined as the operating time of the gallic acid analysis method using UV-Vis spectrophotometry (Nengsih, 2022).

Preparation of a Gallic Acid Standard Curve

Make a series of concentrations of 20, 30, 40, 50, and 60 ppm from a standard solution of gallic acid with a concentration of 500 ppm. Pipette 1, 3, 2, 10, 3 mL of each standard solution of 500 ppm gallic acid, then dissolve with 96% ethanol: distilled water (1:1) into each measuring flask 25, 50, 25, 100, 25 mL to the limit mark. Pipette each of the concentration series solutions as much as 0.5 mL in a test tube. Add 5 mL of Folin Ciocalteu reagent (diluted 1:10 distilled water). Add 4 mL of 7.5% Na2CO3 solution, then shake until homogeneous and leave at room temperature for 30 minutes. Measuring the solution at a maximum absorption wavelength of 758nm with a UV-Vis Spectrophotometer (Fitria, 2020).

Simple brew of bay leaves, cinnamon and black tea

Weigh 0.05 grams each of bay leaf simplicity, cinnamon and black tea in a 100 mL beaker, 3 repetitions, then add warm water (temperature 80ºC) and let sit for 8 minutes. The brew is filtered, and the filtrate is placed into a 25 mL flask, then distilled water is added to the mark.

Determination of Polyphenol Content

Pipette the filtrate of the simplica brewing solution of 0.5 mL each of bay leaf simplica, cinnamon and black tea in a test tube. Add 5 mL of Folin Ciocalteu reagent (diluted 1:10 distilled water). Add 4 mL of 7.5% Na2CO3 solution, then shake until homogeneous and leave at room temperature for 30 minutes. Measuring the solution at a maximum absorption wavelength of 758nm with a UV-Vis Spectrophotometer (Fitria, 2020).

Herbal Preparation Formulation

Upper and lower limits of preparations of bay leaves, cinnamon and black tea leaves in formulation optimization using Expert Design software version 13.0. The lower and upper limits obtained from the experimental design used in this optimization study are D-optimal Mixture Design (DMD) with the help of Design Expert software version 13.0. In this design, the independent variables are bay leaves (X1, %), cinnamon (X2, %) and black tea (X3, %), while the dependent variable is total polyphenol content (Y1). The polynomial equation produced by this experimental design is

$$y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1X_2 + A_5X_2X_3 + A_6X_1X_3 + A_7X^2_1 + A_8X^2_2 + A_9X^2_3 + E \quad (1)$$

where A0 - A9 are the coefficients of each variable and interaction, and E is an error. The model used was selected based on the significance of the p-value of the model, the meaninglessness of the Lack of Fit value and considering the Adj-R2 and R2 values. Numerical optimization was carried out to determine the optimal formulation with the criteria of bay leaves (X1, %), cinnamon (X2, %) and black tea (X3, %) for certain pools with high Total Polyphenol (Y) levels. The optimal formula results are verified, then the optimal formula which produces the lowest response bias between predictions and observations (<10%) will be compared with the optimal formula from high energy using ANOVA statistical analysis.

Based on the analysis results, Formula 14 run was obtained, then the three simple powder ingredients using the dry mixing method using a plastic jar (Dry mixing) (Rusli et al., 2018)

Build Information

File Version	13.0.5.0		
Study Type	Mixture	**Subtype**	Randomized
Design Type	Simplex Lattice	**Runs**	14.00
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	58.00		

Design Constraints

Mixture Coding: Actual

Low Limit		Constraint		High Limit
10.000	≤	A:Daun Salam	≤	80.000
10.000	≤	B:Kayu Manis	≤	80.000
10.000	≤	C:Teh Hitam	≤	80.000
		A+B+C	=	**100.000**

Mixture Components

Component	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Daun Salam		Mixture	10.00	80.00	+0 ↔ 10.00	+1 ↔ 80.00	34.17	25.26
B	Kayu Manis		Mixture	10.00	80.00	+0 ↔ 10.00	+1 ↔ 80.00	34.17	25.26
C	Teh Hitam		Mixture	10.00	80.00	+0 ↔ 10.00	+1 ↔ 80.00	31.67	25.83
				Total =	100.00	**L_Pseudo Coding**			

Figure 1. Design of 14 Run Formula Optimization of Bay Leaves, Cinnamon and Black Tea.

