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Combining Natural Ingredients to Optimise Antioxidant Content in Brewing Herbal Drinks

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Abstract. Herbal drinks are one of the functional foods utilized and needed by the community to maintain health. Herbal drinks consisting of green tea, noni leaves, and butterfly pea flowers can be used to meet the community's needs. The research was conducted by optimizing the combination of natural ingredients to obtain high antioxidant content in herbal drinks. Optimization using RSM Method on Minitab 17, with 2 factors of green tea concentration and maximum concentration of noni leaves 2.4 g, using Central Composite Design with a combination of 13 treatments, one replication. The main parameter tested was DPPH antioxidant activity as a response to the treatments tried. The results obtained were that there was an interaction between the components of green tea and noni leaves on the antioxidant activity of counteracting DPPH free radicals, with the equation: DPPH = 89.8 + 4.2 GT - 23 NL + 5.6 GT * GT + 14.2 NL * NL - 0.58 GT * NL. it can be concluded that there is an interaction between the two ingredients used in the antioxidant activity of the herbal drink.

1 Introduction

Herbal products from the processing of natural resources that are currently being developed are aimed at answering the challenge of obtaining products that provide health benefits, including various natural ingredients for food products that are antidiabetic, anticarcinogenic, and others. This answers the challenges of preventing degenerative diseases or metabolic diseases people suffer today. These natural resources can be processed as a single food ingredient or a combination of several ingredients. To process combined food ingredients into a product can consist of several kinds of ingredients. This product can produce health benefits that synergistically become stronger and more optimal, as well as an effort to increase its added value economically. The natural ingredients used are green tea (*Camellia sinensis*), noni (Morinda sp) leaf, and butterfly

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pea (*Clitoria ternatea* L.) flower, each of which has the ability as an antioxidant as well as other special abilities according to the characteristics of the compounds it contains. Previous research on green tea that has been carried out by other researchers includes in vivo and in vitro and hypoglycemic testing [1], testing with targeted and untargeted metabolomics [2], to recover tea antioxidants [3], for metabolomics testing with other characteristics [4, 5,6]. Meanwhile, research on the butterfly pea flower has been carried out several decades previously and can be traced, namely the effect of butterfly pea flower on free radical hemolysis and oxidative damage [7], phenolic, antioxidant, and color testing [8], amylase binding [9], in vitro testing of antidiabetic properties. and antioxidant [10], anti-obesity metabolomics [11], and antidiabetic properties [12], and research [13] on the bioactive components of butterfly pea flower which showed antioxidant, antihemolytic and antihypertensive effects, inhibiting the activity of α-amylase and α-glucosidase and reducing LDL cholesterol and DNA induced oxidation. Research on noni leaves has also been carried out in parallel with research on noni fruit, including testing the anti-oxidative properties of noni leaves, roots and fruit [14], antioxidant, anti-inflammatory, stress response properties [15], catechins and hypolipidemic activity [16], chemical composition, biological activity and toxicity of noni leaves and fruit [17], and profiling of noni leaf polyphenols and antioxidants [18].

2 Material and method

The ingredients used were green tea obtained from supermarkets and distributed under the premium level quality "KD" brand, originating from Central Java, water content of 6.2% (wet basis). In contrast, butterfly pea flower was obtained from suppliers in Surabaya, which was obtained online with the criteria of plastic packaging, and water content 5.9±0.7% (wet basis). Meanwhile, noni leaves were a natural material obtained around the Keputih Permai housing complex, which was treated with air drying, so that the water content was obtained at 6 ± 0.5% (wet basis). The classification of all chemicals for testing to scavenge free radical DPPH was a chemical for analysis.

2.1 Material

The materials used were weighted according to the RSM design used by rounding up to two digits after the comma. The weight of the green tea and noni leaf treatment ingredients was a minimum of 0.1 and a maximum of 2.4 g, while the butterfly pea flower had a fixed weight of 0.25 g. The mixture was put into a tea bag, then brewed using hot water at 94 C, for 5 minutes. The brewed water was then tested to obtain a value for its ability to ward off DPPH free radicals.

2.2 Method

The input parameters for RSM are DPPH measurement data obtained using the ability to scavenge DPPH free radicals' method [19]. The results of the DPPH analysis were carried out from the average of 4 replications which were read 3 times (*triplo*) and included as 1

replication in this RSM method. The RSM designs displayed are Central Composite Design Model, Factors: 2, Replicates: 1, Base runs: 13, Total runs: 13, Base blocks: 1 Total blocks: 1, Two-level factorial: Full factorial, Cube points: 4, Centre points in cube:5, Axial points: 4, Centre points in axial:0, α: 1.41421.

Table 1. Input data for RSM-Minitab 2017.