Data analysis

In this study, the levels of polyphenolic compounds were determined using the UV-Vis spectrophotometric method using the Folin-Ciocalteu method and gallic acid was used as a standard solution. The resulting polyphenol content data will be processed in statistical form with the Response Surface test using Expert Design software version 13.0. The resulting data can be used to determine optimal polyphenol compounds in several herbal tea formulas, which are described using 3D statistical data.

RESULTS AND DISCUSSION

Determination of Total Polyphenol Content in Bay Leaves, Cinnamon, and Black Tea

Determination of Sample Wavelength

Determination of the maximum wavelength of the sample using a spectrophotometric instrument with a wavelength range of 500 – 800 nm. Determination of the maximum wavelength was carried out to determine the wavelength of gallic acid with the Folin-Ciocalteu reagent which gave the highest wavelength value. To determine the maximum wavelength using a working standard solution of gallic acid with a concentration of 40 ppm. The results of determining the maximum wavelength are 758 nm with a maximum absorbance value of 0.286.

Determination of Absorbance of Working Standards and Calibration Curves

In this research, bioactive polyphenolic compounds were determined using the Folin-Ciocalteu method using UV-Vis spectrophotometry. The results of measuring the absorbance of standard concentrations of gallic acid, 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm, are presented in Table 1. They were used in determining Total Polyphenol levels.

Results and Discussion should be written as a series of connecting sentences, however, a manuscript with a long discussion should be divided into subtitles. Results should be clear and concise.

Table 1. Determination of Absorbance of Gallic Acid Working Standards.

Concentration	Absorbance λ 758 nm
20	0,187
30	0,238
40	0,320
50	0,386
60	0,434

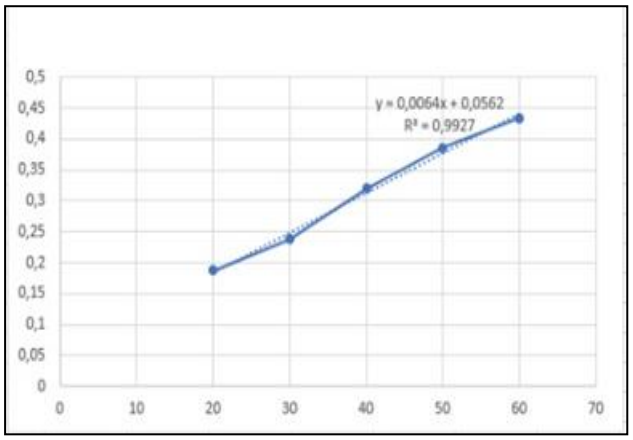

Figure 2. Gallic Acid Standard Curve at Wavelength 758 nm.

Determination of Total Polyphenol Content of Bay Leaves, Cinnamon, and Black Tea

The total polyphenol content was determined based on the linear equation y = 0.0064X + 0.0562 and the absorbance value of bay leaf simplicial extract, cinnamon and black tea, as presented in Table 2.

Table 2. Total Polyphenol Content of Bay Leaves, Cinnamon, and Black Tea.

No.	Description	Total Polyphenol Content (GAE/g)
1.	Bay Leaves	49,36 ± 0,13
2.	Cinnamon	61,36 ± 0,27
3.	Black Tea	61,73 ± 0,39

Herbal Tea Formula from Bay Leaves, Cinnamon, and Black Tea

In this study, the samples used were bay leaves, cinnamon, and black tea. Bay leaves and cinnamon are previously processed into simplicia using oven drying at 50°C, then blended into a coarse powder using a grinder. These three ingredients will be combined in a plastic jar into several formulas. Formulation optimization using the "Design Expert Version 13.0" software obtained 14 runs as presented in Table 3.

Table 3. Design Output of Tea Herbal Formula.

Run	Bay Leaves (A) gram	Cinnamon (B) gram	Black Tea (C) gram
1	1	1	8
2	8	1	1
3	4.5	4.5	1
4	3.4	3.3	3.3
5	1	1	8
6	5.6	2.1	2.1
7	1	4.5	4,5
8	1	8	1
9	4.5	1	4.5
10	8	1	1
11	2.1	5.6	2.1
12	1	8	1
13	4.5	4.5	1
14	2.1	2.1	5.6

Determination of Polyphenol Content in 14 Formulations

The results of polyphenol levels in samples from each formula are presented in Table 5. Based on the data from this table, it can be seen that the highest polyphenol content is formula 8, which consists of 1 gram of bay leaves, 8 grams of cinnamon, and 1 gram of black tea, as shown in Table 4.

Table 4. Total Polyphenol Content of Herbal Teas from Bay Leaves, Cinnamon and Black Tea.

Run	Absorbansi	Total Polyphenol Content (mg GAE/ g)
1	0,380	24,0922
2	0,236	13,2768
3	0,417	25,4859
4	0,468	30,2363
5	0,382	24,7598
6	0,367	21,7184
7	0,408	26,4272
8	0,836	**58,4662**
9	0,210	10,9232
10	0,235	13,1532
11	0,487	33,0611
12	0,838	58,5039
13	0,491	30,6024
14	0,372	23,9998

Data Analysis for Optimizing Polyphenol Levels with "Design Expert 13.0"

The results of the data on polyphenol levels from 14 formulas will then be processed using the "Design Expert 13.0" software to determine the optimization results of polyphenol levels, as shown in Figure 3-7.

Fit Summary

Response 1: Kadar Polifenol

Mixture Component Coding is L_Pseudo.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.0114	0.8523	0.7951	
Quadratic	0.0750	0.0223	0.9103	0.7350	
Special Cubic	0.0130	0.0897	0.9600	0.7783	
Cubic	0.0442	0.3652	0.9839	0.8281	Aliased
Sp Quartic vs Quadratic	**0.0082**	**0.3652**	**0.9839**	**0.8281**	**Suggested**
Quartic vs Cubic	0.3652		0.9841		Aliased
Quartic vs Sp Quartic	0.3652		0.9841		Aliased

Figure 3. Fit Summary.

Model Summary Statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	5.55	0.8750	0.8523	0.7951	556.27	
Quadratic	4.33	0.9448	0.9103	0.7350	719.40	
Special Cubic	2.89	0.9785	0.9600	0.7783	601.91	
Cubic	1.83	0.9938	0.9839	0.8281	466.61	Aliased
Special Quartic	**1.83**	**0.9938**	**0.9839**	**0.8281**	**466.61**	**Suqgested**
Quartic	1.82	0.9951	0.9841		*	Aliased

Figure 4. Summary Statistics Model.

ANOVA for Special Quartic model

Response 1: Kadar Polifenol

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2697.60	8	337.20	100.43	< 0.0001	significant
(1)Linear Mixture	2375.11	2	1187.55	353.70	< 0.0001	
AB	82.75	1	82.75	24.65	0.0042	
AC	52.89	1	52.89	15.75	0.0106	
BC	187.13	1	187.13	55.74	0.0007	
A^2BC	42.99	1	42.99	12.80	0.0159	
AB^2C	10.17	1	10.17	3.03	0.1422	
ABC^2	30.51	1	30.51	9.09	0.0296	
Residual	16.79	5	3.36			
Lack of Fit	3.47	1	3.47	1.04	0.3652	not significant
Pure Error	13.32	4	3.33			
Cor Total	2714.38	13				

Figure 5. Anova and Model Actual Equation.

Final Equation in Terms of L_Pseudo Components

Kadar Polifenol	=
+13.15	* A
+58.42	* B
+24.36	* C
-31.49	* AB
-32.38	* AC
-60.90	* BC
+647.74	* A^2BC
-315.12	* AB^2C
+553.48	* ABC^2

Figure 6. Final Equation.

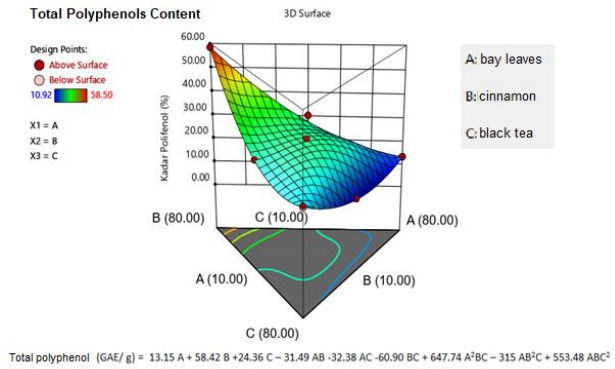

Figure 7. 3D Surface Graphic.