Run Order	Green Tea	Noni Leaf	DPPH (ppm)
1	1.05	1.05	60.18
2	1.05	2.39	92.44
3	1.05	0	76.80
4	2	0.10	86.70
5	1.05	1.05	112.60
6	2	0.1	136.78
7	1.05	1.05	90.08
8	0.10	0.10	102.08
9	0.10	2	114.80
10	1.05	1.05	94.26
11	2	2	128.62
12	0	1.05	62.12
13	1.05	1.05	87.48

3 Result and discussion

The results of the study carried out on mixing green tea, noni leaf, and butterfly pea flower can be seen in the residual plot, response surface regression, contour plot, and surface plot as described below.

3.1 Residual plot

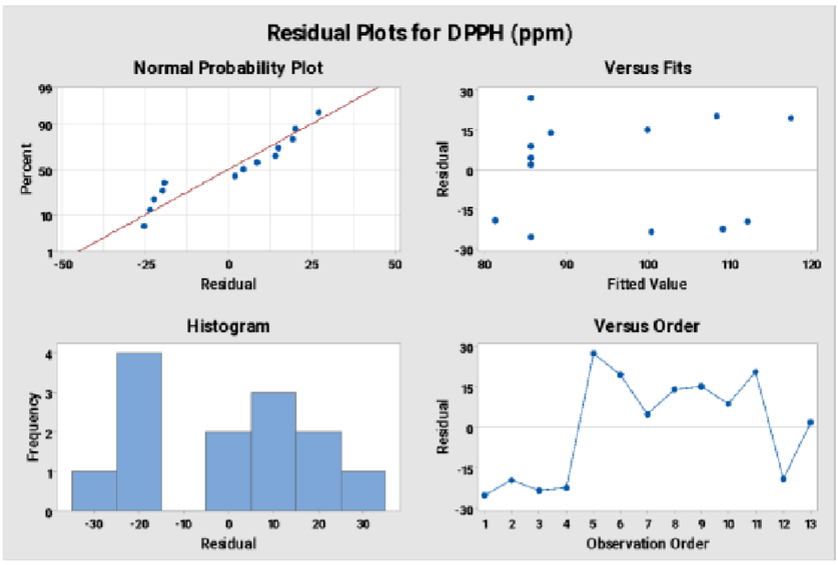

Fig. 1. Residual plot.

From Fig.1. above, from the residual plot test, it can be seen that the residuals are normally distributed, by looking at the graph of residuals versus the fitted value it can be seen that the residuals are randomly distributed, by looking at the histogram you can see that there is a distribution of residuals, but because the data is less than 20, the normal probability plot is used. Meanwhile, by looking at the distribution of residuals versus order, it can be seen that the residuals are independent of each other. From these four things, it can be concluded that the equation model obtained in the response surface regression is as assumed.

3.2 Response surface regression

The results of the analysis of variance for the response surface regression between DPPH (ppm) versus green tea and noni leaf are as follows:

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	1907.11	381.42	0.59	0.707
Linear	2	625.22	312.61	0.49	0.634
Green Tea	1	620.82	620.82	0.97	0.358
Noni leaf	1	3.35	3.35	0.01	0.944
Square	2	1001.22	500.61	0.78	0.495
Green Tea*Green Tea	1	135.43	135.43	0.21	0.660
Noni leaf*Noni leaf	1	859.16	859.16	1.34	0.285
2-Way Interaction	1	108.99	108.99	0.17	0.693
Green Tea*Noni leaf	1	108.99	108.99	0.17	0.693
Error	7	4494.60	642.09		
Lack-of-Fit	3	3075.94	1025.31	2.89	0.166
Pure Error	4	1418.66	354.67		

```
Total                    12   6401.71
Model Summary

      S     R-sq  R-sq(adj)  R-sq(pred)
25.3394  29.79%      0.00%       0.00%

Coded Coefficients
Term                  Effect   Coef  SE Coef  T-Value  P-Value   VIF
Constant                       85.6     11.1     7.70    0.000
Green Tea              18.98   9.49     9.65     0.98    0.358  1.05
Noni leaf               1.39   0.70     9.65     0.07    0.944  1.05
Green Tea*Green Tea     10.2    5.1     11.1     0.46    0.660  1.05
Noni leaf*Noni leaf     25.6   12.8     11.1     1.16    0.285  1.05
Green Tea*Noni leaf    -10.4   -5.2     12.7    -0.41    0.693  1.00


Regression Equation in Uncoded Units

DPPH (ppm) = 89.8 + 4.2 Green Tea - 23.0 Noni leaf
+ 5.6 Green Tea*Green Tea
             + 14.2 Noni leaf*Noni leaf - 5.8 Green Tea*Noni leaf
```

From the calculation of the analysis of variance, the equation is obtained
DPPH (ppm) = 89.8 + 4.2 GT - 23.0 NL + 5.6 GT*GT + 14.2 NL*NL - 5.8 GT*NL
From this equation, it can be seen that noni leaf is a factor in reducing DPPH, but the interaction between the two has a positive sign, and conversely, the interaction between green tea and noni leaf has a negative sign.