Discussion

In determining total polyphenol content, it begins with determining the maximum wavelength, determining the standard curve, and determining the concentration. Determination of the maximum wavelength of the sample using a spectrophotometric instrument with a wavelength range of 500 – 800 nm. Determination of the maximum wavelength was carried out to determine the wavelength of gallic acid with the Folin-Ciocalteu reagent which gave the highest wavelength value. To determine the maximum wavelength, a working standard solution of gallic acid with a concentration of 40 ppm was used. The results of determining the maximum wavelength are 758 nm with a maximum absorbance value of 0.286. Table 1 and Figure 2 show that the absorbance value is directly proportional to the concentration following the linear regression equation and a standard curve for gallic acid is obtained with the equation y = 0.0064X + 0.0562 and a correlation coefficient (R^2) of 0.9927. The determination of polyphenol levels was carried out using the Folin-Ciocalteu method which determines the amount of polyphenols or phenolic compounds contained in the sample. The Folin-Ciocalteu method is generally widely used in testing polyphenols because this method is effective for forming complex compounds with gallic acid as a comparison. The reaction of gallic acid and Folin-Ciocalteu reagent can be seen in Figure 6. Figure 6 shows that complex compound reactions can be formed due to the hydroxyl group and conjugated double bonds in each benzene ring of gallic acid. The phenolic-hydroxy group will reduce the heteropoly acid (phosphomolybdic-phosphotungstic) contained in the Folin-Ciocalteu reagent into a molybdenum-tungsten complex compound to form a blue color that can be detected using UV-Vis spectrophotometry. The addition of 7.5% Na_2CO_3 reagent functions to create an alkaline atmosphere in the solution so that proton dissociation occurs in phenolic compounds into phenolic ions (Atikah Della Putri et al., 2021). Determination of the polyphenol content of 14 herbal tea formulas was determined based on the linear regression equation values obtained from Figure 1.

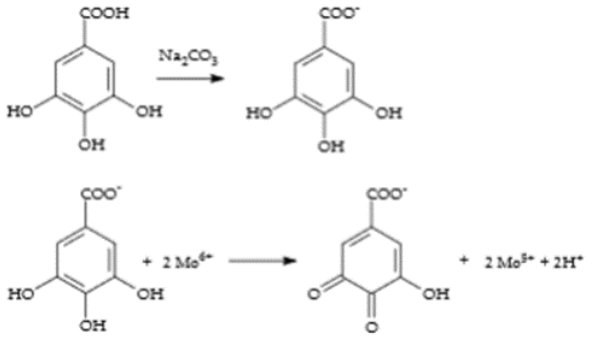

Figure 8. Reaction of Gallic acid with Folin-Ciocalteu reagent.

The results of the analysis of Total Polyphenol levels are shown in Table 2, the content is 40 – 70 mg GAE/g. According to Ilmi et al., 2022, reported polyphenol levels in the cinnamon brew as a functional drink reached 205.5 – 682.17 mg GAE/g, so based on this information it is possible that the formula with cinnamon components is

more dominant than bay leaves and tea. Black produces high levels of polyphenols. Differences in Total Polyphenol levels of plant species and varieties as a result of research and literature, possibly due to differences in environmental and genetic factors, (Prasad et al., 2006; Sarpras et al., 2016).

In this research, 14 formula design outputs were obtained from these three materials, as presented in Table 4, using the "Design Expert Version 13.0" software. The software has designed the formulation. A quantitative test of polyphenol content will be carried out to determine the optimal formula with a high polyphenol content. Data from Table 5, shows that the highest polyphenol content is the formula whose components consist of 1 gram of bay leaves, 8 grams of cinnamon, and 1 gram of black tea. The high levels of polyphenols in this formula are due to the cinnamon component being more dominant than the bay leaf and black tea components. Design Expert analysis, as presented in Figure 3, the output serves to determine the model recommended by Central Composite Design for optimizing polyphenol levels, the recommended model is Special Quartic vs Quadratic. Figure 3 shows the predicted R^2 value in the suggested model of 0.8281 (82.81%), which means the probability of design error is 17.19%. Figure 4, the summary statistics model functions to explain comparing and selecting an appropriate model to explain the relationship between the independent variable and the dependent variable. In the Special Quartic model, an R^2 value of 0.9938 is obtained. If the R^2 value is more significant (closer to 1), the better the model, which means that the herbal tea formula combining bay leaves, cinnamon and black tea (independent variable) influences polyphenol levels (dependent variable). In the Special Quartic vs Quadratic model that has been suggested, an Adjusted R^2 of 0.9839 is obtained. The Adjusted R^2 value is lower than the R^2 value of 0.9938 because the Adjusted R^2 value will only measure independent variables that have a significant influence on the dependent variable. Figure 5, explains the anova test and the actual model equation which functions to determine significant differences between the variations in the data tested. In the recommended Special Quartic model, Anova results were obtained with a p-value <0.0001, this value was <0.05 (p-value 5%). The p-value means that the Special Quartic model is significant, and the possibility of error in the model is slight (< 0.01%). In this model, it is known that the components of bay leaves - cinnamon (X_1X_2), components of bay leaves - black tea (X_1X_3), components of cinnamon - black tea (X_2X_3), repeated components of bay leaves - cinnamon - black tea ($X_1^2X_2X_3$), and the bay leaf – cinnamon – black tea repeat components (X_1,X_2,X_3^2) are significant with p-value <0.05. Meanwhile, the components of bay leaves – cinnamon repetition – black tea ($X_1X_2^2X_3$) are components that are not significant in influencing polyphenol levels because the p-value is 0.1422 (14.22%) which means >0.05 (p-value 5%) . The p-value for Lack of Fit is 0.3652, this value is >0.05 (p-value 5%) which means that the possibility of an error is 36.52%, Lack of Fit in the Special Quartic model is not significant. Figure 6, Final Equation functions to display the appropriate mathematical equation to explain the relationship between the independent variable and the dependent variable. In the Anova analysis there are similarities to the actual model.

$$y = 13{,}15X_1 + 58{,}42X_2 + 24{,}36X_3 – 31{,}49X_1X_2 – 32{,}38X_1X_3 – 60{,}90X_2X_3 +647{,}74X_1^2X_2X_3 - 315{,}12X_1X_2^2\ X3 + 553{,}48X_1X_2X_3^2 \quad (2)$$

or

$$y = 13{,}15\ A + 58{,}42\ B +24.36\ C – 31{,}49\ AB -32{,}38\ AC -60{,}90\ BC + 647{,}74\ A^2BC – 315{,}12\ AB^2C + 553{,}48\ ABC^2 \quad (3)$$

This equation shows that the components of the bay leaf - cinnamon (X_1X_2), bay leaf - black tea (X_1X_3), cinnamon - black tea (X_2X_3), bay leaf - cinnamon - black tea ($X_1X_2^2X_3$) have a decreasing effect on polyphenol content (Y), while the component equations are bay leaves (X_1), cinnamon (X_2), black tea (X_3), repetition of bay leaves – cinnamon – black tea ($X1^2X_2X_3$), bay leaves – cinnamon – repetition black tea ($X_1X_2X_3^2$) has an increasing effect on Total Polyphenol levels (Y). Figure 7 shows the prediction of the optimization point for polyphenol content in several herbal tea formulas from bay leaves, cinnamon and black tea on 3D Surface, namely formula 8 with components consisting of bay leaves (X_1) 10%, cinnamon (X_2) 80% , and black tea (X_3) 10%, with Total Polyphenol Equation (GAE/ g) = 13,15 A + 58,42 B +24.36 C – 31,49 AB -32,38 AC -60,90 BC + 647,74 A^2BC – 315,12 AB^2C + 553,48 ABC^2

Solutions

3 Solutions found

	Number	Daun Salam	Kayu Manis	Teh Hitam	Kadar Polifenol	Desirability	
	1	**10.000**	**80.000**	**10.000**	**58.419**	**0.998**	**Selected**
	2	45.000	45.000	10.000	27.913	0.357	
	3	10.000	10.000	80.000	24.360	0.282	

Figure 9. *Solution of Optimization Herbal Tea Formula*

Optimization results in Figure 9, the recommended solution for optimal conditions for Total Polyphenol content consists of 10% bay leaves, 80% cinnamon, and 10% black tea with a Total Polyphenol content of 58.419

mg GAE/g. Next, conformational determination of total polyphenol content was carried out with this formulation, as presented in Table 5.

Table 5. Total Polyphenol Content in the Conformation Formulation of Bay Leaves 10%, Cinnamon 80%, and Black Tea 10%.

No.	Formula Propose	Total Polyphenol Content (mg GAE/ g)
1.	Replication-1	59.34
2.	Replication-2	60.51
3.	Replication-3	59.06
	Rata-rata	59.63±0.769

The composition of bay leaves, cinnamon and black tea has differences in the amount of polyphenol content, as presented in Table 4.2, where the total polyphenol content in bay leaves is 49.36 ± 0.13, cinnamon 61.36 ± 0.27 and black tea 61.73 ± 0.39 mg GAE/g. It can be seen that the total polyphenol content in the composition of the three single levels is in line with the content in the mixed formulation levels.