3.3 Contour plot

An illustration of the influence between green tea and noni leaf can be seen in Fig. 2. as follows:

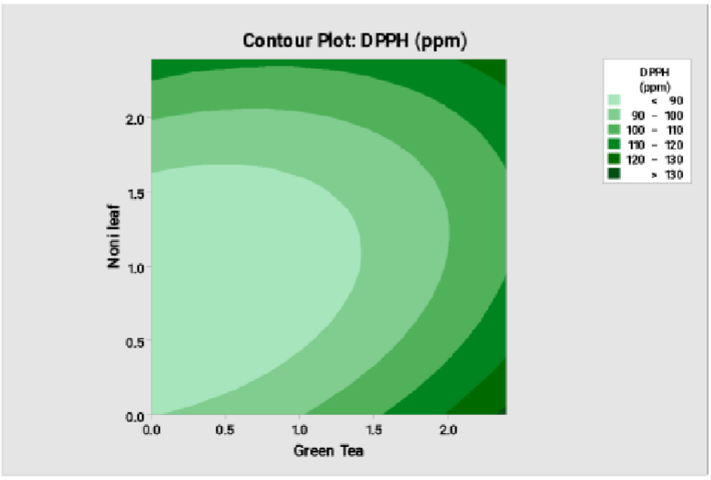

Fig. 2. Contour plot

From Fig. 2., it is visible that there is an interaction between green tea and noni leaf in determining the amount of DPPH from the herbal drink made. Up to a certain value, the DPPH value increases, both the influence of noni leaf and green tea alone and the influence of noni leaf and green tea together. Depicting using a contour plot can help us see the two main influences in the process of mixing herbal drink materials.

3.4 Surface plot

To see more clearly the influence depicted through the contour plot, it can be strengthened by depicting the surface plot in Fig. 3, as follows:

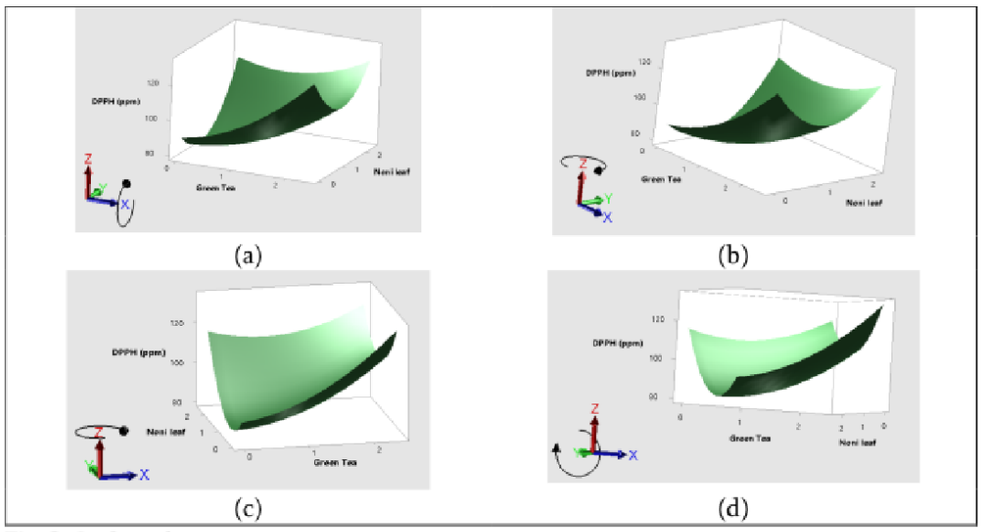

Fig. 3. Surface plot

From Fig. 3, it can be seen that there is a depression that identifies the minimum DPPH value for each concentration of green tea or noni leaf added. The DPPH value that appears in this condition is thought to be the value of the butterfly pea flower's ability to ward off free radicals.

3.5 Prediction

To find out more about the prediction results, calculations were carried out using the RSM which was obtained in Table 2 as follows:

Table 2. Prediction

No.	Green Tea (g)	Noni Leaf (g)	DPPH *) (ppm)
1	0	2	100.581
2	2	0	120.799
3	1	1	85.084

*) Confidence Interval (CI)=95%

By paying attention to the ingredients in herbal drinks other than green tea and noni leaf, namely the presence of butterfly pea flower which is formulated in the same amount for all treatments, then from these predictions, it can be assumed that the presence of butterfly pea flower with noni leaf can provide value which is higher when compared to the presence of the three ingredients together. This also applies to green tea and butterfly pea flowers which provide higher results than a mixture of the three ingredients. This only applies to the concentrations used in this experiment. If there are plans to apply these three ingredients, it is also necessary to optimize these three natural ingredients at once. The presence of noni leaves with tea is also expected to produce bone regeneration benefits as per research [20].

4 Conclusion

Mixing natural ingredients to make herbal drinks can make their abilities synergize with each other, up to certain concentration limits. When mixing green tea with noni leaf in the same amount of butterfly pea flower, it shows a relationship with the equation: DPPH (ppm) = 89.8 + 4.2 GT - 23.0 NL + 5.6 GT*GT + 14.2 NL*NL - 5.8 GT*NL. The potential of noni leaf and butterfly pea flowers can be optimized not only for their antioxidant properties but also for their other unique properties.

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