Bay leaves were detected to contain polyphenolic compounds consisting of caffeic acid, gallic acid, and triterpene squalene. Of these three compounds, gallic acid was proven to have potential antihypertensive action. Gallic acid is trihydroxybenzoic acid with a hydroxy group that has antioxidant activity and therapeutic effects, one of which is cardiovascular. However, according to previous literature studies (Parawati & Mulyanti, 2022), brewing has a total polyphenol content of 163.27 mg GAE/g. Apart from that, research (Palupi & Widyaningsih, 2015) stated that the total polyphenol content in the bay leaf tea functional drink was 134.02 mg GAE/g. Apart from bay leaves, Total Polyphenol compounds can be found in other natural ingredients such as black tea. Black tea is tea leaves that are processed using a fermentation process. Black tea contains total polyphenol levels of 165 mg GAE/g. In general, black tea contains derivatives of polyphenol compounds which have antioxidant effects. These compounds include catechins with phenolic hydroxyl groups, gallic acid and theaflavins with their hydroxyl groups. According to (Sudaryat et al., 2016), black tea contains total polyphenol levels of 165 mg GAE/g, while according to (Paramita et al., 2020), the results of determining polyphenol levels in black tea leaves are 150 mg GAE/g. Apart from containing polyphenolic compounds, bay leaves and black tea contain phenolic acid compounds consisting of p-hydroxybenzoic acid, vanillic acid, syringic acid, caffeic acid, cinnamic acid, p-coumaric acid, ferulic acid and gallic acid. Apart from being found in bay leaves and black tea, polyphenol compounds can also be found in cinnamon. Cinnamon contains polyphenol compounds consisting of cinnamaldehyde, catechin, protocatheuic acid, quercetin, epicatechin, p-coumaric acid, p-hydoxybenzoic acid, syringic acid, and caffeic acid. According to research (Sholihah et al., 2023), cinnamon contains total polyphenol levels of 682 mg GAE/g, while according to (Chan et al., 2014) the polyphenol content in boiled cinnamon water is 315.02 mg GAE/g. Apart from that, the cinnamaldehyde compound is the main compound derived from the polyphenol group in cinnamon with an aldehyde group which has a protective role against cardiovascular disease, one of which has an anti-platelet aggregation effect and in vitro can function as a vasodilator (Das et al., 2022).

The optimal formula consists of 1 gram of bay leaves, 8 grams of cinnamon and 1 gram of black tea to produce polyphenol levels of 58.419 mg GAE/g. Polyphenol levels in cinnamon decrease when combined with bay leaves and black tea, this occurs because of the acid standard. The gallate used is influenced by phenolic acid group compounds such as gallic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, caffeic acid, cinnamic acid, p-coumaric acid, and ferulic acid.

The presence of phenolic compounds which are polyphenols and phenolic acids will influence the polyphenol levels in this study. The optimization results are influenced by the content of the three natural ingredients being optimized. Total Polyphenol Equation (GAE/g) = 13,15 A + 58,42 B +24.36 C – 31,49 AB - 32,38 AC -60,90 BC + 647,74 A^2BC – 315,12 AB^2C + 553,48 ABC^2

CONCLUSIONS

Based on the research that has been carried out, it can be concluded that herbal tea from bay leaves, cinnamon and black tea obtained optimal levels of total polyphenols in a formula consisting of 10% bay leaf, 80% cinnamon and 10% black tea components with the results Total polyphenol content was 58.419 mg GAE/g, with Total Polyphenol Equation (GAE/g) = 13,15 A + 58,42 B +24.36 C – 31,49 AB -32,38 AC -60,90 BC + 647,74 A2BC – 315,12 AB2C + 553,48 ABC2.

***Acknowledgements*:** We would like to thank the institutions of the Putra Indonesia Health Polytechnic Malang and the Materia Medika Batu Malang for helping and assisting in the implementation of this research.

***Authors' Contributions*:** Sentot Joko Rahajo's concept and designed the study. Devita Nur Azizah carried out the laboratory work. Ayu Ristamaya Yusuf analyzed the data. Sentot and Devita wrote the manuscript. All authors read and approved the final version of the manuscript.

***Competing Interests*:** The authors declare that there are no competing interests.

***Funding*:** The authors gratefully acknowledge financial support by the Directorate of Research, Technology and

Community Service, Directorate General of Vocational Studies, Ministry of Education and Culture, Research and Technology, Republic of Indonesia, in accordance with Contract Agreement Number: 104/SPK/D.D4/PPK.01.APTV /III/2024, dated 19 March 2024, Number: 025/SP2H/PPKM-PTV/LL7/2024, dated 20 March 2024, Number: 365.1/PLK/PN/2024, dated 22 March 2024, Fiscal Year

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